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Utilization of Tigernut Milk in Yoghurt Production: Physicochemical Properties and Growth of *Lactobacillus bulgaricus* and *Streptococcus thermophiles* in Tigernut Yoghurt

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Abstract

This study investigated the physicochemical properties and growth of *Lactobacillus bulgaricus* and *Streptococcus thermophiles* in tigernut yoghurt. Yoghurt was produced from 3 sets of milk namely 100% tigernut milk, 50:50% of tigernut milk and cow milk; and 100% of cow milk. A set coded: TMYs, TCMYs and CMYs was sweetened with sugar and another set coded: TMYd TCMYd and CMYd was sweetened with date. Pasteurized milk samples were inoculated with the starter culture (1% w/v) and incubated at 43 °C for 6 and 12 h. Sample were analyzed using standard methods. pH decreased significant (P<0.05) after 6 and 12 h of fermentation from 6.44 – 3.57 and 3.52 for samples with sugar and from 6.46 –3.67 and 3.62 for samples with date. Level of pH decrease was significantly (P<0.05) highest in 100% tigernut yoghurt (TMYs and TMYd). Significant increase in TTA was >1.10 %lactic acid for all samples except for CMYd and TCMYd after 6 h. Viscosity ranged from 0.70 - 0.73 and 0.71 – 0.72 Pa.s, while TSS (ºBrix) varied from 7.00 - 11.00 and 6.00 - 11.00 for samples with sugar and date, after 6 and 12 h respectively. Growth rate (/h) of *L. bulgaricus* ranged from 0.26 - 0.52 and 0.14 - 0.27 for sugar sweetened samples after 6 and 12 h and 0.24 - 0.49 and 0.12 - 0.25 for date sweetened samples. *Strep. thermophilus* had growth rate (/h) of 0.39 - 0.40 and 0.32 - 0.60 in sugar and date sweetened samples after 6 h, while it was 0.26 - 0.37 and 0.26 - 0.31 after 12 h. Tigernut milk supported the growth of the fermenting microbes with pH, TTA, viscosity and TSS that was comparable with those of cow milk yoghurt. Tigernut milk can therefore be utilized as a substrate in yoghurt production.

Keywords: *Tigernut, Yoghurt, Lactobacillus bulgaricus, Streptococcus thermophilus, physicochemical properties and Growth rate*

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1. Introduction

The consumption of yoghurt is widespread not just for its refreshing taste but the consciousness of the health benefits conferred by the supposed probiotics used in the fermentation. Yoghurt is a lactic acid bacteria fermented milk product (Aktar, 2022). It is produced by inoculating pasteurized milk with a starter culture of lactic acid bacteria (LAB) and allowed to ferment at a warm temperature (42ºC) for about 8 h. Commonly used starter is made of a mixture *of Lactobacillus debrueckii subsp. Bulgaricus* or *Lactobacillus bulgaricus*, or *Lactobacillus acidophilus* and *Streptococcus thermophiles.* A combination of other LAB such as *Bifidobacterium animalis* and *Enterococus faecium* has also been used (Crittenden *et al* 2003). Depending on the type of LAB, various short chain fatty acids and other metabolites are product from the metabolism of the milk sugar (lactose) (Onyimba *et al.*, 2022**)**. *Strep. thermophiles* is associated with the flavour development from the production of diacetyl, and acetaldehyde and create the yoghurt texture through the production of exopolysaccharides (Purwandari *et al.*, 2007) while the *Lactobacillus* spp. are responsible for acidity required for the coagulation of the milk. There are different types of yoghurt depending on the substrate, consistency and additional ingredients, hence there is drinking (liquid), stirred, strained, set, frozen, sweetened or flavoured yoghurt.

Conventionally, milk is the secretion from the mammary gland for the nourishment of young ones with the exception of colostrum. Predominantly, animal milk, particularly cow milk is used for yoghurt production. However, the continuous increase in the price of animal milk in Nigeria coupled with the quest for lactose free products for those allergic to dairy and dairy products calls for alternatives to animal milk and milk products. Plant based extracts such as soybean, melon seed, walnuts, tigernut etc. offers such alternative to milk beverages and yoghurt production.

Tigernut (*Cyperus esculentus*) is a rhizome spherical crop grown in large quantities in many West-African countries and Spain (Yu *et al*., 2022). The tuber can be oval, ovoid or oblong (Asare *et al.,* 2020). There are three cultivars of tiger nut; yellow, brown and black cultivars. The cultivars possessed different physicochemical (Ayo *et al.,* 2016; Nina *et al.,* 2019; Ayaşan *et al.,* 2020) and functional properties (Nina *et al.*, 2019; Ismaila *et al.*, 2020), attributable to genetic makeup and environment (Duman, 2019; Ihenetu *et al.*, 2021). Tigernut is mostly hawked by street vendors where they are purchased in small portions and consumed raw as a snack or processed into a refreshing beverage called tigernut milk. Washed fresh tubers or dry tubers that have been soaked for 48 -72 h are used in the production of milk. The process involves blending and wet sieving to obtain an aqueous viscous cream coloured extract.

The milk is very popular in Nigeria and some studies have established the quality and effect of packaging on tigernut milk (Obinna-Echem and Torporo, 2018, Obinna-Echem, *et al*., 2019a and 2019b). The milk has a very short shelf-life (48 h) due to its low acidity and rich nutrient content that supports microbial growth. Fermentation of the milk with probiotic lactic acid bacteria may not only aid preservation but the production of a yoghurt like product for value addition to the less utilized tubers. These short chain organic acids produced during fermentation, would improve on keeping qualities of the product due to decrease in pH and also confers unique sensory characteristics. Igwebuike *et al*. (2022) demonstrated the physicochemical, sensory properties and the ability of partially hydrolysed tigernut milk and beetroot beverage to support the growth of probiotic *Lactobacillus acidophilus*.

Tigernut is abundant and underutilized in Nigeria, its extract that have nutritious and medicine qualities would be a potential resource for cheaper production of alternatives to yoghurt product. This was therefore aimed at evaluation of the physicochemical properties of tigernut yoghurt and effect of tigernut extract on the growth of the fermenting microorganisms.

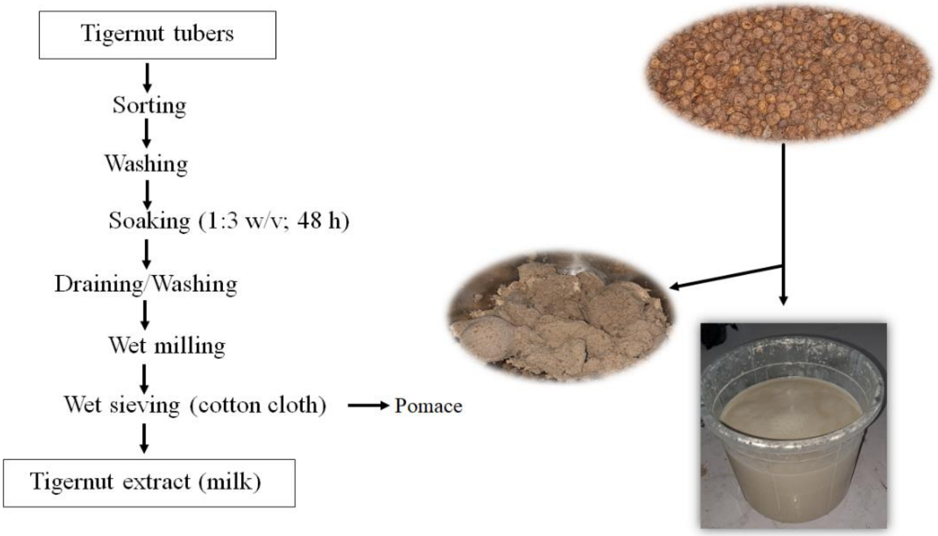

Figure 1: Extraction of Tigernut milk

2. Materials and Methods

1.1. Materials

Dry tigernut tubers, date and sugar used in this study were purchased from Mile 1 market in Port Harcourt, Rivers State Nigeria. Starter culture used was composed of *Lactobacillus bulgaricus* and *Streptococcus thermophiles* manufactured by NPSelection LTD, London, UK. The microbial media and chemicals of analytical grade were obtained from the Department of Food Science and Technology, Rivers State University.

2.2 Methods

2.2.1 Extraction of Milks from Tigernut

The milk from tigernut was extracted by wet sieving of sorted, soaked and wet milled tigernut, as described by Obinn-Echem and Torporo (2018) and shown in Figure 1. About 1 kg of tigernut tubers were sorted to remove stones and broken tubers, washed and soaked in clean warm water to soften the tubers. After 48 h, the soaked tubers were washed, wet milled and sieved through a clean muslin cloth to obtain the milk.

2.2.2 Production of Tigernut Yoghurt

The flow chart for the production of the tigernut yogurt is shown in Figure 2. Two of three sets of milk made of 100% tigernut milk, 50:50% of tigernut milk and cow milk; and 100% of cow milk were used in the yoghurt production. One set with sample codes: TMYs TCMYs and CMYs was sweetened with sugar and another set with sample codes: TMYd TCMYd and CMYd was sweetened with date. For each sample, three sets of 200 mL in conical flask was heated at 72 °C for 15 min and then cooled to 43°C in a water bath. The starter culture was prepared following the manufacturers instruction and inoculated (1% w/v) into the pasteurized milk samples followed by incubation at 43 °C for 6 and 12 h. The initials and samples after each time duration, were removed for analysis. Samples for sensory evaluation were stored in the refrigerator.

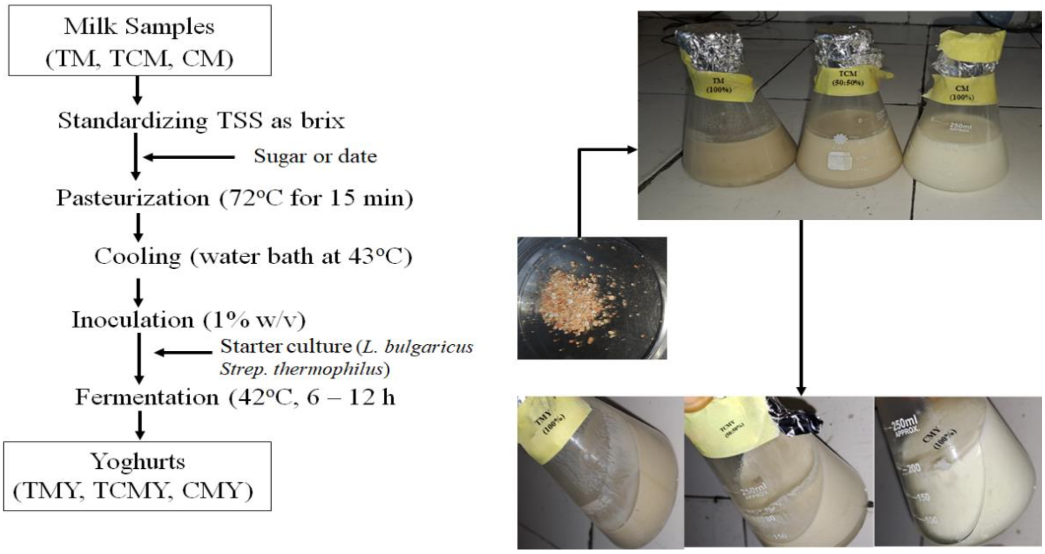

Figure 2: Yoghurt Production from Tigernut milk

2.2.3. Determination of pH, Titratable Acidity as % Lactic Acid, Viscosity and Total Soluble Solid in ºBrix

The determination of pH, TTA and Viscosity properties was as described by Obinn-Echem and Torporo (2018) with some modifications. Briefly, pH of 10 ml of the sample was determined with the aid of a pH meter (TS 652, Germany) that had been calibrated using pH buffers 4 and 7. Thereafter, 1 ml of the sample was diluted to 10 ml with distill water and titrated against 0.1 mol L-1 NaOH with phenolphthalein as indicator to determine the total titratable acidity (TTA). The result was expressed as % lactic acid. Viscosity of the samples was determined with the aid of a Rotary Digital Viscometer (NDJ-85, China) at 20ºC. The rate of flow of 150 mL of sample in a beaker introduced directly unto the rotating spindle was displayed on the LCD screen in Pa.s was recorded as the viscosity. Sugar as ºBrix was determined following the standard AOAC (2012) using Abbe hand refractometer. The prism of the refractometer was cleaned and a drop of the sample was introduced on the prism and the result read off from the scale of the refractometer when held close to the eye.

2.2.4. Enumeration of the Fermenting Microorganisms

Conventional microbiological method was used in the enumeration of *Lactobacillus bulgaricus* on MRS agar and *Streptococcus thermophilus* on nutrient agar. A serial dilution of up to 10^6 was prepared from the stock made of 10 ml of the yoghurt sample homogenized in 90 ml of sterile peptone water. Aliquot of 0.1 ml of the dilutions were spread plated on MRS agar at pH 6.3 and incubated at 37 °C for 24 h for *L. bulgaricus* count and nutrient agar incubated in anaerobic gas jars at 42 °C for 24 h for *Strep. thermophilus* count. Colonies were counted and the number of bacteria expressed as:

Number of bacteria (CFU/ml) = $(N_c \times Df)/V$ …(Eq 1)

Where: Nc = Number of colonies, Df = Dilution plated and V = Volume plated.

Values obtained were converted to Log_{10} CFU/ml for statistical analysis.

2.2.5. Computation of the Generations and Growth Rate of the fermenting Microorganisms in Tigernut Yoghurt

The number of generations, the generation time and growth rate of the fermenting microorganisms in the tigernut yoghurt were computed using the relevant formula.

$G = (N_x – N_0)/\text{Log } 2$ ……….…...(Eq 2)

$GT = (60 \text{ min x time in h})/G$ ……(Eq 3)

$GR = \text{Log } 2/GT$ ………...……….(Eq 4)

Where: G = Number of Generation, N_x and N_0= Final and initial viable counts respectively, GT = Generation time, and GR = Growth rate

3. Results and Discussion

3.1 Physicochemical Properties of Tigernut Milk Yoghurt Sweetened with Sugar and Date

Shown in Table 1, is the pH and of the level of decrease in pH of tigernut yoghurt samples.

The initial pH of the samples before inoculation ranged from 6.46 – 5.89 with the cow milk having the highest. There was significant (P<0.05) decrease in pH after 6 and 12 h of fermentation from 6.44 – 3.57 and 3.52 for samples with sugar and from 6.46 –3.67 and 3.62 for samples with date. The initial pH of the samples before inoculation was in line with the report by Onyimba *et al*. (2022) except for the cow milk which could be attributed to the type of milk used. The decrease in pH after fermentation for 6 and 12 h was similar to the report by Bristone *et al.* (2015) for yoghurts produced from cow milk, tiger nut milk, soybean milk and their combinations but higher than 4.0 – 4.5 reported by Makut *et al.* (2018) for tigernut milk yoghurt and a commercially sold yoghurt. The rate of decrease in pH after 6 h for the samples with sugar was 0.91 – 2.56 and for date it was 2.11 – 2.67 while after 12 h, the rate of decrease was 0.86 – 2.75 and 2.21 – 2.68 respectively. There were wide variations amongst samples fermented with sugar than with dates. The level of decrease in pH was significantly (P<0.05) highest in 100% tigernut yoghurt (TMys and TMYd). This is an indication of more fermentable substrate in the tigernut milk. Decrease in the pH of the fermenting milk samples was accompanied by increase in titratable acidity (TTA).

TTA (%Lactic acid) of the samples before inoculation ranged from 0.23 – 0.55. There was significant (P<0.05) increase in TTA from 0.45 – 1.69 and 1.62 for sample with sugar after 6 and 12 h of fermentation with levels of increase in the range of 0.45 - 1.17 and 0.72 – 0.90 while samples with date had TTA of 0.54 – 1.10 and 1.37 after 6 and 14 h with levels of increase ranging from 0.27 – 0.37 and 0.54 – 0.82. The level of increase in TTA was significantly (P<0.05) highest in 50:50 tigernut (TCMYs and TCMYd) and cow milk yoghurt CMYs and CMYd) after 6 and 12 h. The TTA range after fermentation was higher than 0.50 – 0.65 and 0.91 – 0.95 % lactic acid reported by Akoma *et al.* (2000) and Makut *et al.* (2018) but comparable with the report by Bristone *et al.* (2015). Variations could be attributed to differences in fermentation time, type of substrate and the starter culture used in the yoghurt production. The pH of the yoghurt samples (3.48 - 3.80) after 6 and 12 of incubation, was lower than the specification of 4.2 – 4.6 given by EAC (2018). The TTA for all the samples except CMYd and TCMYd (0.90 and 0.90 %lactic acid, after 6 h) were > than 1.0 %lactic acid recommended by EAC, 2018. This implies that with the substrates' ability to support growth of the starter evidenced in increased metabolic activities, the incubation time can be reduced to when the pH and TTA will falls with the recommended level.

Viscosity of the samples before and after inoculation did not vary significantly (P>0.05) except for CMYd and TCMYd samples respectively. The values ranged from 0.69 - 0.72 and 0.70 – 0.74 respectively before and after inoculation. CMYd had the highest viscosity before inoculation and TCMYd had the least after inoculation. The viscosity after 6 and 12 h of fermentation ranged from 0.70 - 0.73 and 0.71 – 0.72. There was no significant (P>0.05) variation after 12 h of fermentation. Viscosity is affected by the strength and number of bonds between casein micelles in yoghurt, as well as their structure and spatial distribution (Izadi *et al.*, 2014). It is also an important parameter that correlates with the consistency, texture and flow of the yoghurt. There was no significant (P<0.05) differences in the samples viscosity this implies that the yoghurt from the tigernut milk is as good as the cow milk yoghurt.

Total soluble solid (ºBrix) of the yoghurt samples are shown in Figure 3. TSS (ºBrix) of the samples before and after inoculation ranged from 9.00 - 12.00 and 10.0 -12.00 respectively, for the sugar and date sweetened samples. There was no significant difference (P>0.05) between the sweeteners but amongst the samples CMYd and TMYd had the least and highest values respectively. After 6 and 12 h of incubation, TSS (ºBrix) ranged from 7.00 - 11.00 and 6.00 -11.00 respectively, were TMYd had the least. These values are in line with the report by Ezeonu *et al* (2016) for different plant based yoghurt. The use of sugar or date made no significant difference (P>0.05) in the TSS of the samples except after 6 and 12 h of fermentation where there was significant (P<0.05) variation in the TSS content of the 100% tigernut yoghurt. The TSS of TMYd (100% tigernut yoghurt with date) was the least after 6 and 12 h of fermentation. This could imply faster utilization of the soluble sugars in the substrate by the fermenting microorganisms. The TSS of the tigernut milk yoghurt with the exception of 100% tigernut with date were comparable with the cow milk yoghurt.

Table 1: pH and Its Level of Decrease in Tigernut Milk Yoghurt Samples

Sweetener	Sample Code	Initials		Fermentation time (h)		Level of decrease	
		Before inoculation	After inoculation	6	12	i - 6	i - 12
Sugar	**CMYs**	5.96±0.06^{a}	5.38±0.01^{a}	3.48±0.01^{e}	3.52±0.03^{a}	0.91±0.02^{d}	0.86±0.04^{e}
	TMYs	6.40±0.07^{b}	6.44±0.04^{e}	3.88±0.05^{a}	3.69±0.04^{c}	2.56±0.08^{a}	2.75±0.07^{a}
	TCMYs	6.34±0.08^{b}	5.43±0.01^{b}	3.57±0.00^{d}	3.54±0.01^{a}	1.86±0.01^{c}	1.89±0.02^{d}
Date	**CMYd**	5.89±0.03^{a}	5.83±0.04^{c}	3.72±0.01^{bc}	3.62±0.00^{b}	2.11±0.04^{b}	2.21±0.04^{c}
	TMYd	6.46±0.01^{b}	6.46±0.07^{e}	3.80±0.01^{ab}	3.79±0.01^{d}	2.67±0.08^{a}	2.68±0.06^{ab}
	TCMYd	6.28±0.03^{b}	6.17±0.04^{d}	3.67±0.01^{c}	3.68±0.01^{c}	2.50±0.02^{b}	2.49±0.04^{b}

Values are means ±standard deviation of duplicate samples. Values with the same superscript are not significantly different (p>0.05)

Table 2: Titratable Acidity (%Lactic Acid) and Its Level of Increase in Tigernut Milk Yoghurt Samples

Sweetener	Sample Code	Initials		Fermentation time (h)		Level of increase	
		Before inoculation	After inoculation	6	12	i – 6 h	i – 12 h
Sugar	**CMYs**	0.47±0.03^{a}	0.45±0.00^{c}	1.28±0.03^{b}	1.24±0.03^{c}	0.83±0.03^{b}	0.79±0.03^{b}
	TMYs	0.23±0.00^{b}	0.90±0.00^{a}	1.35±0.00^{b}	1.62±0.00^{a}	0.45±0.00^{c}	0.72±0.00^{c}
	TCMYs	0.50±0.00^{a}	0.52±0.03^{bc}	1.69±0.03^{a}	1.42±0.03^{b}	1.17±0.00^{a}	0.90±0.00^{a}
Date	**CMYd**	0.52±0.03^{a}	0.54±0.00^{b}	0.91±0.02^{d}	1.35±0.00^{b}	0.37±0.02^{d}	0.81±0.00^{b}
	TMYd	0.29±0.03^{d}	0.83±0.03^{a}	1.10±0.00^{c}	1.37±0.03^{b}	0.27±0.03^{e}	0.54±0.00^{d}
	TCMYd	0.55±0.02^{b}	0.55±0.02^{b}	0.90±0.00^{d}	1.37±0.03^{b}	0.35±0.02^{d}	0.82±0.02^{b}

Values are means ±standard deviation of duplicate samples. Values with the same superscript are not significantly different (P>0.05)
CMYs= 100% Cow milk yoghurt with Sugar
TMYs = 100% Tigernut milk yoghurt with sugar
TCMYs= 50% Tigernut and 50% Cow milk yoghurt with sugar
CMYd = 100% Cow milk yoghurt with date
TMYd = 100% Tigernut milk yoghurt with date
TCMYd = 50% Tigernut and 50% Cow milk yoghurt with date'

Table 3: Viscosity (P.aS) of Tigernut Milk Yoghurt Samples

Sweetener	Sample Code	Initials		Fermentation time (h)	
		Before inoculation	After inoculation	6	12
Sugar	**CMYs**	$0.69{\pm}0.01^{b}$	$0.72{\pm}0.00^{a}$	$0.71{\pm}0.01^{ab}$	$0.71{\pm}0.01^{a}$
	TMYs	$0.69{\pm}0.00^{b}$	$0.74{\pm}0.00^{a}$	$0.70{\pm}0.00^{ab}$	$0.71{\pm}0.01^{a}$
	TCMYs	$0.69{\pm}0.00^{b}$	$0.73{\pm}0.00^{a}$	$0.70{\pm}0.00^{b}$	$0.72{\pm}0.01^{a}$
Date	**CMYd**	$0.72{\pm}0.00^{a}$	$0.73{\pm}0.00^{a}$	$0.73{\pm}0.00^{a}$	$0.72{\pm}0.01^{a}$
	TMYd	$0.69{\pm}0.00^{b}$	$0.73{\pm}0.00^{a}$	$0.72{\pm}0.00^{ab}$	$0.72{\pm}0.00^{a}$
	TCMYd	$0.68{\pm}0.00^{b}$	$0.70{\pm}0.00^{b}$	$0.72{\pm}0.01^{ab}$	$0.71{\pm}0.00^{a}$

Values are means ±standard deviation of duplicate samples. Values with the same superscript are not significantly different (P>0.05)
CMYs= 100% Cow milk yoghurt with Sugar
TMYs = 100% Tigernut milk yoghurt with sugar
TCMYs= 50% Tigernut and 50% Cow milk yoghurt with sugar
CMYd = 100% Cow milk yoghurt with date
TMYd = 100% Tigernut milk yoghurt with date
TCMYd = 50% Tigernut and 50% Cow milk yoghurt with date

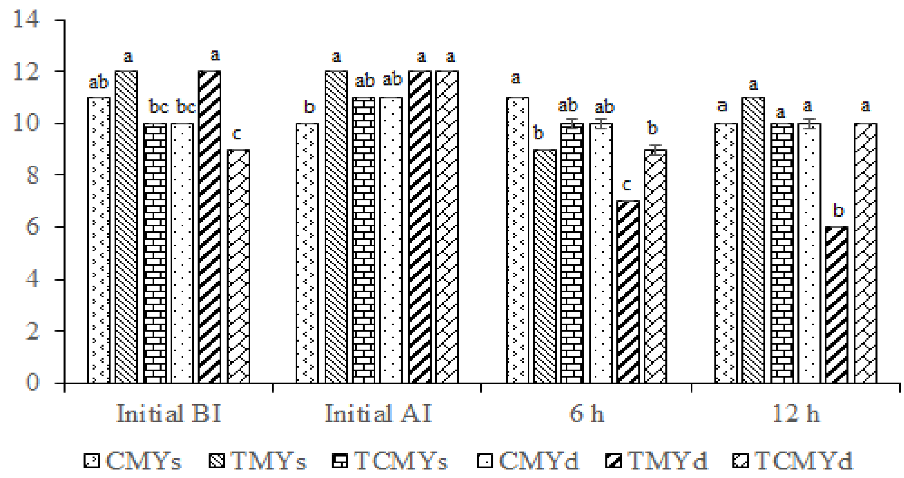

Figure 1: Total soluble solids (°Brix) of tigernut yoghurt.
Bars and error bars are means ±standard deviation of duplicate samples. Means with the same superscript for each sample are not significantly different (P>0.05)
CMYs= 100% Cow milk yoghurt with Sugar
TMYs = 100% Tigernut milk yoghurt with sugar
TCMYs= 50% Tigernut and 50% Cow milk yoghurt with sugar
CMYd = 100% Cow milk yoghurt with date
TMYd = 100% Tigernut milk yoghurt with date
TCMYd = 50% Tigernut and 50% Cow milk yoghurt with date.

3.2. Viable Count and Growth Rate of *L. bulgaricus* in Tigernut Yoghurt

L. bulgaricus count for the yoghurt samples and the growth rates are shown in Table 4 and Figure 2 respectively. The viable count after inoculation did not differ significantly (P>0.05) and the values ranged from 4.45 – 5.54 and 4.52 - 5.64 Log_{10} CFU/mL for the sugar and date sweetened samples respectively. There was significant (P<0.05) increase in *L. bulgaricus* count after 6 and 12 h of incubation. Viable counts in Log_{10} CFU/mL ranged from 6.00 – 8.67 and 7.04 – 7.44 for the sugar and date sweetened samples after 6 h. After 12 h the viable counts were in the range of 7.02 – 8.76 and 7.04 – 8.04 respectively for sugar and date sweetened samples. The increase in viable number is indicative of growth of the organism in the samples. There was significant (P>0.05) differences among the sugar sweetened samples. TCMYs had the least count after 6 and 12 h with TMYs having the highest count. There was no significant (P<0.05) difference among the date sweetened samples after 6 h but after 12 h, TCMYd had the highest count while TYMd had the least.

Computation of the rate of growth (/h) of *L. bulgaricus* in the yoghurt samples using the viable counts showed values ranging from 0.26 - 0.52 and 0.24 - 0.49 for sugar and date sweetened samples after 6 h while it ranged from 0.14 -0.27 and 0.12 - 0.25 after 12 h. CMYs and CMYd had the highest growth rate at both times while TMY had the least. Growth rate of the organisms was higher after 6 h than 12 h. This is a function of time as evidenced in the generation time despite that the number of divisions was higher after 12 h. TMYd had the least number of divisions, highest generation time and the least growth rate.

Table 4: Viability of *L. bulgaricus* in Tigernut Milk Yoghurt Samples

Sweetener	Sample codes	Viability (Log$_{10}$ CFU/ml)			Generation		Generation Time (min)	
		Initial	**6 h**	**12 h**	**6 h**	**12 h**	**6 h**	**12 h**
Sugar	CMYs	5.31±0.11^{a}	7.39±0.10^{ab}	7.02±0.03^{d}	10.40±4.75^{a}	10.71±2.00^{a}	38.60±17.60^{b}	68.44±12.80^{b}
	TMYs	5.54±0.60^{a}	8.67±0.83^{a}	8.76±0.01^{a}	6.89±0.35^{ab}	5.67±0.49^{b}	52.29±2.67^{ab}	127.47±11.03^{a}
	TCMYs	4.45±0.21^{a}	6.00±0.00^{c}	7.56±0.02^{c}	5.14±0.71^{ab}	10.31±0.65^{a}	70.65±9.71^{a}	69.95±4.41^{b}
Date	CMYd	5.60±0.08^{a}	7.06±0.03^{bc}	7.04±0.06^{d}	9.70±2.41^{ab}	10.04±2.45^{a}	38.31±9.52^{b}	73.90±18.00^{b}
	TMYd	4.52±0.73^{a}	7.44±0.01^{ab}	7.54±0.00^{c}	4.84±0.37^{b}	4.77±0.09^{b}	74.59±5.67^{a}	150.93±2.94^{a}
	TCMYd	5.21±0.19^{a}	7.04±0.00^{bc}	8.04±0.00^{b}	6.08±0.62^{ab}	9.40±0.62^{a}	59.50±6.05^{ab}	76.73±5.05^{b}

Values are means ±standard deviation of duplicate samples. Values with the same superscript along each column are not significantly different (P>0.05)

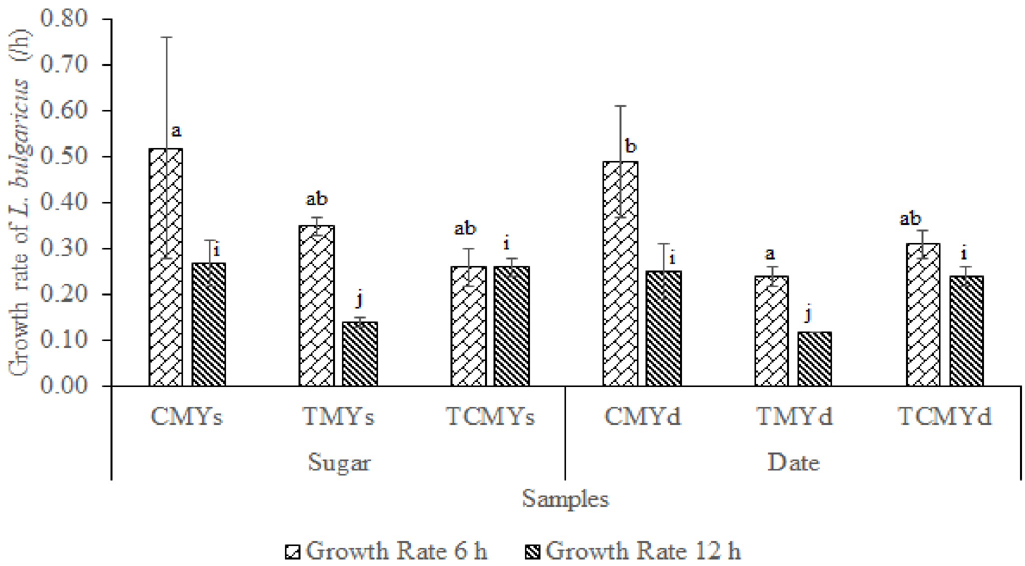

Figure 3: Growth Rate of *L. bulgaricus* in Tigernut Milk Yoghurt Samples

Bars and error bars are means ± standard deviation of duplicate samples. Means with the same superscript for each sample are not significantly different (P>0.05)

CMYs= 100% Cow milk yoghurt with Sugar
TMYs = 100% Tigernut milk yoghurt with sugar
TCMYs= 50% Tigernut and 50% Cow milk yoghurt with sugar
CMYd = 100% Cow milk yoghurt with date
TMYd = 100% Tigernut milk yoghurt with date
TCMYd = 50% Tigernut and 50% Cow milk yoghurt with date

Streptococcus thermophiles count for the yoghurt samples and the increase in count are shown in Table 5 and Figure 3 respectively. There was no significant (P>0.05) difference in the initial count after inoculation, the initial viable counts ranged from 5.23 – 5.60 Log$_{10}$ CFU/mL for yoghurt sweetened with sugar and 5.20 – 5.65 Log$_{10}$ CFU/mL for those sweetened with date. After 6 h of incubation, the count in the samples sweetened with sugar did not vary significantly (P>0.05), it ranged from 7.11 - 7.92 Log$_{10}$ CFU/mL for CMYs and TMYs respectively, but there was significant (P<0.05) difference with the date sweetened samples, the count varied from 6.83 – 8.81 Log$_{10}$ CFU/mL for CMYd and TCMYd respectively. After 12 h of inoculation, the count differed significantly (P<0.05), ranging from 8.31 – 9.46 and 8.48 – 9.32 Log$_{10}$ CFU/mL for the sugar and date sweetened yoghurt samples respectively. TMYs and TMYd had significantly (P<0.05) the highest total bacteria count.

The growth rate (/h) of *Strep. thermophilus* in the yoghurt samples ranged from 0.39 - 0.40 and 0.32 - 0.60 for sugar and date sweetened samples after 6 h while it ranged from 0.26 - 0.37 and 0.26 - 0.31 after 12 h. There was no significant difference in the growth rate of the organisms in the sugar sweetened samples after 6 h. TCMYd had the highest growth rate at both times while TMY had the least. Growth rate after 6 h was higher than 12 h. This is also a function of time as evidenced in the generation time despite that the number of divisions was higher after 12 h. TMYd had the least number of divisions, highest generation time and the least growth rate.

The least number of divisions, highest generation time and lower growth rate of the fermenting microorganisms in the tigernut samples could be attributed to the substrate. The usual environment of the fermenting microorganisms is dairy and dairy products, however, tighernut milk was able to support the growth of the organism. The increase in viable count in tigernut milk yoghurt with incubation time is indicative of the tigernut milk's capability to support the growth of the fermenting microbes as do the cow milk and can be utilized as a substrate in yoghurt production

Table 5: Viability of *Strep. thermophilus* in Tigernut Milk Yoghurt Samples

Sweetener	Sample codes	Viability (Log_{10} CFU/ml)			Generation		Generation Time (min)	
		Initial	6 h	12 h	6 h	12 h	6 h	12 h
Sugar	CMYs	5.29±0.72[a]	7.11±0.05[b]	9.18±0.04[c]	7.87±0.05[b]	12.83±0.00[b]	45.75±0.32[b]	56.14±0.00[c]
	TMYs	5.60±0.00[a]	7.97±0.02[ab]	9.46±0.00[a]	7.73±0.12[b]	14.58±0.17[a]	46.57±0.75[b]	49.37±0.57[b]
	TCMYs	5.23±0.00[a]	7.63±0.05[ab]	8.31±0.02[e]	7.96±0.17[b]	10.24±0.05[d]	45.23±0.96[b]	70.34±0.34[a]
Date	CMYd	5.38±0.09[a]	6.83±0.75[b]	8.48±0.01[d]	7.08±0.25[bc]	12.19±0.14[b]	50.90±1.77[ab]	59.08±0.69[c]
	TMYd	5.65±0.01[a]	7.78±0.06[ab]	9.32±0.03[b]	6.47±0.44[c]	10.30±0.33[d]	55.79±3.76[a]	69.96±2.22[a]
	TCMYd	5.20±0.08[a]	8.81±0.05[a]	8.56±0.01[d]	12.00±0.41[a]	11.17±0.23[c]	30.03±1.04[c]	64.48±1.32[b]

Values are means ±standard deviation of duplicate samples. Values with the same superscript along each column are not significantly different (P>0.05)

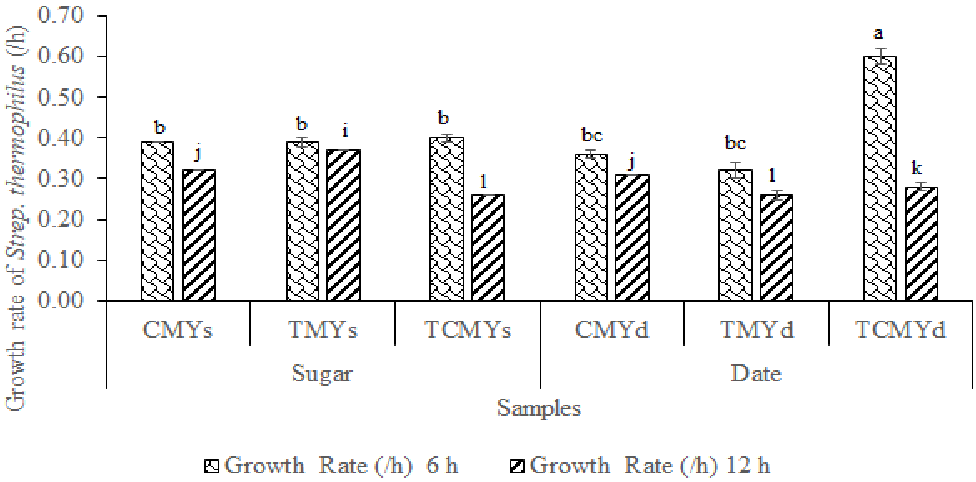

Figure 4: Growth Rate of *Strep. thermophilus* in Tigernut Milk Yoghurt Samples

Bars and error bars are means ± standard deviation of duplicate samples. Means with the same superscript for each sample are not significantly different (P>0.05)

CMYs= 100% Cow milk yoghurt with Sugar
TMYs = 100% Tigernut milk yoghurt with sugar
TCMYs= 50% Tigernut and 50% Cow milk yoghurt with sugar
CMYd = 100% Cow milk yoghurt with date
TMYd = 100% Tigernut milk yoghurt with date
TCMYd = 50% Tigernut and 50% Cow milk yoghurt with date

4. Conclusion

The physicochemical properties of yogurt produced from tigernut milk, cow milk and a blend of tigernut milk and cow milk with the viability of the fermenting microorganism in the yoghurt was investigated. Significant (P<0.05) decrease in the pH of the fermenting milk samples was accompanied by increase in titratable acidity (TTA). There was no significant (P<0.05) differences in the viscosity of the yoghurt samples. There was no significant difference (P>0.05) in the total soluble solid content of the samples for except TMYd that had the least after TSS 6 and 12 h of fermentation. The pH, TTA, viscosity and TSS of the tigernut yoghurts were comparable with those of cow milk yoghurt. Tigernut milk as a substrate was able to support the growth of the fermenting microorganisms evidenced in the viable counts. This is responsible for increased metabolic activities as indicated in the increase in acidity. Tigernut milk can therefore be utilized in yoghurt production but the incubation time can be reduced to when the pH and TTA will falls with the recommended level of 4.2.

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