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Microbiological Analysis, Nutritional Composition and Sensory Evaluation of Cow Milk Yoghurt Flavoured with Soursop (*Annona muricata*) Pulp

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A B S T R A C T

This study is aimed at evaluating the quality of cow milk yoghurt (CMY) flavoured with soursop pulp (SSP) in varied proportions at 0 and 24 hr after production. The different proportions of yoghurt samples prepared include CMY 300: SSP 0, CMY 225: SSP 75, CMY 150: SSP 150 and CMY75: SSP 75. Microbiological analysis and nutritional composition of the yoghurt preparations was carried out using standard procedures while sensory evaluation involved the use of 9 point Hedonic scale. Total bacterial count (TBC) and lactic acid bacterial (LAB) count of the yoghurt samples was within the range 4.0 - 5.85 and 4.0 – 6.35 $\log_{10}$CFU/ml, respectively. The pH and total titratable acidity of the samples was within the range 4.8-6.4 and 0.52-0.93 %, respectively. Proximate analysis revealed that moisture, crude ash, crude fat, and carbohydrate content of the yoghurt samples was within the range 77.09 - 83.02, 5.16 - 6.28, 4.16 - 7.54, 0.55 - 0.86, 4.78 - 11.51 %, respectively. In terms of proximate compositions and sensory properties of the yoghurt preparations, there was significant difference ($p<0.05$) among the samples with the exception of ash content and aroma, respectively. According to the sensory report, cow milk yoghurt flavoured with soursop pulp (CMY 225: SSP 75) was the most prepared product. In conclusion, cow milk yoghurt flavoured with soursop is recommended to everyone because the product is safe and nutritious with potential health benefits.

Introduction

Yoghurt is regarded as the most popular and the oldest fermented milk product. Its origin is traced to the Middle East and Asia as far back as 6000 B.C. (Desai *et al.*, 2013; Gahruie *et al.*, 2015; Badawi *et al.*, 2020). The United States Food and Drug Administration (FDA) describe yoghurt as a type of food produced by culturing one or more of the optional dairy ingredients which include cream, milk, partially skimmed milk, and skim milk, used alone or in combination with a characteristic bacterial culture collectively known as lactic acid bacteria (LAB) which contain *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (Weerathilake *et al.*, 2014).

The source of milk largely used for yoghurt production is cow. However, milk from other animals such as goat, sheep, buffalo etc have also been used to produce yoghurt (Bilgin and Kaptan, 2016). Globally, there has been an increase in demand for yoghurt by consumers due to its health benefits (Afolabi *et al.,* 2017). Health benefits associated with yoghurt consumption include lactose digestion, cholesterol reduction, intestinal microflora modulation, stimulation of the immune system and prevention of cancer. The consumption of yogurt could also help in the improvement of dental and bone health, prevention of hypertension and liver disease (Desobry-Banon *et al.,* 1999). According to Gahruie *et al.,* (2014), yoghurt is more nutritious than milk. Whole milk yoghurt per 100 g contain protein (5.7 g), carbohydrate (7.8 g), fat (3.0 g), thiamin (0.06 mg), riboflavin (0.27 mg), niacin (0.2 mg), vitamin B_6 (0.10 mg), vitamin B_{12} (0.2 mg), folate (18 µg), carotene 21 µg), potassium (280 mg), calcium (200 mg) and phosphorus (170 mg) (Weerathilake *et al.,* 2014). Higher protein content of commercial yoghurt compared with milk is as a result of nonfat dry milk solid added to yoghurt during processing (Gómez-Gallego *et al.,* 2018).

Addition of different flavours from natural sources such as fruits into plain yoghurt is aimed at improving its sensory properties as well as nutritional value. This has resulted in high demand for yoghurts available in different flavours (Tan and Korel, 2007). Several studies on yoghurts flavoured with fruits, coffee and other food materials have been reported (Tan and Korel, 2007; Mbaeyi-Nwaoha *et al.,* 2017). Flavouring of yoghurts sold in commercial quantity usually involves adding exotic fruits which include banana, raspberry, vanilla, strawberry and peach (Mbaeyi-Nwaoha *et al.,* 2017). Due to high cost of exotic fruits in many developing countries, it has become necessary to source cheaper fruits locally available as a good alternative.

Soursop (*Annona muricata*) is a slightly sweet underutilized tropical fruit not with standing the fact that its origin is traced to Central America (Sanusi and Abu Bakar, 2018; Sanusi *et al.,* 2022). Soursop is rich in sugar. It contain minerals which include sodium (0.63-0.65 %) and potassium (1.39-1.41 %). Badrie and Schauss (2010) reported that soursop also contain phosphorus (27.7 mg), iron (0.64 mg), thiamine (0.11 mg), riboflavin (0.05 mg), niacin (1.28 mg), ascorbic acid (29.6 mg), tryptophan (11 mg), methionine (7 mg) and lysine (60 g). According to Afzaal *et al.,* (2022), soursop fruit contain carbohydrate (14.88 - 14.91 %), ash (0.89-0.90 %), crude protein (1.20-1.24 %), moisture (78.49-78.68 %) and dry matter (19.15 -19.35 %). The fruit of soursop produces a rich, creamy and fruity flavour. When soursop is ripe, the highly aromatic fruit has a slightly acidic taste which brought about the name, soursop. The soursop tree is found growing in the tropics and frost-free subtropics (Mbaeyi-Nwaoha, 2014; Ani *et al.,* 2019).

Studies have shown that phytochemicals found in soursop possess therapeutic properties. Soursop is useful in the treatment of fever, hyperglycemia, inflammation, hypertension, cancer and anxiety. Bacterial or parasitic infections can be treated using soursop. Extract from soursoup have demonstrated antiprotozoan, anti-inflammatory, antitumor and antioxidant properties (Afzaal *et al.,* 2022). Soursop has different applications in the food processing industry which include the production of alcoholic beverages, ice cream, jam, among others (Costa *et al.,* 2014). The soursop-yoghurt and soursop-frozen dessert prepared by Virgen-Ceceña *et al.,* (2019) was well acceptable. The variety of soursop and geographical location where the fruit was harvested could influence the properties of yoghurt prepared using the soursop pulp and cow milk. Therefore, this study is aimed at using locally available soursop in different ratios to add flavour to cow milk yoghurt and evaluate the physicochemical properties, microbiological quality, nutritional composition and sensory evaluation of the product.

Materials and Methods

Peak® full cream evaporated milk and starter culture (Yogourmet by LyosanIncorp., England)

were obtained from Oyibo market, Rivers state. Fresh and ripe soursop was obtained from Rumuomasi market. The starter culture comprise of *Streptococcus thermophilus, Lactobacillus acidophilus* and *Lactobacillus bulgaricus*. Carboxyl methyl cellulose (C. M. C.) was bought from a cake shop in Rumuomasi, Port Harcourt, Rivers State.

Processing of soursop fruit pulp

Five (5) big, fresh and ripe soursop fruits were washed with potable water, peeled with sterilized knife and deseeded. Five hundred grams (500 g) of the fruit was blended with 300 ml of sterilized water using a clean Breville smoothie maker sterilized with 70 % ethanol. Thereafter, it was pasteurized for 85 ^{o}C for 3 minutes and allowed to cool.

Production of soursop yoghurt

Cow milk yoghurt flavoured with soursop pulp was produced using the method described by Ihekoronye (1999) with a slight modification. Eight hundred millilitre (800 ml) of cow milk was mixed with 0.5g carboxyl methyl cellulose. The mixture was pasteurized at 85℃ for 5 minutes and stirred continuously to avoid the formation of clumps, then allowed to cool to 40℃. Eight grams (8g) of the starter culture (yogourmet) was mixed with a small amount of milk, and then introduced into the bulk portion which subsequently resulted in cow milk yoghurt (CMY).

Three hundred millilitre (300 ml) of CMY was not blended with pasteurized soursop pulp (SSP). Seventy-five millilitre (75 ml) of SSP was blended with 225 ml of CMY inoculated with starter culture; 150 ml of SSP was blended with 150 ml of CMY; 225 ml of SSP was blended with 75 ml of CMY. Each portion of the yoghurt samples -CMY 300: SSP 0, CMY 225: SSP 75, CMY 150: SSP 150 and CMY 75: SSP 225 was further divided into two: 0 hour (unfermented) and 24 hour (fermented) at 40 - 45^{o}C. The fermented portion of cow milk yoghurt flavoured with soursop pulp was allowed to cool (4^{o}C).

Microbiological analysis

Serial dilution

Ten-fold serial dilution from 10^{-1} to 10^{-5} were carried out. The process involved mixing 1 ml of the sample with 9 ml of sterile diluent (peptone water broth). Subsequent dilutions were carried out by stepwise transfer of 1 ml solution into test tubes containing 9 ml of sterile diluent using a sterile pipette for each transfer.

Total heterotrophic bacterial count

Following the serial dilution of the sample, 0.1 ml of dilution 10^{-5} was inoculated onto different media. The media which include de Man, Rogosa and Sharpe (MRS) agar, plate count agar and MacConkey (MCA) agar were prepared according to manufacturer's instruction. Autoclaving of the media was done at 121^{o}C for 15 minutes at 15 psi. The inoculums was spread immediately using a sterilized glass rod (flame sterilized using 70 % ethanol). After spreading the sample on the agar, the glass rod was finally sterilized.

The inoculated culture plates were incubated at 37^{o}C for 24 hours. After the incubation period, the number of colonies on the Petri dishes were counted manually and recorded. The colonial characteristics of the bacterial growth on the culture media was noted.

Obtaining pure culture

The colonies on the culture plates were subcultured into freshly prepared agar plates to obtain pure and discrete colonies. Preservation of discrete colonies was done by transferring the isolates into slants and stored at 4^{o}C.

Characterization of lactic acid bacteria (LAB) isolates

The LAB isolated from MRS agar were subjected to Gram staining and catalase test. Other biochemical

tests (indole, methyl red, Voges - Proskauer, citrate, and triple sugar iron) and sugar fermentation tests were also carried out on the bacterial isolates.

Physicochemical analysis

Determination of pH

The pH of cow milk yoghurt flavoured with soursop pulp in different proportions was determined using the method described by AOAC (1990). The pH E-201 meter was calibrated by rinsing the electrode with de-ionized water. Afterwards, the electrode was wiped dry before using it for analysis by inserting the electrode in the sample and left for 2-4 minutes until a stable value was noticed on the digital display of the pH meter.

Determination of total titratable acidity

The total titratable acidity (TTA) of cow milk yoghurt flavoured with soursop pulp in different proportions was determined using AOAC (2005) method. Exactly 2.5 ml of the sample was measured into a conical flask and diluted with 7.5ml of distilled water. Three (3) drops of phenolphthalein indicator was added to the sample and the diluted yoghurt sample was titrated against 0.1N sodium hydroxide (NaOH) until a pink end point was observed. The titre value at the end point was noted. The TTA was calculated as percentage (%) using the acid factor of lactic acid for each soursop flavoured yoghurt sample (0.009).

$$\% \text{ TTA} = \frac{M\ (NaOH)\ X\ N\ (NaOH)\ X\ 0.09\ X\ 100}{Volume\ of\ sample}$$

NaCl Test

The presumptive lactic acid bacterial (LAB) isolates were tested for tolerance to varying concentrations of NaCl. One percent (1 %) of overnight culture of each of the isolates was inoculated into MRS broth containing NaCl and incubated at 37℃ for 24-48 hours and checked for presence of turbidity.

Determination of dry matter

Two grams (2 g) of soursop flavoured yoghurt was weighed into previously washed, weighed and dried crucibles in duplicates. The samples were placed inside an oven for 5 hours at 105℃until a constant weight for the sample was obtained. The crucibles were removed from the oven using a crucible thong and kept inside a dessicator to cool and reweighed. The weight of dry matter of the samples were determined by calculation and the results were expressed as a percentage (AOAC, 2005).

Proximate analysis

This test was carried out to determine the major constituents of a food and to verify if the food is within its specified parameters. This analysis partition food into six (6) different components: crude protein, crude fiber, moisture, ash content, crude fat and total solid.

Determination of crude protein

The AOAC (2005) method was adopted. Two grams (2 g) of the yoghurt sample was placed in a Kjeldahl digestion flask containing a catalyst (selenium) and 25ml of concentrated H_2SO_4 which was added in a fume chamber. The setup was continuously heated until a clear solution was observed within 5 hours after which digestion of the sample was completed. The clear digest was cooled and transferred to a 100ml volumetric flask and made up to 100 ml with distilled water. A digest of about 10 ml was collected for distillation and put in the steam distillation unit. Thereafter, 18 ml of 40% sodium hydroxide (NaOH), 25ml of 2% boric acid was pipette into a conical flask and 2 drops of bromocresol green methyl red indicator was put into solution inside the distillation flask. Distillation was allowed to take place for 3 minutes. The ammonia distilled off was absorbed by boric acid indicator; then titrated against 0.1M hydrochloric acid (HCl). The titre value of the end point at which the colour changed from green to pink was noted. The crude protein was then calculated using a factor 6.25.

$$\% \text{ Crude protein} = \frac{0.000140\ X\ T\ X\ 100\ X\ 6.25}{W\ x\ 5}$$

Determination of moisture content

The moisture content of the soursop flavoured yoghurt was determined using the method described by Oladipo and Jadesimi (2012). Two gram (2 g) of the sample was weighed in duplicate and put inside a clean, dried and pre-weighed crucible.

The samples inside the crucible were placed inside a thermostatically controlled oven at 105℃ for 4 hours and dried until a constant weight of the samples were achieved. The crucibles and its content were removed from the oven and cooled in a desiccator and re-weighed. The moisture content of the samples was calculated by difference in weight and expressed as a percentage.

$$\% \text{ Moisture content} = \frac{W2-W3}{W2-W1} \text{ x } 100$$

Where: W_1= Initial weight of empty crucible

W_2= Weight of crucible + sample before drying

W_3= Final weight of crucible + sample after drying

Determination of ash content

The method described by Oladipo and Jadesimi (2012) with slight modifications was used to determine the crude ash content of soursop flavoured yoghurt. Two gram (2g) of the homogenized soursop flavoured yoghurt was weighed into a previously dried and weighed porcelain crucible in duplicates. The sample was heated for 20 minutes over a boiling water-bath until it appeared visibly dried. The crucibles with its contents were transferred into a muffle furnace at 600°C and left for 2 hours until whitish-grey colour was observed. It is an indication that all organic matter in the sample had been destroyed. The crucibles together with its content were removed from the furnace, placed inside a desiccator to cool and re-weighed. The ash content of the sample was calculated and expressed as a percentage (%).

$$\% \text{ Ash content} = \frac{C-A}{B-A} \text{ x } 100$$

Where: A= weight of empty crucible

B= weight of empty crucible+ sample before ashing

C= weight of crucible+ ash

Determination of crude fat content

Slight modifications in the solvent extraction method described by Oladipo and Jadesimi (2012) was used to determine the crude fat content of soursop flavoured yoghurt. Five grams (5g) of the sample was poured into a previously weighed petri dish and boiled over a water-bath to enable reasonable quantity of water to evaporate. The sample was then transferred to an oven and dried further until a constant weight was obtained. Two grams (2 g) of the sample was weighed into an extraction thimble and returned to the soxhlet apparatus. The washed flask was filled with 150ml hexane and fat extraction was carried out for four (4) hours. Thereafter, hexane was recovered leaving only small amount mixed with the oil in the flask at the end of fat extraction. The oil inside the extraction flask was dried inside the oven, leaving the door of the oven partially closed for hexane to completely evaporate. The flasks were cooled inside a dessicator and finally reweighed. The fat content was expressed as a percentage (%) of wet per weight basis using the water content determined after drying the wet sample.

$$\% \text{ Crude fat content} = \frac{C-A}{A} \text{ x } 100$$

Where: A= weight of sample

B= weight of empty flask

C= weight of flask+ oil

Determination of carbohydrate content

The method described by Matela *et al.,* (2019) was used to determine the carbohydrate content of soursop-flavoured yoghurt as the nitrogen free extraction and expressed as a percentage (%) calculated by difference. The formula below was used:

% Carbohydrate = 100 % - (protein + fat + fiber + ash + moisture).

Sensory evaluation

The method described by Olugbuyiro and Oseh (2011) was adopted. Three (3) coded samples of soursop flavoured yoghurt and plain yoghurt sample were presented toten (10) sensory panelists to evaluate the samples on a 9 point Hedonic scale based on their personal opinion on each sensory attribute of the flavoured and non-flavoured yoghurt. In the sensory evaluation form, there is provision for additional comments which is optional. Potable water inside a transparent glass cup was provided for the sensory panelists to rinse their mouth before evaluating each sample.

Statistical analysis

Data analysis involved the use of Two-way Analysis of variance (ANOVA) and significance was accepted at ($p<0.05$). Separation of the means involved the use of Duncan's multiple range test. The performances of statistical analyses were carried out using SPSS (version 20.0) software.

Results and Discussion

The result presented in Table 1 shows the total viable count (TVC) and lactic acid bacterial (LAB) count in cow milk yoghurt flavoured with varied portion of soursop pulp is within the range 4.0–5.85 and 4.0 - 4.60 $\log_{10}$CFU/ml, respectively. The result indicate that TVC of the yoghurt samples reduced as the proportion of soursop pulp added to it increased. This could be as a result of low pH of soursop pulp which reduced the population of bacteria in the flavoured yoghurt. It was observed that LAB population in the samples at 0 hr were relatively stable.

Table 2 shows the total viable count (TVC) and lactic acid bacterial (LAB) count in cow milk yoghurt flavoured with varied portion of soursop pulp at 24 hr. The result shows that TVC and LAB count of the samples was within the range 5.91–6.29and 5.98 – 6.35$\log_{10}$CFU/ml, respectively. The TVC and LAB counts encountered in the yoghurt samples at 24 hr is higher than the values reported in the samples at 0 hr. To have allowed the cow milk yoghurt flavoured with soursop pulp to ferment for 24 hr could be responsible for higher TVC and LAB counts of the samples.

Table 3 shows the result of Gram reaction, biochemical reaction, NaCl tolerance and Sugar fermentation tests carried out to identify the lactic acid bacterial isolates 1-10. The test results indicate that Isolate 1, 4 and 7 are *Lactobacillus* spp., Isolate 2 is *Leuconostoc* spp., Isolate 3, 5, 8, and 10 are *Streptococcus* spp. while Isolate 6 and 9 are *Enterococcus* spp. All the isolates tolerated 1 %, 1.5 % and 2 % NaCl concentration. However, only *Leuconostoc* spp. could not tolerate 6.5 % NaCl concentration contrary to what was observed in other isolates.

Depicted in Figure 1 is the pH of cow milk yoghurt flavoured with varied portions of soursop pulp at 0 and 24 hr. The pH values within the range 5.2 - 6.4 and 4.8 - 5.6 was reported in the samples of cow milk yoghurt flavoured with varied portion of soursop pulp at 0 and 24 hr, respectively. Plain cow milk yoghurt served as control, CMY + SSP (300: 0). The results indicate that pH of each yoghurt preparation allowed to ferment for 24 hrwas lower than the values reported in the sample at 0 hr.

Depicted in Figure 2 is the total titratable acidity (TTA) of cow milk yoghurt flavoured with varied

portion of soursop pulp at 0 and 24 hr. Plain cow milk yoghurt served as control, CMY + SSP (300: 0). The results shows that TTA within the range 0.52 – 0.59 and 0.59 – 0.93 % was reported in the samples at 0 and 24 hr, respectively. The TTA of each yoghurt preparation at 24 h was higher than the value encountered in the yoghurt sample at 0 hr.

Figure 3 below depicts the total dry matter in cow milk yoghurt flavoured with varied portions of soursop pulp at 0 and 24 hr. Plain cow milk yoghurt served as control, CMY + SSP (300: 0). Total dry matter within the range 19.77-22.11 and 16.98-20.82 % was reported in the yoghurt samples at 0 and 24 hr, respectively.

Presented in Table 4 is the proximate composition of cow milk yoghurt flavoured with varied portions of soursop at 0 hr. Plain cow milk yoghurt served as control, CMY + SSP (300: 0). The results indicate that moisture, crude ash, crude fat, and carbohydrate content of the samples was within the range 77.09 - 80.23, 5.68 - 6.28, 4.16 - 7.54, 0.55 - 0.81, 5.75 - 11.51 %, respectively. The values of each proximate parameter with regards to the cow milk yoghurt preparations was significantly different ($p<0.05$) with the exception of crude ash content.

Table 5 shows the proximate composition of cow milk yoghurt flavoured with varied portion of soursop at 24 hr. Plain cow milk yoghurt served as control, CMY + SSP (300: 0). The results indicate that moisture, crude ash, crude fat, and carbohydrate content of the samples was within the range 79.11-83.02, 5.16-6.09, 4.22-6.31, 0.71-0.86, 4.78-10.18 %, respectively. The values of each proximate parameter with regards to the cow milk yoghurt preparations was significantly different ($p<0.05$) with the exception of crude ash and moisture content.

Presented in Table 6 is the sensory scores for each sensory parameter assigned to cow milk yoghurt flavoured with soursop pulp in different proportions by the panelists. The result obtained from this study shows that total viable count in cow milk yoghurt flavoured with varied portions of soursop pulp was within the range 4.0-5.85 $\log_{10}$CFU/ml. According to Farinde *et al.,* (2010), total aerobic count in cowmilk yoghurt flavoured with orange, pineapple, grape and banana stored at ambient temperature (0 hr) is 20.2±0.7, 21.2±1.1, 10.0±1.1 and 32.5±0.6 cfu/ml, respectively. Food Safety and Standard Act 2006 which governs the establishment of Food Safety and Standards Authority of India stipulate that total plate count of yoghurt/dahi is <6.0 $\log_{10}$CFU/g (Pal *et al.,* 2015). According to FAO standard, bacterial count in yoghurt should not go beyond 5.0 $\log_{10}$CFU/ml (Omola *et al.,* 2014). Based on the standards recommended by the two agencies, only 2 out of 6 samples of yoghurt flavoured with soursop met the requirement.

High population of *Lactobacillus* sp. in the yoghurt samples flavoured with soursop pulp within the range 4.0–6.35 $\log_{10}$ CFU/ml is a strong indication that the products were rich in yoghurt starter cultures. High population of starter culture in fermented yoghurt was reported by Afolabi *et al.*, (2017). In a related study, Guevarra and Barraquio (2015) reported that the population of *S. thermophilus* and *L. bulgaricus* in yoghurt samples obtained from different grocery stores in Philippines is within the range 8.18±0.02 – 10.20±0.06 and 8.15±0.09 – 9.96±0.01 $\log_{10}$CFU/ml, respectively. According to Degnon *et al.,* (2020), a good yoghurt should contain a minimum of 6.0 $\log_{10}$CFU/g of two (2) lactic acid bacteria (*Streptococcus thermophilus* and *Lactobacillus bulgaricus*) still alive. Based on the results obtained from this study, only the yoghurt samples left for 24 hr met the minimum lactic acid bacterial population. Findings from this study showed that pH of cow milk yoghurt flavoured with soursop pulp at 0 hr and 24 hr was within the range of 5.2 - 6.4 and 4.8 - 5.6, respectively. It was observed that pH of the fruit-flavoured yoghurt samples decreased with increase in quantity of soursop pulp. The acidity of soursop could also have contributed to low pH values of the yoghurt samples flavoured with soursop. This is in agreement with the trend of results reported by Virgen-Ceceña *et al.,* (2019) from a similar study.

Table.1 Total viable count and lactic acid bacteria count in cow milk yoghurt flavoured with varied portions of soursop pulp at 0 hr.

Samples	Total viable count (CFU/ml)	Presumptive lactic acid bacterial count (CFU/ml)
CMY + SSP (300:0)	5.85	4.60
CMY + SSP (225: 75)	5.81	4.0
CMY + SSP (150:150)	4.85	4.0
CMY + SSP(75:225)	4.0	4.60

Key: CMY = Cow milk yoghurt; SSP = Soursop pulp

Table.2 Total viable count and lactic acid bacteria count in cow milk yoghurt flavoured with varied portions of soursop pulp at 24 hr.

Samples	Total viable count (CFU/ml)	Presumptive lactic acid bacterial count(CFU/ml)
CMY + SSP (300:0)	6.29	6.35
CMY + SSP (225: 75)	6.04	6.98
CMY + SSP (150:150)	6.24	6.37
CMY + SSP(75:225)	5.91	6.03

Key: CMY = Cow milk yoghurt; SSP = Soursop pulp

Table.3 Gram reaction, biochemical reaction, NaCl tolerance and Sugar fermentation for identification of lactic acid bacterial isolates

Characteristics	Isolate codes									
	1	2	3	4	5	6	7	8	9	10
Gram reaction	+	+	+	+	+	+	+	+	+	+
Catalase	-	-	-	-	-	-	-	-	-	-
Growth in NaCl										
1 %	+	+	+	+	+	+	+	+	+	+
1.5 %	+	+	+	+	+	+	+	+	+	+
2 %	+	+	+	+	+	+	+	+	+	+
6.5 %	+	-	+	+	+	+	+	+	+	+
Citrate	-	-	-	-	-	-	-	-	-	-
Glucose	AG	A	AG	A	A	A	A	A	A	A
Lactose	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+	+	+	+	+

Key: AG - Acid and gas; A – Acid

Table.4 Proximate composition of cow milk yoghurt flavoured with varied portions of soursop pulpat0 hr

Samples	Moisture (%)	Crude ash (%)	Crude protein (%)	Crude fat (%)	Carbohydrate (%)
CMY + SSP (300:0)	78.93±0.87^{b}	6.28±0.32^{a}	4.16±0.27^{a}	0.63±0.10^{ab}	10.00±0.27^{c}
CMY + SSP (225: 75)	77.09±0.79^{a}	5.68±0.28^{a}	4.91±0.23^{b}	0.81±0.18^{b}	11.51±0.51^{d}
CMY + SSP (150:150)	79.53±0.63^{b}	6.02±0.45^{a}	5.31±0.34^{b}	0.77±0.10^{ab}	8.37±0.48^{b}
CMY + SSP(75:225)	80.23±0.85^{b}	5.93±0.16^{a}	7.54±0.48^{c}	0.55±0.08^{a}	5.75±0.44^{a}

Key: CMY-Cow milk yoghurt; SSP-Soursop pulp
Values show means of triplicate analysis ±SD. Values with different superscript down the column are significantly different (P = 0.05).

Table.5 Proximate composition of cow milk yoghurt flavoured with varied portions of soursop pulp at 24hr

Samples	Moisture (%)	Crude ash (%)	Crude protein (%)	Crude fat (%)	Carbohydrate (%)
CMY + SSP (300:0)	81.11±2.21^{a}	6.09±0.22^{a}	4.27±0.67^{a}	0.84±0.08^{ab}	7.69±0.53^{b}
CMY + SSP (225: 75)	79.11±2.95^{a}	5.71±0.56^{a}	4.22±0.81^{a}	0.71±0.03^{a}	10.18±0.55^{c}
CMY + SSP (150:150)	81.35±2.39^{a}	5.48±0.41^{a}	5.11±0.73^{ab}	0.86±0.09^{b}	7.28±0.53^{b}
CMY + SSP(75:225)	83.02±1.37^{a}	5.16±0.83^{a}	6.31±0.52^{b}	0.73±0.08^{ab}	4.78±0.40^{a}

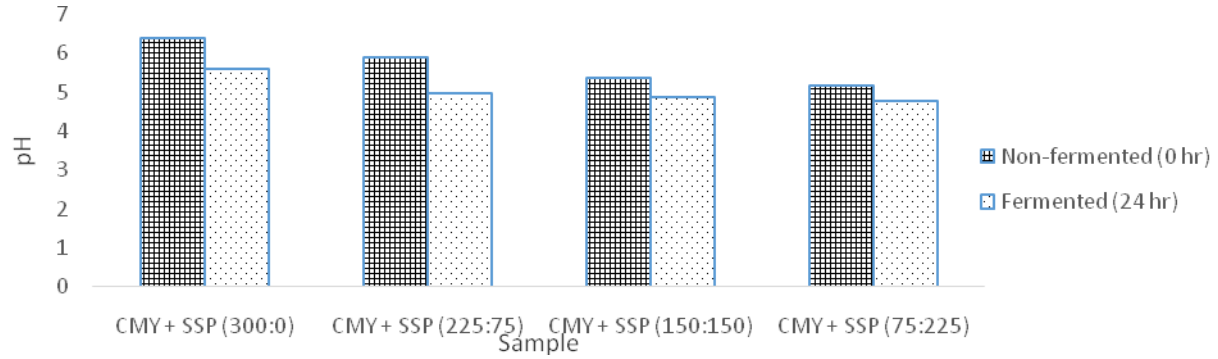
Key. CMY- Cow milk yoghurt; SSP – Soursop pulp
Values show means of triplicate analysis ±SD. Values with different superscript down the column are significantly different (P = 0.05).

Table.6 Sensory scores of cow milk yoghurt flavoured with varied levels of soursop pulp at 24 h

Samples	Colour	Appearance	Aroma	Taste	Mouthfeel	Overall acceptability
CMY + SSP (300:0)	7.00±0.82^{c}	7.00±1.05^{b}	5.80±1.23^{a}	4.40±0.97^{ab}	5.10±0.99^{bc}	5.40±0.97^{b}
CMY + SSP (225: 75)	7.30±1.06^{c}	6.70±1.16^{b}	6.30±0.82^{a}	4.90±0.57^{b}	5.50±0.85^{c}	6.00±1.05^{b}
CMY + SSP (150:150)	5.00±0.94^{b}	4.50±0.85^{a}	5.60±0.84^{a}	4.80±0.92^{b}	4.50±0.97^{ab}	4.00±0.67^{a}
CMY + SSP (75:225)	3.60±1.07^{a}	3.80±0.63^{a}	5.50±0.97^{a}	3.90±0.74^{a}	4.00±0.67^{a}	3.88±0.88^{a}

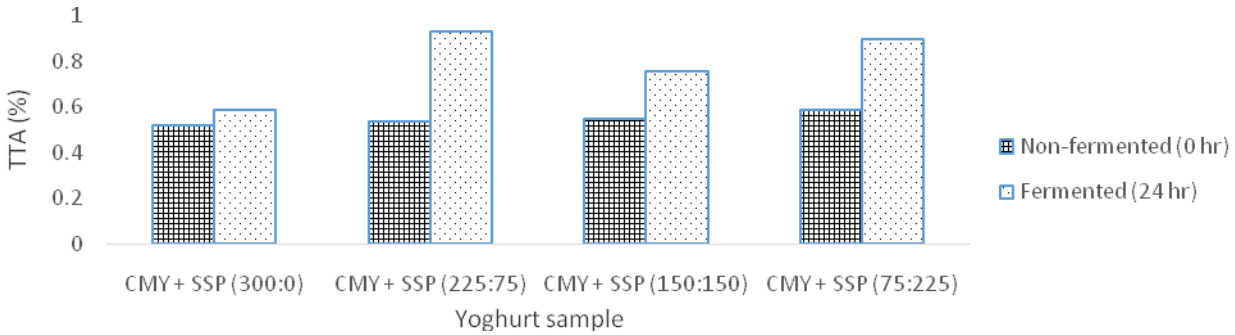
Key. CMY- Cow milk yoghurt; SSP–Soursop pulp
Values show means of sensory score of ten panelists ±SD. Values with different superscript down the column are significantly different (P = 0.05).
Sensory scores: 9 - Like extremely; 8 - Like very much; 7 - Like moderately; 6 - Like slightly; 5 - Neither liked nor disliked; 4 – Disliked slightly; 3 – Disliked moderately; 2 –Disliked very much; 1- Disliked extremely

Fig.1 pH of cow milk yoghurt flavoured with varied portions of soursop pulp



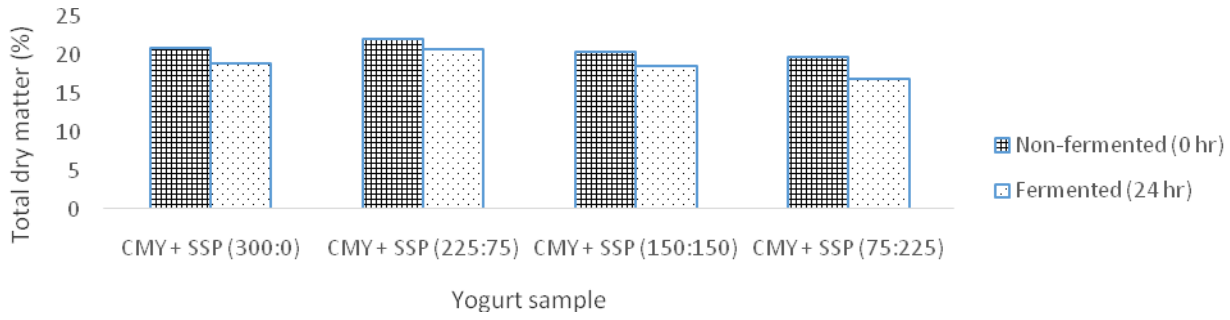
Key. CMY- Cow milk yoghurt; SSP – Soursop pulp

Fig.2 Total titratable acidity of cow milk yoghurt flavoured with varied portions of soursop pulp



Key. CMY- Cow milk yoghurt; SSP – Soursop pulp

Fig.3 Total dry matter in cow milk yoghurt flavoured with varied portions of soursop pulp



Key. CMY- Cow milk yoghurt; SSP – Soursop pulp

According to Omola *et al.*, (2014), pH of 4.4 is the least internationally acceptable value a yoghurt should have. The pH values within the range 4.8 – 6.4 reported in this study met the requirement. Virgen-Ceceña *et al.*, (2019) reported that pH of soursop yoghurt is 4.08. The pH values reported by Amal *et al.*, (2016) and Mbaeyi-Nwaoha *et al.*, (2017) in yoghurts flavoured with different quantities of pawpaw (*Carica papaya*) and African bush mango (*Irvingia gabonensis*) was within the

range 4.63-4.74 and 4.69±0.12-5.01±0.21, respectively.

The pH of the yoghurt samples reported in this study is suitable for marketability of the product in Nigeria and other tropical regions where temperatures are usually high which could affect the acidity of yoghurts during storage (Olugbuyiro and Oseh, 2011).

The total titratable acidity (TTA) of cow milk yoghurt flavoured with varied portions of soursop pulp including the control was within the range 0.52 – 0.59 and 0.59 – 0.93 %at 0 and 24 hr, respectively. The results indicate that each yoghurt preparation at 24 hr had a higher TTA compared with the values encountered in the sample at 0 hr. Many researchers have previously reported that titratable acidity of yoghurt increases with storage time after its preparation. This could be attributed to conversion of lactose present in the milk into lactic acid by *S. thermophilus* and *L. bulgaricus* which is part of the starter culture. Titratable acidity of 0.6 % is the minimum value required by Food Standard Code for plain yoghurt (Ityotagher and Julius, 2020). Based on this standard, only three (3) samples of yoghurt flavoured with soursop left for 24 hr met the requirement. However, all the samples of yoghurt did not meet the TA standards of USA which is 0.9 % nor that of Australia and New Zealand which ≥ 0.7 % (Thompson-Witrick *et al.,* 2019).

Total dry matter in the yoghurt samples decreased with increase in concentration of the soursop pulp added to it. This observation is in agreement with the report of Teshome *et al.,* (2017) from a related study. Yoghurt preparation in the ratio CMY+SSP (225: 75) had the highest total dry matter. At 0 hr and 24 h, the total dry matter was 21.11 and 20.82 %, respectively. On the contrary, yoghurt preparation in the ratio CMY + SSP (75: 225) had the lowest total dry matter. The values reported in the sample at 0 hr and 24 h was 19.77 and 16.98 %, respectively.

Proximate analysis of plain cow milk yoghurt and the samples flavoured with varied portions of soursop indicate that moisture, crude ash, crude fat, and carbohydrate content of the product was within the range 77.09 – 83.02, 5.16 – 6.28, 4.16 – 7.54, 0.55 – 0.86, 4.78 – 11.51 %, respectively. In a related study, Virgen-Ceceña *et al.,* (2019) reported that crude protein, fat, ash, moisture and soluble carbohydrate content of soursop yoghurt is 3.14±0.03, 5.15±0.24, 0.61±0.05, 83.69±0.01 and 7.73±0.01 g/100 g Fresh weight, respectively. A separate study carried out by Mbaeyi-Nwaoha (2014) reported that moisture, carbohydrate, crude protein, fats and ash content of plain yoghurt flavoured with soursop pulp in different ratios was within the range 74.57±0.27 – 79.26±0.17, 9.41±0.25 – 19.33±0.35, 2.68±0.30 – 5.83±0.14, 2.21±0.18 – 4.12±0.23 and 1.21±0.25 -1.38 ±0.17 %, respectively. Both reports are considerably at variance with the results obtained in this study. This could be attributed to differences in geographical location where soursop was harvested and used in production of soursop yoghurt, breed of cattle which is the source of cowmilk and procedure used in producing the yoghurts.

According to Codex regulations, the least amount of protein in concentrated yoghurt is 5.6 % while 2.7 % protein content is applicable to normal yoghurt. The regulation further stipulate that 15 % fat content of yoghurt is the maximum quantity permitted in the product (Lee and Lucey, 2010).

Going by FAO standard, fat content of yoghurt within the range 0.5 - 10 is good. When the value is 3.0, it is regarded as the best (Omola *et al.,* 2014). The fat content of the yoghurt samples were within the range 0.55 – 0.86 %. USDA (2001) standards stipulate that yoghurt which has 0.5- 2.0 % fat content before bulky is added is categorized as ‘low fat yoghurt’. A minimum of 5.6 % protein content in whole milk yoghurt is recommended by the Turkish Food Codex Communiqué of fermented dairy product (Bilgin and Kaptan, 2016).

The ash content of the yoghurt samples flavoured with varied portions of soursop was within the range 5.16 - 6.28 %.It is an indication of the level of

minerals present in the yoghurt preparations. The values encountered in the yoghurt preparations is contrary to the report by Ihemeje *et al*., (2015) which stated that ash content of yoghurt flavoured with carrot, pineapple; yoghurt spiced with ginger and pepper fruit is very low. In most of the samples of yoghurt flavoured with soursop, it was observed that ash content of the product decreased as the proportion of soursop pulp added to the product increased. This observation was also reported by Mbaeyi-Nwaoha (2014) from a related study that involved production of yoghurt flavoured with different portions of soursop pulp.

High moisture content of the yoghurt samples reported in this study is in agreement with the results obtained by many authors that evaluated the proximate composition of yoghurt flavoured with different types of fruits. This could be as a result of the fact that yoghurt is a liquid fermented product and fruits generally have high moisture content.

Sensory analysis of the yoghurt samples indicate that the panelist's preference for the product decreased as the proportion of soursop pulp added to cowmilk yoghurt increased. This observation is in agreement with the report of Mbaeyi-Nwaoha (2014). Going by the interpretation of the sensory scores, the yoghurt sample flavoured with soursop pulp in the ratio 225:75 was the most preferred product. The yoghurt preparation was liked slightly based on overall acceptability. There was no significant difference ($p>0.05$) between the most preferred yoghurt flavoured with soursop and plain cow milk yoghurt in terms of colour, appearance, aroma, and overall acceptability. It is worthy to note that cow milk yoghurt (CMY) flavoured with soursop pulp (SSP) in the ratio 225:75 was preferable than plain cow milk yoghurt based on overall acceptability of both products. The sensory panelists neither liked nor disliked the plain cow milk yoghurt whereas CMY 225: SSP 75 preparation was liked slightly. On the contrary, the least preferred product comprise of cow milk yoghurt and soursop pulp mixed in the ratio 75:225. The yoghurt preparation was disliked moderately by the sensory panelists based on overall acceptability.

Cow milk yoghurt flavoured with varied portions of soursop pulp demonstrated that the medium is suitable for growth and multiplication of yoghurt starter culture leading to production of a well acceptable fermented milk product. The yoghurt preparations contain reasonable quantities of protein, ash and carbohydrate, but low in fat content. Interestingly, cow milk yoghurt flavoured with soursop pulp in the ratio 225:75 was preferable than plain cow milk yoghurt in terms of overall acceptability.

References

Afolabi, L. O., Adelowo, M. O. and Onilude, H. A. (2017). Assessment of the microbiological qualities of locally and industrially produced yoghurt in Lagos. Dutse J. Pure Appli. Sci. 3(1), 188-200.

Afzaal, M., Saeed, F., Asghar, A., Shah, Y. A., Ikram, A., Ateeq, H., Hussain, M., Ofoedu, C. E. and Chacha, J. S. (2022). Nutritional and therapeutic potential of soursop. J. Food Quality, 1-9. https://doi.org/10.1155/2022/8828358

Amal, A. M., Eman, A. M. M. and Zidan, N. S. (2016). Fruit flavoured yoghurt: chemical, functional and rheological properties. Intl. J. Environ. Agric.Resear. 2(5), 57-66.

Ani, I. F., Ajuzie, N. C., Adeoye, B. K., Ngozi, E. O. and Bassey, V. E. (2019). Comparative nutritional quality and sensory properties of pure soursop juice and its blend with orange and/or pineapple. Global Sci. J.7(6), 470-479.

AOAC. (1990). Official Methods of Analysis. (15thed.). Association of Analytical Chemist. Washington DC.

AOAC. (2005). Official methods of Analysis of Association of Official Analytical Chemists International 15th Edition. AOAC International, Maryland, USA.

Badawi, R. M., Kebary, K. M. K., Husien, S. A. and Habib, M. A. M. (2020). Quality of yoghurt

made from cow's milk fortified with whey protein isolate. Egyptian J. Dairy Sci. 48, 77-87.

Badrie, N. and Schauss, A. G. (2010). Soursop (*Annona muricata* L.): composition, nutritional value, medicinal uses, and toxicology. Bioactive Foods in Promoting Health: Fruits and Vegetables, 621-643.

Bilgin, B. and Kaptan, B. (2016). A study on microbiological and physicochemical properties of homemade and small scale diary plant buffalo milk yoghurts. Intl. J. Pharm.Resear. Allied Sci. 5(3), 29-36.

Costa, J. P., Rocha, E. M. F. F. and Costa, J. M. C. (2014). Study of the physicochemical characteristics of soursop powder obtained by spray-drying. Food Sci. Tech. 34(4), 663-666.

Degnon, R. G., Konfo, C. T. R., Kpatinvoh, B., Bossa, H. C. and Dagnon, Y. N. (2020). Evaluation of the quality of a yogurt produced by partial substitution of cow milk by soybean milk in Benin. Int. J.Curr.Resear.Biosci. Plant Bio. 7(2), 1-6. https://doi.org/10.20546/ijcrbp.2020.702.001

Desai, N. T., Shepard, L. and Drake, M. A. (2013). Sensory properties and drivers of liking for Greek yoghurts. J. Dairy Sci. 96, 7454-7466.

Desorby-Banon, S., Vetier, N. and Hardy, J. (1999). Health benefits of yoghurt consumption. A review. Intl. J. Food Prop. 2(1), 1-12.

Farinde, E. O., Obatolu, V. A., Oyarenkua, M. A., Adeniran, H. A., Ejoh, S. I. and Olanipekun, O. T. (2010). Physical and microbial properties of fruit flavoured fermented cowmilk and soy milk (yoghurt-like) under different temperature storage. Afri. J. Food Sci. Tech. 1(5), 120-127.

Gahriue, H. H., Eskandari, M. H., Mesbahi, G. and Hanifpour, M. A. (2015). Scientific and technical aspects of yoghurt fortification: a review. Food Sci. Human Wellness, 4, 1-8.

Gómez-Gallego, C., Gueimonde, M. and Salminen, S. (2018). The role of yogurt in food-based dietary guidelines. Nutri. Rev. 78(S1), 29-39.

Guevarra, R. B. and Barraquio, V. L. (2015). Viable counts of lactic acid bacteria in Philippine commercial yogurts. Intl. J. Dairy Sci. Proc. 2(5), 24-28.

Ihekoronye, A. I. (1999). Manual of Small-Scale Food Processing. Macmillan Publishers Ltd, London, pp. 108-212.

Ihemeje, A., Nwachukwu, C. N., and Ekwe, C. C. (2015). Production and quality evaluation of flavoured yoghurts using carrot, pineapple, and spiced yoghurts using ginger and pepper fruit. Afri. J. Food Sci. 9(3), 163-169.

Iyotagher, A. P. and Julius, A. (2020). Physiochemical composition, sensory properties and keeping quality of functional yoghurt produced from milk-soy flour blends. J.Nutri. Health Food Engr. 10(1), 5-12.

Lee, W. J., and Lucey, J. A. (2010). Formation and physical properties of yogurt. Asian-Australian J. Animal Sci. 23(9), 1127-1136.

Matela, K. S., Pillai, M. K., Matebesi-Ranthimo, P. and Ntakatsane, M. (2019). Analysis of proximate compositions and physiochemical properties of some yoghurt samples from Maseru, Lesotho. J. Food Sci.Nutri. Res. 2(3), 245-252.

Mbaeyi-Nwaoha, E. K. S. (2014). Production and evaluation of flavoured yoghurt from graded levels of soursop (*Annona muricata*) pulp. Innovare J. Food Sci. 2(1), 14-21.

Mbaeyi-Nwaoha, I. E., Umeh, L. C., Igbokwe, C. J., Obodoechi, C. M. and Okoronkwo, N. C. (2017). Production and quality evaluation of flavouredyoghurt from graded levels of sweet variety of African bush mango "ugiri' (*Irvingia gabonensis*) juice and pulp. Food Sci. Tech. 5(2), 56-69.

Oladipo I. C. and Jadesimi P. D. (2012). Microbiological analysis and nutritional evaluation of West African soft cheese (wara) produced with different preservatives. Am. J. Food Nutri. 3(1), 366-369.

Olugbuyiro, J. A. O. and Oseh, J. E. (2011). Physico-chemical and sensory evaluation of market yoghurt in Nigeria. Pakistan J.Nutri. 10(10), 914-918.

https://doi.org/10.3923/pjn.2011.914.918

Omola, E. M., Kawo, A. H. and Shamsudden, U. (2014). Physico-chemical, sensory and microbiological qualities of yoghurt brands sold in Kano metropolis, Nigeria. Bayero J.Pure Appli. Sci. 7(2), 26-30. https://doi.org/10.4314/bajopas.v7i2.6

Pal, M., Tefera, M., Tasew, A., Jergefa, T. and Deressa, A. (2015). Hygienic and microbial quality of yoghurt. Beverage and Food World, 42(4), 25-31.

Sanusi, M. S., Sunmonu, M. O., Raji, A., Alaka, A., Abdulazeez, A. and Joshua, V. A. (2022). Impact of pasteurization temperature, preservatives, storage duration and soursop puree substitution for sugar on physicochemical, antioxidant and microbial properties of set-type yoghurt. Research Square, 1-12.

Sanusi, S. B. and Abu Bakar, M. F. (2018). Soursop-*Annona muricata*. Exotic Fruits Reference Guide, 391-395.

Tan, G. and Korel, F. (2007). Quality of flavoured yoghurt containing added coffee and sugar. J. Food Quality 30, 342-356.

Teshome, G., Keba, A., Assefa, Z., Agza, B. and Kassa, F. (2017). Development of fruit flavoured yoghurt with mango (*Mangifera indica* L.) and Papaya (*Carica papaya* L.) fruit juices. Food Sci. Quality Mgt. 67, 40-45.

Thompson-Witrick, K. A., Das, K., Choudhary, R., Fisher, D. J. and Chai, T. (2019). Physicochemical and sensory characteristics of made-in-transit yogurt. Int. J. Food Sci.Nutri. Engr. 9(2), 37- 44. https://doi.org/10.5923/j.food.20190902.02

USDA (2001). Specifications for Yogurt, Nonfat Yogurt and Lowfat Yogurt. Document 21CFR, Part 131.200-203.

Virgen-Ceceña, L. J., Anaya-Esparza, L. M., Coria-Téllez, A. V., García-Magaña, M. L., García-Galindo, H. S., Yahia, E. and Montalvo-González, E. (2019). Evaluation of nutritional characteristics and bioactive compounds of soursop-yoghurt and soursop-frozen desert. Food Sci. Biotech. 1-14. https://doi.org/10.1007/s10068-019-00584-x

Weerathilake, W. A. D. V., Raskia, D. M. D., Ruwanmali, J. K. U. and Munasinghe, M. A. D. D. (2014). The evolution, processing, varieties and health benefits of yoghurt. Int. J. Sci.Resear. Pub. 4(4), 1-10.

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