DEVELOPMENT OF STARTER CULTURE FOR THE PRODUCTION OF COCONUT YOGHURT

Victor E**. Edem,** Aniekpeno I. **Elijah***
Department of Food Science and Technology, University of Uyo, P.M.B. 1017, Uyo, Akwa Ibom State, Nigeria
*E-mail: anielijah@gmail.com

Abstract
To our knowledge, there seem to be no report of the use of endogenous isolates from coconut milk as starter culture for the production of coconut yoghurt. This study was therefore designed to select appropriate microorganisms from coconut milk for use as starter culture for reproducible production of coconut yoghurt and to evaluate the quality of the yoghurt produced. Predominant lactic acid bacteria isolated from spontaneously fermented coconut milk were characterized and tested as potential starter cultures based on their ability to produce lactic acid and hydrogen peroxide. Lactobacillus acidophilus, Lactobacillus bulgaricus and Lactobacillus plantarum were selected and used singly and as mixed cultures for coconut yoghurt production. Starter culture fermented coconut yoghurt (SFCY) were assessed for pH, viscosity, total solid content, total titratable acidity, proximate composition and were subsequently evaluated for consumer acceptability. The pH of the coconut milk sample decreased with increase in fermentation period ranging from 4.71 to 5.72 after 8 h of fermentation. Total titratable acidity, viscosity and total solid content of SFCY ranged from 0.60 to 1.66 %, 3.00 to 8.50 cp and 14.02 to 15.50 % respectively. Consumer sensory analysis showed varying degrees of acceptability for SFCY samples. Overall sensory analysis showed that coconut milk fermented with Lactobacillus acidophilus, Lactobacillus bulgaricus and Lactobacillus plantarum combined starter culture was most preferred.

Keywords: Coconut yoghurt, Starter Culture, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Lactobacillus plantarum*

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1. INTRODUCTION

Yoghurt is commonly referred to as a fermented dairy product obtained from the fermentation of milk by lactic acid producing bacteria such as *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (Fadela *et al*., 2009). The fermentation process results in the conversion of the milk sugar (lactose) to lactic acid, by the LAB, and as well leads to changes in physicochemical and rheological properties that contribute to the unique quality of the product.
Traditionally, yoghurt is obtained from fermentation of cow milk and other dairy products. However, in developing countries such as Nigeria, dairy products are scarce and relatively expensive for low income earners. In addition, strict vegetarians and lactose intolerant people are limited to consuming animal based yogurt. It is of utmost importance therefore, to consider other non-dairy sources as alternative substrates for yogurt production. An inexpensive, readily available milk substitute extracted from locally available plant food such as coconut could play important role in alleviating protein malnutrition in the developing world. It has been reported that the nutrition content of coconut milk is higher compared to cow milk (Yaakob *et al*., 2012).
Production of yoghurt from coconut milk as the only substrate has been attempted by several researchers (Yaakob *et al*., 2012), but none has reported the use of endogenous isolates from coconut milk as starter culture. According to Hati *et al*. (2013), starter culture is defined as an active microbial preparation deliberately added to initiate desirable changes during preparation of fermented products. Starter cultures are of great industrial significance in that they play a vital role in the manufacturing, flavor, and texture development of fermented

dairy foods. Furthermore, additional interest in starter bacteria has been generated because of the data accumulating on the potential health benefits of these organisms (Cogan *et al*., 2007).

The use of appropriate starter culture preferably isolates from spontaneously fermented coconut milk or mixed culture, under optimum conditions could yield coconut milk yoghurt of good quality, which could compete favourably with yoghurt from dairy milk. Development of yoghurt product from coconut milk would help reduce the problem of hunger and malnutrition in the country as well as serve as alternative diet for lactose intolerant people. It would also diversify coconut utilization other than eating as snacks.

The quality and reproducibility of fermented milks and processes are ensured by using industrial starters. Nevertheless, Wouters *et al.* (2002) reported that consumers prefer traditional fermented milks since artisanal starters give these products more typical flavours as compared to industrial starters. The objective of the present study was therefore to develop starter culture for the production of coconut milk yoghurt using endogenous isolate(s) and to evaluate the quality of the yoghurt.

2. MATERIALS AND METHODS

2.1. Sample collection

Matured coconut (dwarf variety) used for the study was obtained from EmVic farm in Ibesikpo Asutan Local Government Area, Akwa Ibom State, Nigeria.

2.2 Extraction of Coconut Milk

Coconut was broken using knife and the coconut water stored in a sterile plastic bottle for further use. The brown layer was carefully removed with knife and the remaining coconut meat (3.5 Kg) washed and grated using manual grater (fabricated by the Department of Food Engineering, University of Uyo, Nigeria. Grated coconut meat (particle size $\leq$ 1617 µm) was mixed in a ratio of 1:1 with a solution containing 75 and 25 % of distilled water and coconut water respectively and allowed to stand in a water bath at 40 ^{o}C for 15 min (Edem, 2016). Thereafter, it was sieved using cheese cloth (folded four times) to obtain the milk. The milk was pasteurized at 90 ^{o}C for 30 min and allowed to assume room temperature (37 ^{o}C).

2.3. Isolation and characterization of bacterial isolates

Nester *et al.* (1995) pure plate method was used for isolation of the microorganisms. A container (plastic) of fresh coconut milk covered with cheesecloth was left on the laboratory bench at room temperature for 3-5 days. One milliliter (1.0 ml) of the 3-5 days spontaneously fermented coconut milk was serially diluted to the 7th factor and plated out on Man Rogosa Sharpe (MRS) agar for LAB species and nutrient agar (NA) for other species. MRS agar plates were incubated anaerobically using the Gas Pack system (Merck Anaerocult type A) at 42, 35 and 30°C for 3 days, in order to provide an optimal temperature for thermophilic *Lactobacilli*, mesophilic *Lactobacilli* and *Leuconostoc*, respectively (Ali, 2011). NA plates were incubated at 37 ^{o}C for 24 h. Distinct colonies of the microorganisms were isolated based on predominance and sub cultured to obtain pure culture of each isolate and stored on appropriate agar slants. Morphological and biochemical characterization was carried out using standard methods (Harrigan and McCane, 1976), while the isolates were identified by reference to Bergey´s Manual of Systematic Bacteriology (Holt et al., 2000).

2.4. Screening of isolates

Predominant isolates from spontaneous fermented coconut milk were screened for their ability to produce organic acid and hydrogen peroxide following the AOAC method (AOAC, 2005). This was used as the basis for selection of isolates for development of starter culture.

2.5. Development of starter culture

Selected bacterial isolates were used singly and as mixed culture at equal proportion for production of coconut milk yoghurt. The starter culture for the production of coconut yoghurt was prepared in Tryptone Soy Broth (TSB) amended with coconut milk. About 50 ml of TSB was autoclaved at 121 ^{o}C for 15 min. When cooled to a lukewarm temperature, a loopful of the pure isolate was inoculated in sterilized TBS and incubated at 37 ^{o}C for 24 h. Thereafter, the mixture was then used to ferment the coconut milk.

2.6. Production of coconut milk yoghurt

The method of Yaakob *et al*. (2012) was adopted with slight modification. Coconut milk was sterilized at 90 ^{o}C for 30 min, cooled to 45 ^{o}C, inoculated with starter culture (3 %) and incubated at 37 ^{o}C for 8 h to obtain coconut yoghurt.

2.7. Physicochemical and proximate analysis of coconut milk yoghurt

pH, viscosity, total solid content, total titratable acidity and proximate composition of coconut yoghurt was determined according to the AOAC (2005) method.

2.8. Microbial count

Aliquots (0.1 ml) of appropriate dilutions of coconut yoghurt samples were pour plated in duplicates on plate count agar (PCA, Oxoid, UK), MRS agar (Oxoid, UK) and MacConkey agar (Oxoid, CM 115) and incubated at 30°C for 24 h, 30°C for 48 h and 37°C for 24 h for total bacterial, coliforms and lactic acid bacteria count.

2.9. Sensory evaluation of coconut milk yoghurt

Starter culture fermented coconut yoghurt (SFCY) were subjected to sensory evaluation using a 9 – point Hedonic scale where 1 represents dislike extremely while 9 represents like extremely. Samples were presented to a panel of 20 - trained members drawn from staff and students of the University of Uyo, Uyo, Akwa Ibom State, Nigeria. Panelists were asked to evaluate product characteristics such as appearance, taste, aroma, consistency and general acceptability. Panelists were also served with a glass of water to neutralize taste after analyzing each sample. Commercial yoghurt was used as control.

3.0. Statistical analysis

Data obtained were subjected to analysis of variance. Differences between the means were evaluated by Duncan's multiple range test using the IBM SPSS version 20. Significant differences were expressed at 5% level.

3. RESULTS AND DISCUSSION

Biochemical characteristics and percentage occurrence of predominant bacterial species isolated from spontaneously fermented coconut milk are presented in Table 1. Most of the identified microorganisms (*Lactobacillus cellobiosus, Lactobacillus acidophilus, Lactobacillus bulgaricus, Flavobacterium meningosepticum, Lactobacillus fermentum* and *Lactobacillus plantarum*) belong to the genus *Lactobacillus*. Kolapo and Olubamiwa (2012) also isolated *Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus fermentum* and *Lactobacillus plantarum* from naturally fermented soymilk while Wakil *et al*. (2014), isolated *Lactobacillus plantarum* from naturally fermented tiger nut milk. *Lactobacillus plantarum* had the highest percentage occurrence (28.1%), followed by *Lactobacillus acidophilus* (23.8%), and *Lactobacillus bulgaricus* (22.4%).

The isolates were further screened for lactic acid and hydrogen peroxide production (Table 2). The result showed that *Lactobacillus acidophilus* produced the highest concentration (0.9008 %) of lactic acid, followed by *Lactobacillus plantarum* and *Lactobacillus bulgaricus,* which were not significantly different ($p>0.05$) from one another. However, *Lactobacillus cellobiosus* produced the least concentration (0.3333 %) of lactic acid. On the other hand, *Lactobacillus bulgaricus* produced the highest concentration (0.122 g/l) of hydrogen peroxide. There was no significant difference ($p>0.05$) in hydrogen peroxide production by the other bacterial species. The difference in amount of antimicrobial compounds may be ascribed to the specificity of strain and species (Badis *et al*., 2004).

Table 1. Characterisation and percentage occurrence of predominant bacterial species isolated from pontaneous fermented coconut milk

Isolate	Cell shape	Gram reaction	Catalase test	Methyl red	Motility	Citrate test	Oxidase test	Spore test	Indole test	Urase test	Glucose	Lactose	maltose	Manitol	Sucrose	Probable dentity	Number of occurrence	% occurrence
A	Rod	+	-	-	-	+	-	-	-	-	A0	00	A0	00	A0	*Lactobacillus cellobiosus*	15	7.1
B	Rod	+	-	-	-	+	-	-	-	-	AG	A0	A0	00	A0	*Lactobacillus acidophilus*	50	23.8
C	Rod	+	-	-	-	+	-	-	-	-	A0	A0	00	00	A0	*Lactobacillus bulgaricus*	47	22.4
D	Rod	-	+	-	-	-	+	-	+	-	A0	A0	A0	A0	00	*Flavobacterium meningosepticum*	8	3.8
E	Rod	+	-	+	-	-	+	-	-	-	A0	A0	A0	00	A0	*Lactobacillus fermentum*	31	14.8
F	Rod	+	-	-	-	-	-	-	-	-	A0	A0	A0	A0	A0	*Lactobacillus plantarum*	59	28.1

+ = positive reaction, - = Negative reaction, A0 = Acid without gas production , AG = Acid with gas production, 00 = No fermentation

Table 2: Screening of bacterial Isolates for lactic acid and hydrogen peroxide production

Isolates	Lactic acid (%)	Hydrogen peroxide (g/L)
Lactobacillus cellobiosus	0.3333 ± 0.11^{c}	0.108 ± 0.001^{b}
Lactobacillus acidophilus	0.9008 ± 0.20^{a}	0.122 ± 0.002^{b}
Lactobacillus bulgaricus	0.8100 ± 0.10^{ab}	0.142 ± 0.000^{a}
Flavobacterium meningosepticum	0.6035 ± 0.20^{b}	0.108 ± 0.001^{b}
Lactobacillus fermentum	0.666 ± 0.11^{b}	0.116 ± 0.010^{b}
Lactobacillus plantarum	0.846 ± 0.10^{ab}	0.122 ± 0.000^{b}

Table 3: Changes in pH of coconut yoghurt samples with fermentation period

Treatments		pH	
	0 h	4 h	8 h
A	6.66 ± 0.040^{a}	6.22 ± 0.120^{a}	5.72 ± 0.100^{f}
B	6.68 ± 0.020^{a}	5.94 ± 0.06^{ab}	5.20 ± 0.200^{d}
C	6.65 ± 0.140^{a}	6.00 ± 0.02^{ab}	5.46 ± 0.260^{e}
D	6.66 ± 0.030^{a}	6.10 ± 1.00^{ab}	4.90 ± 0.300^{c}
E	6.66 ± 0.030^{a}	6.01 ± 0.08^{ab}	4.94 ± 0.120^{c}
F	6.65 ± 0.050^{a}	6.00 ± 0.00^{ab}	4.92 ± 0.220^{c}
G	6.66 ± 0.040^{a}	5.90 ± 0.30^{b}	4.71 ± 0.090^{b}
H (Control)	-	-	3.93 ± 0.000^{a}

Different superscript letters in the same column denote significant difference.
A= coconut yoghurt fermented with *Lactobacillus acidophilus*, B= coconut yoghurt fermented with *Lactobacillus bulgaricus*, C=coconut yoghurt fermented with *Lactobacillus plantarum* D=coconut yoghurt fermented with *Lactobacillus acidophilus* and *L. bulgaricus*, E= coconut yoghurt fermented with *Lactobacillus acidophilus* and *Lactobacillus plantarum*, F= coconut yoghurt fermented with *Lactobacillus bulgaricus* and *Lactobacillus plantarum* , G=coconut yoghurt fermented with *Lactobacillus acidophilus* , *Lactobacillus bulgaricus* and *Lactobacillus plantarum*, H= control (commercial yoghurt).

The acid is responsible for development of characteristic body and texture of the fermented milk products, contributes to the overall flavor of the products, and enhances preservation. Hydrogen peroxide also plays an important role in the shelf stability of fermented dairy products. Lactic acids bacteria (LAB) produce hydrogen peroxide through oxidation of reduced nicotinamide adenine dinucleotide (NADH) by flavin nucleotide, which reacts rapidly with gaseous oxygen and produce an antibiotic effect on other organisms that might cause food spoilage (Steinkraus, 2015). Based on the quantity of lactic acid and hydrogen peroxide produced, *Lactobacillus acidophilus, Lactobacillus bulgaricus* and *Lactobacillus plantarum* were selected and used singly and as mixed cultures for the production of coconut yoghurt.

Changes in pH of starter culture fermented coconut yoghurts (SFCY) with fermentation period are presented in Table 3. The pH of SFCY decreased with increase in fermentation time. During fermentation, Lactic acid bacteria produce lactic acid, thus lowering the pH (Eke *et al*., 2013). At the beginning of fermentation, there was no significant difference ($p>0.05$) in pH among SFCY.

At the end of fermentation (8 h), SFCY had significantly higher ($p<0.05$) pH values than the control sample. This was followed by coconut yoghurt fermented by a mixed culture of *Lactobacillus acidophilus, Lactobacillus bulgaricus* and *Lactobacillus plantarum*. The difference in pH values might be due to the metabolic activities of the lactic acid bacteria in the yoghurt culture (Obi *et al*., 2010). Food Standard Code requires that the pH of yoghurt be a maximum of 4.50 in order to prevent the growth of any pathogenic organisms (Donkor *et al*., 2006).

The results of total titratable acidity, viscosity and total solid contents of SFCY and the control sample are presented in Table 4. Total titratable acidity values of SFCY ranged from 0.60-2.20 %. These values are within the average of 0.6% acidity recommended for plain yoghurts (Eke *et al*., 2013). The control sample (commercial yoghurt) had a significantly higher ($P < 0.05$) total titratable acidity (2.20 %) than SFCY, followed by the sample fermented with a mixed culture of coconut yoghurt fermented with *Lactobacillus acidophilus, Lactobacillus bulgaricus* and *Lactobacillus plantarum*. This may be due to the differences in composition of the raw material. Cow milk (used in the control sample) contains lactose which the fermenting organisms readily convert to lactic acid thus leading to high acidity of the control sample.

Viscosity of SFCY ranged from 3 to 8 cp. Coconut yoghurt fermented with mixed culture of *Lactobacillus acidophilus*, *Lactobacillus bulgaricus* and *Lactobacillus plantarum* had a significantly higher viscosity ($p < 0.05$) than other samples.

Table 4. Total titratable acidity, Viscosity and Total solid contents of starter culture fermented yoghurt samples

Treatments	Titratable acidity (%)	Viscosity (cp)	Total solid (%)
A	$0.65 \pm 0.\ 100^{d}$	3.00 ± 0.200^{e}	14.60 ± 0.60^{ab}
B	0.77 ± 0.100^{d}	4.00 ± 0.50^{d}	15.00 ± 0.500^{ab}
C	0.60 ± 0.500^{d}	3.00 ± 0.60^{e}	14.02 ± 1.00^{c}
D	1.27 ± 0.100^{c}	6.00 ± 0.50^{b}	15.50 ± 0.50^{a}
E	1.15 ± 0.150^{c}	5.00 ± 0.00^{c}	15.15 ± 0.150^{a}
F	1.17 ± 0.070^{c}	5.00 ± 0.20^{c}	15.00 ± 0.200^{ab}
G	1.66 ± 0.100^{b}	8.50 ± 0.50^{a}	15.22 ± 0.220^{a}
H (Control)	2.20 ± 0.200^{a}	6.60 ± 0.20^{b}	13.50 ± 0.500^{c}

Different superscript letters in the same column denote significant difference.

A= coconut yoghurt fermented with *Lactobacillus acidophilus*, B= coconut yoghurt fermented with *Lactobacillus bulgaricus*, C=coconut yoghurt fermented with *Lactobacillus plantarum* D=coconut yoghurt fermented with *Lactobacillus acidophilus* and *L. bulgaricus*, E= coconut yoghurt fermented with *Lactobacillus acidophilus* and *Lactobacillus plantarum*, F= coconut yoghurt fermented with *Lactobacillus bulgaricus* and *Lactobacillus plantarum* , G=coconut yoghurt fermented with *Lactobacillus acidophilus* , *Lactobacillus bulgaricus* and *Lactobacillus plantarum*, H= control (commercial yoghurt).

This was followed by the control sample which was not significantly different from coconut yoghurt fermented with mixed culture of *Lactobacillus acidophilus* and *L. bulgaricus*. Total solid content of SFCY ranged from 14.02±1.00 to_15.50± 0.5 %. Total solid is an indication of the dry matter content of the yoghurt samples (Belewu *et al*., 2010; Khalifa *et al*., 2011). Except for sample C (coconut yoghurt fermented with *Lactobacillus plantarum*), SFCY had significantly higher (P<0.05) total solid content than the control sample. The higher total solid content could be attributed to the fact that the starter organisms may secret exopolysaccharide (EPS) materials during fermentation. The genus *Lactobacillus* has been reported to produce EPS and has gained increasing attention in recent time because of their contribution to the rheology and texture of fermented milk and food products (Cerning and Marshall, 1999). EPS produced by LAB contribute to the quality of fermented milk, especially to the texture, flavour and viscosity.

Table 5 shows the proximate composition of the yoghurt samples evaluated in this study. The results indicated that moisture content of the yoghurt samples ranged from 79.51 - 82.28 %. There was no significant difference (p>0.05) in moisture content among the yoghurt samples. Moisture contents of SFCY were within the range (80-86%) obtained for most commercial yoghurts (Ndife *et al*., 2014).

The protein content of SFCY ranged from 3.89 – 5.5 %. These values were above the minimum protein content of 3.5 % for commercial yoghurt (Akoma *et al*., 2000). There was no significant difference (P>0.05) in protein content between the control sample (commercial yoghurt) and coconut yoghurt fermented with a mixed culture of *Lactobacillus acidophilus*, *Lactobacillus bulgaricus* and *Lactobacillus plantarum*. The protein content of coconut yoghurt samples fermented with mixed culture was significantly higher (P<0.05) than protein content of coconut yoghurt samples fermented with single culture. Previous studies showed that yoghurt fermented with mixed starter had higher protein content than those fermented with single starter (Wakil *et al*., 2014).

The fat content of SFCY ranged from 7.42 - 9.18 %. Similar results have been reported (Belewu *et al*., 2010). It has been reported that commercially yoghurt should have a minimum fat content of 3.25% (Akoma *et al*., 2000). SFCY samples were significantly higher (P < 0.05) in fat content than the commercial yoghurt. The fat in coconut though high is unique and different from most all other fats and possesses many health giving properties and is gaining recognition (CRC, 2015). Besides, fat generally contributes to the sensory characteristics of food.

The ash content of the yoghurt samples was low, ranging from 0.08 - 0.23%. This implies that SFCY has low in mineral content. This could warrant fortification of SFCY to make up for the low mineral content. There was no significant difference (P > 0.05) in ash content between SFCY and the control sample. Starter culture fermented coconut yoghurt contained no fiber. Others (Tansakul and Chaisawang, 2006; Law *et al*., 2009; Minh, 2014) also reported that coconut milk had no fibre. However, the control sample contained 0.20 % fibre.

The carbohydrate content of the yoghurt samples ranged from 6.0 - 8.10 %. Coconut yoghurt sample fermented with *Lactobacillus bulgaricus* had a significantly higher (P < 0.05) carbohydrate content. The energy content of the coconut yoghurts ranged from 107.54 to 122.99 Cal/kg. SFCY were significantly higher (P < 0.05) in energy content that the control sample, indicating that they would provide more energy than the commercial yoghurt when consumed. The higher energy content could be as a result from the higher fat content of the starter culture fermented yoghurt samples. Belewu and Belewu (2007) reported that total energy value of milk is from the fat, therefore higher fat content indicates more total energies available. This shows that SFCY provides more available energy than the commercial yoghurt.

Table 5: Proximate composition of coconut milk yoghurt

Parameter (%)	Sample							
	A	B	C	D	E	F	G	H
Moisture	80.10±1.000^{a}	82.28±2.00^{a}	79.73±1.00^{a}	80.65±1.00^{a}	80.48±1.000^{a}	80.46±2.000^{a}	79.51±1.00^{a}	81.00±2.00^{a}
Protein	3.90±020^{c}	3.89±1.000^{c}	3.92±1.000^{c}	4.09±1.000^{bc}	4.05±1.00^{bc}	4.10±0.4000^{bc}	5.50±0.50^{ab}	6.18±1.000^{a}
Fat	8.71±0.710^{ab}	7.42±1.00^{c}	8.10±0.500^{abc}	9.18±0.500^{a}	9.11±0.566^{a}	8.23±0.460^{abc}	7.80±0.770^{bc}	5.33±0.090^{d}
Ash	0.09±0.528^{a}	0.11±0.30^{a}	0.15±0.30^{a}	0.08±0.20^{a}	0.16±0.20^{a}	0.23±0.100^{a}	0.19±0.40^{a}	0.38±0.10^{a}
Fibre	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.00^{b}	0.20±0.46^{a}
Carbohydrate	7.20±0.200^{ab}	6.30±1.000^{bc}	8.10±0.500^{a}	6.00±0.690^{c}	6.20±0.620^{bc}	6.98±0.490^{bc}	7.00±0.330^{bc}	6.97±0.610^{bc}
Energy (Cal/kg)	122.79+2.00^{a}	107.54+1.00^{d}	120.98+0.00^{a}	122.98+0.00^{a}	122.99+2.00^{a}	118.39+2.00^{c}	120.20+1.00^{b}	100.33+0.00^{e}

Different superscript letters in the same row denote significant difference.
A= coconut yoghurt fermented with *Lactobacillus acidophilus*, B= coconut yoghurt fermented with *Lactobacillus bulgaricus*, C=coconut yoghurt fermented with *Lactobacillus plantarum* D=coconut yoghurt fermented with *Lactobacillus acidophilus* and *L. bulgaricus*, E= coconut yoghurt fermented with *Lactobacillus acidophilus* and *Lactobacillus plantarum*, F= coconut yoghurt fermented with *Lactobacillus bulgaricus* and *Lactobacillus plantarum* , G=coconut yoghurt fermented with *Lactobacillus acidophilus* , *Lactobacillus bulgaricus* and *Lactobacillus plantarum*, H= control (commercial yoghurt).

Table 6. Microbial count of coconut yoghurt

Count ($x10^8$ cfu/ml)	Sample							
	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**
TBC	7.6 ± 0.50^{e}	8.8 ± 0.40^{d}	7.6 ± 0.00^{e}	10.4 ± 0.40^{c}	10.0 ± 0.23^{c}	10.4 ± 0.00^{c}	13.6 ± 0.23^{b}	23.6 ± 0.23^{a}
LAB	7.2 ± 0.31^{f}	8.0 ± 0.40^{f}	6.8 ± 0.40^{e}	8.8 ± 0.00^{d}	9.2 ± 0.40^{cd}	9.6 ± 0.40^{c}	12.0 ± 0.40^{b}	20.4 ± 0.40^{a}
Coliform count	NP	NP	NP	NP	NP	NP	NP	2.8 ±0.40^{a}

Different superscript letters in the same row denote significant difference.
NP; not present, A= coconut yoghurt fermented with *Lactobacillus acidophilus*, B= coconut yoghurt fermented with *Lactobacillus bulgaricus*, C=coconut yoghurt fermented with *Lactobacillus plantarum* D=coconut yoghurt fermented with *Lactobacillus acidophilus* and *L. bulgaricus*, E= coconut yoghurt fermented with *Lactobacillus acidophilus* and *Lactobacillus plantarum*, F= coconut yoghurt fermented with *Lactobacillus bulgaricus* and *Lactobacillus plantarum* , G=coconut yoghurt fermented with *Lactobacillus acidophilus* , *Lactobacillus bulgaricus* and *Lactobacillus plantarum*, H= control (commercial yoghurt).

The microbial counts of SFCY are presented in Table 6. Total bacterial count (TBC) and lactic acid bacteria count (LABC) of SFCY ranged from 7.6 ± 0.00 to 13.6 ± 0.23 $x10^8$ cfu/ml and 6.8 ± 0.40 to 12.0 ± 0.04 $x10^8$ cfu/ml respectively. International standards for yoghurt require minimum total viable microorganisms of 10^7 cfu mL^{-1} in the finished product (El Bakri, 2009).

LABC in starter culture fermented coconut yoghurt samples was more than 10 cfu/ml required minimum level for yoghurt (FDA, 2013). The control samples (commercial yoghurt) had significantly higher ($p< 0.05$) total bacteria and lactic acid bacteria counts than the SFCY. No coliform was detected in SFCY, indicating that adequate hygienic measures were taken during the production process and by implication, the microbiological safety of the SFCY. However, coliform count was 2.8 ±0.40 $x10^8$ cfu/ml for the control sample. This is more than 10^2 cfu/ml which is the maximum level for yoghurt, which when exceeded indicate potential health hazard or eminent spoilage (FDA, 2013). Contamination of the commercial sample might be from contaminated water source, equipment used or probably as due to contamination at storage and display/sale outlet. Coliforms have been used as indicator organisms for bacteriological quality of milk and its products (ICMSF, 1986).

Results of sensory evaluation of the SFCY as compared with the commercial yoghurt are presented in Table 7. The result showed that the control sample (commercial yoghurt) was rated significantly higher ($p < 0.05$) in appearance, taste, aroma and general acceptability.

Table 7: Sensory attributes of coconut milk yoghurt

Sensory Attribute	Sample A	B	C	D	E	F	G	H
Appearance	7.30± 0.979^{b}	6.45± 1.468^{c}	6.75± 1.0699^{bc}	6.300± 1.302^{c}	6.900± 1.447^{bc}	6.70± 1.625^{bc}	7.40± 0.883^{b}	8.65± 0.489^{a}
Taste	6.75± 0.630^{b}	6.15± 1.268^{b}	6.25± 1.209^{b}	6.15± 0.875^{b}	6.35± 1.137^{b}	6.850± 1.089^{b}	6.90± 1.518^{b}	8.70± 0.657a
Aroma	5.50± 1.433^{bc}	4.60± 1.729^{c}	5.60± 1.273^{b}	5.55± 1.432^{bc}	5.850± 1.725^{b}	5.25± 1.446^{bc}	6.15± 1.424^{b}	8.600± 0.503^{a}
Consistency	6.40± 0.681^{b}	6.30± 1.559^{b}	6.75± 0.786^{b}	6.65± 1.308^{b}	6.70± 1.081^{b}	6.80± 0.894^{b}	8.5003± 0.889^{a}	6.500± 1.573^{b}
General Acceptability	6.50± 1.395^{cd}	5.90± 1.518^{d}	6.30± 0.571^{cd}	6.45± 0.945^{cd}	6.70± 0.979^{c}	6.90± 0.912^{c}	7.50± 0.688^{b}	8.850± 0.366^{a}

Different superscript letters in the same row denote significant difference.

A= coconut yoghurt fermented with *Lactobacillus acidophilus*, B= coconut yoghurt fermented with *Lactobacillus bulgaricus*, C=coconut yoghurt fermented with *Lactobacillus plantarum* D=coconut yoghurt fermented with *Lactobacillus acidophilus* and *L. bulgaricus*, E= coconut yoghurt fermented with *Lactobacillus acidophilus*

This was followed by coconut yoghurt fermented with a mixed culture of *Lactobacillus acidophilus, Lactobacillus bulgaricus* and *Lactobacillus plantarum*, which was rated significantly higher (P < 0.05) in consistency(flowing nature).

4. CONCLUSION

This study showed that a mixed culture of *Lactobacillus acidophilus, Lactobacillus bulgaricus* and *Lactobacillus plantarum* isolated from spontaneously fermented coconut milk could be used to produce nutritious, organoleptically acceptable and microbiologically safe yoghurt from 100% coconut milk.

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