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Production and analyses of yogurts obtained from cow's milk and soy milk blended with banana (*Musa paradisiacal*)

Jimoh MO
Department of Chemical and Food Sciences, Bells University of Technology, OTA, Nigeria

Abstract
The consumption of imported dairy products is high especially in developing countries such as Nigeria, where little or no milk is produced locally. Soy milk is known to be a good source of protein and a good alternative to cow's milk. The objective of this work is to compare proximate and microbial analyses of yogurts produced using cow's milk and soy milk blended with banana. The yogurt was processed by the inoculation of cow's milk and soymilk with freeze-dried cultures of Lactobacillus bulgaricus and Streptococcus thermophilus. The moisture content, crude protein, crude fat, crude fibre, ash content, and carbohydrate in the yoghurt were: 86.38%, 3.76%, 3.76%, 0.02%, 0.18% and 5.90% respectively for dairy; 82.87%, 3.60%, 4.17%, 0.04%, 0.84% and 8.48% respectively for dairy-banana; 87.02%, 5.96%, 1.44%, 0.02%, 1.53% and 4.03% respectively for soy; and 82.94%, 4.82%, 2.84%, 0.06%, 1.80% and 7.54% respectively for soy-banana. Highest lactic acid bacteria count, total viable count, and yeast and mould count were found in soy-banana to be 8.4×10^3 CFU/g, 3.3×10^3 CFU/g and 4.9×10^3 CFU/g respectively at ambient temperature. At refrigeration condition, total viable count, yeast and mould count and total coliform count were found to be nil. A test of the significance of correlation in proximate analysis and sensory evaluation confirms that there exists a trend between the samples at 95% significance level. Respondents' preference in yoghurt was in the order; Dairy > Dairy banana > Soy banana > Soy. The inclusion of banana in the production of yogurt is a means of value addition to banana which also reduces wastage by preventing postharvest losses.

Keywords: analysis, animal, plant, temperature, yoghurt

1. Introduction

Plant-milk yogurt products are varieties of yogurts produced from plant milk which are typically made from legume, nut, grain or seed. These yogurts are suitable for vegan's people with intolerance to dairy milk, and those who prefer plant milk (Jayeola *et al*., 2010; Height, 2014) [18, 16]. Soy yogurt is the most produced variety of plant-milk yogurt (Shi *et al*., 2015) [32]. The immense benefits of soya beans must have stipulated lot of research on incorporating soya beans or any of its products into indigenous diet. Soymilk is an aqueous extract produced by soaking dried soy beans and grinding. Soya beans, Glycine max are of considerable interest to nutritionist as possible substitute for cow milk (Bolla, 2015; Atli, 2015) [12, 8]. Soybean is one of the few plants that provide high quality protein with minimum saturated fat with high amount of poly-unsaturated fat and is a readily available source of essential fatty acids, lecithins, isoflavones, mineral substances, free amino acids and polypeptides (Yang and Li, 2010; Adeyemo and Onilude, 2013; Ma *et al*., 2015) [36, 5, 21]. Soybeans contain antioxidants and phytonutrients that have been linked with various health benefits, it also prevent cardiovascular disease and cancer as well as bone disease such as Osteoporosis in elderly people (Schneeman, 2002; Ahmed *at al*., 2015) [31, 6].

Dairy yogurt is a popular cultured dairy product that can be made from whole, low-fat or skim milk, including reconstituted non-fat dry milk powder (Jost, 2005) [19]. Yogurt comes in many flavours and varieties which appeals to everyone's taste buds. Cow milk makes a significant contribution to the required nutrient intakes for calcium, magnesium, selenium, riboflavin, vitamin B12 and pantothenic acid (Ehirim and Onyeneke, 2013; Nkhata *et al*., 2015) [15, 25]. It contains no fibre. The composition of milk from cows may vary due to differences in the relative rates of synthesis and secretion of milk components by mammary gland. Conditions affecting cows which lead to the variation of milk composition may include the weather or season, breed, diet, stage of lactation (Igbabul *et al*., 2014) [17].

The global consumption of dairy product is high especially in developing countries. Nigeria as a major consumer of dairy products produces little or no milk. 98% of all the milk and dairy products consumed in Nigeria are imported (Adekoya, 2016) [2]. The substitution of soy milk in the production of yogurt will reduce the importation of milk as raw material for production. Plant-milk yogurt is a cultured plant based, cholesterol free product. Soy milk generally has a longer shelf life than dairy milk, and some types of packaged soy milk can be stored at room temperature for months, which eliminates waste caused by food spoilage (Tremblay, 2014) [33]. The consumption of soy in place of meat and other foods high in saturated fat is said to reduce blood cholesterol (Messina and Messina, 2010) [23].

Yogurt production from both plant and animal origin is a process that involves fermentation, the conversion of large molecules to small molecules or molecular oxidation or reduction mechanisms mediated by selected microorganisms (Abiola and Oyetayo, 2015) [1]. The mechanism of food fermentation is essentially the conversion of carbohydrates to alcohols and carbon dioxide or organic acids by yeasts, bacteria or a combination thereof, under anaerobic conditions (Offor *et al*., 2014) [27]. Fermented foods are defined as those foods which have been subjected to the

action of microorganisms or enzymes so that desirable biochemical changes cause significant modification (Adesulu and Awojobi, 2014) [3]. Over the years, fermentation has become part of the culture and tradition of most indigenous communities in developing countries especially in Africa (Nwachukwu *et al.,* 2018) [26]. Nigeria as well as other countries in Africa depends on fermented foods for nutritional and organoleptic satisfaction (Okechukwu *et al.,* 2015) [28].

Banana is an edible fruit, botanically a berry, produced by several kinds of large herbaceous flowering plants in the genus Musa. Banana is one of the most popular seasonal foods in the world prone to post harvest losses (Brian *et al*., 2013; Bolarin and Bosa, 2015) [13, 11]. It contain various health promoting flavonoids and poly-phenolic; such as lutein, zeaxanthin, beta and alpha carotenes, acting as free radical-globing antioxidants (Adewunmi *et al*., 2009; Badertscher *et al*., 2007) [4, 9]. Ripe banana is a good source of vitamin A and contains medium quantities of vitamin B and minerals like phosphorus, iron and potash. It also contains sugars; sucrose, fructose and glucose. Banana contains protein known as tryptophan, which the body converts into serotonin that helps to relax (Mohapatra, 2010) [24]. Fresh and creamy bananas mix well with other fruits or food which makes it an excellent complement (Ahmed *et al*., 2015) [6]. In view of these potentials, it is imperative to compare proximate and microbial analyses of yogurt produced using dairy and soy milk blended with banana.

2. Materials and Methods

Fresh soy bean and partially skimmed Dano milk powder were purchased from Ota market, Ota, Ogun State, Nigeria. The Yoghurtmet freeze dried yoghurt starter, Lyo-San Inc. Canada was purchased at Oke-arin Market, Lagos Island, Lagos state, Nigeria.

2.1 Yoghurt production from soy milk

Soy milk was prepared by soaking cleaned soybean overnight. The water used for soaking was consistently changed. Soy yogurt was produced using fresh soy bean. The soybean is then dehulled manually. The bean seeds were boiled for 30 minutes at 90 °C – 100 °C and wet milled. The slurry was cooked and filtrated through a muslin cloth to remove the cake, Okara in order to obtain clear soy milk as shown in Figure 1. Soy milk was reconstituted by the addition of 360 g of clean milk to 3 liters of hot water. It was homogenised before it was pasteurized at 80 °C – 85 °C for 30 minutes. The mixture was cooled to 43 ºC – 45 °C and inoculated with starter cultures (Lactobacilusdelbrueckiissp. bulgaricus and Streptococcus thermophilus) at 42 °C – 45 °C. After incubation, fermentation was halted by cooling. Sucrose was added to the yoghurt and stirred, packaged and stored at 4 ºC for 21 days as shown in Figure 2 (Weerathilake *et al*., 2014) [35]. Banana yogurt was produced by the addition of blended banana to curd at 15% after fermentation of milk as described in Figure. 3. Then it was homogenized thoroughly before packaging and storage.

2.2 Yogurt production from cow milk

Yogurt was produced using partially skimmed Dano milk powder. Milk powder was reconstituted in a similar way to that of soy milk as described in Figure 2 by the addition of 360 g of milk powder to 3 liters of hot water. It was homogenised before it was pasteurized at 80 °C – 85 °C for 30 minutes. The mixture was cooled to 43 ºC – 45 °C and inoculated with starter cultures (Lactobacilusdelbrueckiissp. bulgaricus and Streptococcus thermophilus) at 42 °C – 45 °C. After incubation, fermentation was halted by cooling. Sucrose was added to the yoghurt and stirred, packaged and stored at 4 ºC for 21 days (Weerathilake *et al*., 2014) [35]. Dairy-banana yogurt was produced by the addition of blended banana to curd at 15% after fermentation of milk similar to soy-banana yogurt as shown in Figure 3. Then it was homogenized thoroughly before packaging and storage.

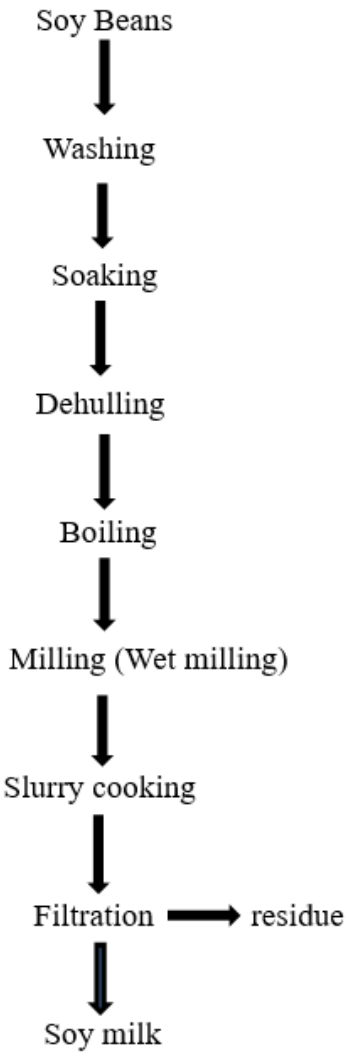

Fig 2: Flowchart for both soy yogurt and dairy yogurt production

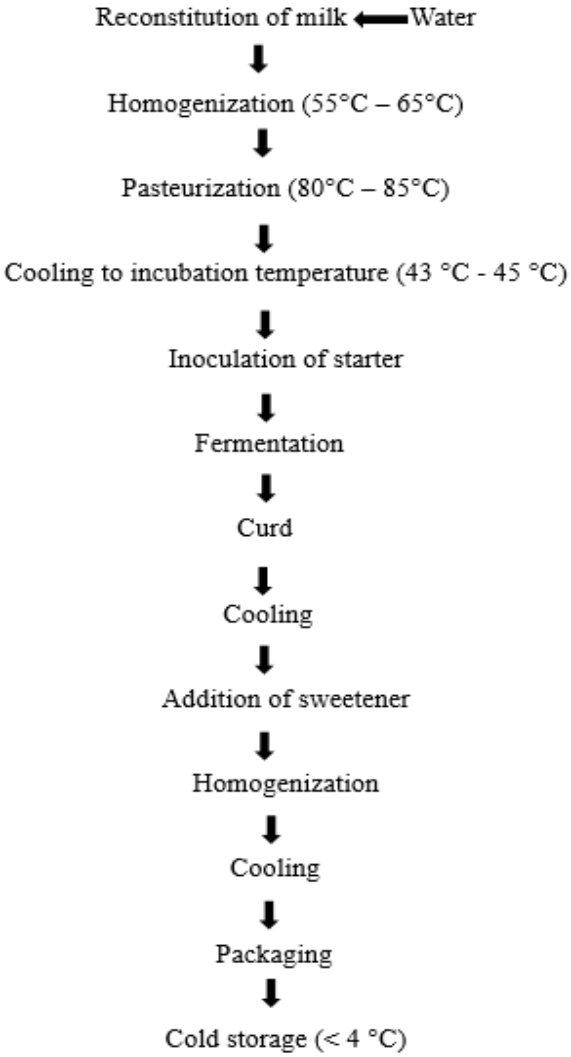

Fig 3: Flowchart for both soy-banana yogurt and dairy-banana yogurt production

2.3 Proximate analysis

Standard methods of the Association of Official Analytical Chemists (AOAC, 2005) [7] were used to determine the crude protein content, total ash, crude fat, crude fibre and moisture content of the samples. Crude protein content (Total nitrogen (%) x 6.25) was determined by Kjedahl method, using 2 g of sample. Crude fat was obtained by exhaustively extracting 5 g of sample in Soxhlet apparatus using n-hexane as the extractor. Ash content was determined by the incineration of 2 g sample in a Muffle furnace maintained at 550 ºC for 5 hours. Crude fibre was determined by weighing 2 g of sample and added 1.25% H_2S0_4, the mixture was boiled under reflux for 30 minutes. The residue was rinsed thoroughly with hot water and 1.25% NaOH was added and boiled to achieve neutral filtrate, oven dried at 100 ºC for 8 hours. Moisture content was determined by heating 2 g of sample to constant weight in a crucible placed in an oven maintained at 105 ºC. The total carbohydrate content was calculated by difference in protein, fat, ash, fibre and moisture from 100.

2.4 Microbial analysis

Normal saline was prepared by dissolving 0.85 g of NaCl in every 100 ml of water to make 0.85% saline. Serial dilution was done by mixing 1 g of each yogurt sample with 9 ml of normal saline to make a dilution of 10^{-1}. Further dilution was made until a dilution of 10^{-5} was obtained and the saline was autoclaved at 121 ºC for 15 minutes to effect sterilization.

Total viable counts of bacteria: The total aerobic counts of bacteria were determined using the method described by (Bergey and Holt, 1994) [10]. One (1 ml) of each serially diluted sample was inoculated into sterile petri dish and Nutrient agar (prepared according to manufacturer's specification) was poured into the petri dish. It was then allowed to solidify and incubated at 37 ºC for 24 hours. This was done on yogurt samples on the 1, 3, 7, and 14 days of storage at refrigeration temperature and 1, 2 and 3 days of storage at ambient temperature.

Lactic acid bacteria counts: The lactic acid bacteria count was determined using the method described by (Bergey and Holt, 1994) [10]. One (1 mil) of each serially diluted sample was inoculated into sterile petri dish and De Man Rogosa and Sharpe agar (MRS) (prepared according to manufacturer's specification) was poured on the dish, allowed to solidify and incubated at 37 ºC for 48 hours. This was done on yogurt samples on the 1, 3, 7 and 14 days of storage at refrigeration temperature and 1, 2, and 3 days of storage at ambient temperature.

Yeasts and moulds count: The yeasts and molds count was determined using the method described by (Bergey and Holt, 1994) [10]. One (1 mil) of each of serially diluted samples was inoculated into sterile petri dish and acidified Potato Dextrose Agar (PDA) which was prepared according

to the manufacturer's specification was poured into the dish. It was allowed to solidify and incubated at 25 ºC for 72 hours. This was done on yogurt samples on the 1, 3, 7 and 14 days of storage at refrigeration temperature and 1, 2 and 3 days of storage at ambient temperature.

Total coliform count: One (1 ml) of each of serially diluted samples was inoculated into sterile petri dish and MacConkey agar (prepared according to the manufacturer's specification) was poured on the dish. It was allowed to solidify and incubated at 37 ºC for 48 hours. This was done on yogurt samples on the 1, 3, 7 and 14 days of storage at refrigeration temperature and 1, 2 and 3 days of storage at ambient temperature.

2.5 Evaluation of Sensory attributes

Consumer assessment of overall acceptability of the yogurt was done, according to (Sanni *et al*., 2016) [30]. Forty (40) staff of Bells University of Technology, Ota, Nigeria was chosen. These are regular consumers of yogurt and were randomly selected for the evaluation. Four samples were provided coded with 148, 914, 579, and 221. Each sample of the yogurt was placed in identical tight insulated stainless cup and placed on a clean table. A questionnaire was designed and distributed among the respondents to score five textural characteristics namely appearance, colour, taste, aroma, and mouth feel on a Hedonic scale of 9 points: Like extremely----9, Like very much---8, Like moderately---7, Like slightly---6, Neither like nor dislike---5, Dislike slightly---4, Dislike moderately---3, Dislike very much---2 and Dislike extremely---1. Each of the four samples was presented at different times to each of the forty respondents to avoid bias of judgement. The responses were collated to compare the consumer preferences of the yogurt produced.

2.6 Statistical analysis

Statistical analysis was carried out using SAS statistical software, version 9.4. For each experiment, one way analysis of variance (ANOVA) was used to study significant differences among treatments using Tukey's multiple comparison tests. The level of significance was set as 0.05 and mean values in row with different superscripts were significantly difference ($p<0.05$).

3. Results

Proximate composition of the various yogurt samples are shown in table 1. Lactic acid bacteria count, total coliform count, yeast and mould count and total viable count at ambient temperature are shown in Table 2. Microbial analysis of yogurt samples at refrigeration temperature on days 1, day 3, day 7 and day 14 are shown in Table 3. Table 4 presented the results of sensory evaluation of yogurt samples during the experiment.

Table 1: Proximate composition of the various yogurt samples

Sample	Moisture content (%)	Crude Protein (%)	Crude fat (%)	Crude fibre (%)	Ash Content (%)	CHO (%)
Dairy Yogurt	86.38±0.10^{c}	3.76±0.10^{b}	3.76±0.10^{c}	0.02 ±0.01^{a}	0.18±0.01^{a}	5.90±0.10^{b}
Dairy-Banana Yogurt	82.87±0.10^{a}	3.60±0.10^{a}	4.17±0.10^{d}	0.04±0.01^{c}	0.84±0.01^{b}	8.48±0.10^{d}
Soy Yogurt	87.02±0.10^{d}	5.96±0.10^{c}	1.44±0.10^{a}	0.02±0.01^{b}	1.53±0.01^{c}	4.03±0.10^{a}
Soy-Banana Yogurt	82.94±0.10^{b}	4.82±0.10^{d}	2.84±0.10^{b}	0.06±0.01^{d}	1.80±0.01^{d}	7.54±0.10^{c}

Table 2: Microbial analysis at ambient temperature

Samples	Parameters	Day 1 (CFU/g)	Day 2 (CFU/g)	Day 3 (CFU/g)
DY	LAB	4.1 x 10^2	4.8 x 10^2	5.1 x 10^2
	TVC	NIL	1.0 x 10^3	1.9 x 10^3
	Y/M	NIL	NIL	4.1x 10^3
	COLI	NIL	NIL	NIL
BDY	LAB	1.0 x 10^2	1.8 x 10^2	2.4 x 10^2
	TVC	NIL	1.3 x 10^3	2.3 x 10^3
	Y/M	NIL	2.3 x 10^3	4.7 x 10^3
	COLI	NIL	NIL	NIL
SY	LAB	1.0 x 10^2	3.7 x 10^2	3.3 x 10^2
	TVC	NIL	2.3x 10^3	2.7 x 10^3
	Y/M	NIL	NIL	3.0 x 10^3
	COLI	NIL	NIL	NIL
BSY	LAB	7.0 x 10^3	7.0 x 10^3	8.4 x 10^3
	TVC	NIL	2.2 x 10^3	3.3x 10^3
	Y/M	NIL	3.2 x 10^3	4.9 x 10^3
	COLI	NIL	NIL	NIL

Legend: DY- Dairy Yogurt TVC- Total Viable Count
BDY- Banana Dairy Yogurt Y/M- Yeast and Mould Count
SY- Soy Yogurt COLI- Total Coliform count
BSY- Banana Soy Yogurt LAB- Lactic Acid Bacteria Count

Table 3: Microbial analysis at refrigeration temperature

Samples	Parameters	Day 1 (CFU/g)	Day 3 (CFU/g)	Day 7 (CFU/g)	Day 14 (CFU/g)
DY	LAB	4.1 x 10^2	4.7 x 10^2	5.0 x 10^2	7.4 x 10^2
	TVC	NIL	NIL	NIL	NIL
	Y/M	NIL	NIL	NIL	NIL
	COLI	NIL	NIL	NIL	NIL
BDY	LAB	1.0 x 10^2	2.1 x 10^2	2.5 x 10^2	3.3 x 10^2
	TVC	NIL	NIL	NIL	NIL

	Y/M	NIL	NIL	NIL	NIL
	COLI	NIL	NIL	NIL	NIL
SY	LAB	1.0×10^2	3.0×10^2	3.4×10^2	5.1×10^2
	TVC	NIL	NIL	NIL	NIL
	Y/M	NIL	NIL	NIL	NIL
	COLI	NIL	NIL	NIL	NIL
BSY	LAB	7.0×10^3	7.5×10^3	8.2×10^3	9.3×10^3
	TVC	NIL	NIL	NIL	NIL
	Y/M	NIL	NIL	NIL	NIL
	COLI	NIL	NIL	NIL	NIL

Legend: DY- Dairy Yogurt Y/M- Yeast and Mould count BDY- Banana Dairy Yogurt
COLI- Total Coliform count SY- Soy Yogurt LAB- Lactic Acid Bacteria Count
BSY- Banana Soy Yogurt TVC- Total Viable count

Table 4: Sensory evaluation of yogurt samples

Sample ID	Colour	Appearance	Taste	Aroma	Mouth Feel	Overall Acceptability
Dairy Yogurt	8.55^d	8.35^d	8.15^d	8.25^d	8.10^d	8.40^d
Daily-Banana Yogurt	6.10^b	6.05^b	7.00^c	5.95^b	6.35^c	6.45^c
Soy Yogurt	6.70^c	6.30^c	5.10^a	6.01^c	5.20^a	5.15^a
Soy-Banana Yogurt	5.95^a	6.00^a	5.55^b	4.95^a	5.40^b	5.65^b

4. Discussion

Proximate composition of yogurt The moisture content, crude protein, crude fat, crude fibre, ash content and carbohydrate was: 86.38%, 3.76%, 3.76%, 0.02%, 0.18% and 5.90% respectively for dairy yogurt; 82.87%, 3.60%, 4.17%, 0.04%, 0.84% and 8.48% respectively for dairy-banana yogurt; 87.02%, 5.96%, 1.44%, 0.02%, 1.53% and 4.03% respectively for soy yogurt; and 82.94%, 4.82%, 2.84%, 0.06%, 1.80% and 7.54% respectively for soy-banana yogurt. The moisture content was in accordance with 86.38% reported by (Igbabul et al., 2014) [17]. Reduced moisture content in dairy-banana yogurt was likely to be as a result of the addition of banana in replacement of certain percentage of dairy product which could have made it less viscous (Denin et al., 2001) [14]. Increased in crude protein of soy yogurt might be linked to the fact that soy bean contains all the nine essential amino acids (Osundahunsi et al., 2007) [29]. Increased in crude fat, crude fibre and carbohydrate level in dairy-banana and soy-banana yogurts was most likely be as a result of banana inclusion. Ripe banana is reached in fibre, saturated and unsaturated fat (Adewunmi et al., 2009) [4]. The results showed that the values in all the compositions were significantly different ($p<0.05$) in the samples analysed.

Lactic acid bacteria count for dairy yogurt was 4.1×10^2, 4.8×10^2 and 5.1×10^2 at days 1, 2 and 3 respectively during ambient storage. Dairy-banana yogurt for day 1, day 2 and day 3 was 1.0×10^2, 1.8×10^2 and 2.4×10^2 respectively. The lactic acid bacteria count for soy yogurt was 1.0×10^2, 3.7×10^2, and 3.3×10^2 for day 1, day 2 and day 3 respectively. Soy-banana yogurt lactic acid bacteria count for day 1 and 2 was 7.0×10^3 and 8.4×10^3 for day 3. Total viable count of dairy yogurt ranged from nil to 1.9×10^3, dairy-banana yogurt ranged from nil to 2.3×10^3, soy yogurt and soy-banana yogurt ranged from nil to 2.7×10^3 and 3.3×10^3 respectively. Total yeast and mould count of dairy yogurt, dairy-banana yogurt, soy yogurt and soy-banana yogurt, ranged from nil to 4.1×10^3, 4.7×10^3, 3.0×10^3 and 4.9×10^3 respectively at ambient temperature for days 1, day 2 and day 3. Total coliform count was observed to be nil from day 1 to day 3 at ambient storage. All the microbial tests carried out were nil at day 1 except lactic acid bacteria. This could be as a result of fermentation (Mariangeles *et al*., 2012) [22]. The value increased as the fermentation increases. Samples resistance to total viable count, yeast and mould count and total coliform count at day 1 was high and this might likely be contributed to the nil value.

Lactic acid bacteria count for dairy yogurt was 4.1×10^2, 4.7×10^2, $5.0 \times 10^2$and 7.4×10^2 for day 1, day 3, day 7 and day 14 respectively. Total viable count, yeast and mould count were nil for dairy yogurt during storage at 4ºC. Dairy-banana yogurt lactic acid bacteria count was 1.0×10^2, 2.1×10^2, 2.5×10^2 and 3.3×10^2 at days 1, day 3, day 7 and day 14 respectively. The lactic acid bacteria for soy yogurt ranged from 1.0×10^2 to 5.1×10^2 and soy-banana yogurt ranged from 7.0×10^3 to 9.3×10^3. Lactic acid bacteria count increased during refrigeration storage. This could be that the culture grown slowly to adequately acidified milk to achieve a good texture as a result of low temperature (Wakil and Onilude, 2009; Lahtinen *et al*., 2012) [34, 20]. Total viable counts, yeast and mould counts and total coliform count were observed to be nil at days 1, day 3, day 7 and day 14 during refrigeration storage. This was perhaps because cold storage retards microbial activities.

The sensory evaluation showed significant difference ($p < 0.05$) in colour, appearance, mouth feel, taste, aroma and overall acceptability for all yogurt samples as shown in Table 3.4. Dairy yogurt has the highest mean value in the colour (8.55), appearance (8.35), taste (8.15), aroma (8.25), mouth feel (8.10), and overall acceptability (8.40). In dairy-banana yogurt, the values are colour (6.10), appearance (6.05), taste (7.00), aroma (5.95), mouth feel (6.35), and overall acceptability (6.45). In soy yogurt, the values are colour (6.70), appearance (6.30), taste (5.10), aroma (6.01), mouth feel (5.20), and overall acceptability (5.15). In soy-banana yogurt, the values are colour (5.95), appearance (6.00), taste (5.55), aroma (4.95), mouth feel (5.40), and overall acceptability (5.65).

5. Conclusion

1. Dairy yogurt produces variety of taste substantially stimulated and thereby influences consumers' perception on textural quality and perhaps general acceptability of the product. Thus, dairy yogurt is most preferred and suitable to the panelist.
2. Yogurt blended with banana (fruit yogurt) significantly

influences both the proximate and organoleptic properties of dairy and soy yogurt. The inclusion of banana in the production of yogurt is a means of value addition to banana which also reduces wastage by preventing postharvest losses.

3. Inclusion of banana has not in any way made the product to be prone to microbial activities. However, yogurt can be stored up to 14 days under refrigeration condition.
4. Soy yogurt has the highest protein content among the samples even though it has the least acceptability because of initial assessment particularly in the area of colour, taste, aroma and mouth feel. This challenges demands urgent attention in the subsequent research. The substitution of soy milk in the production of yogurt will apparently reduce the importation of dairy milk as raw material in developing countries for yogurt production and improve the shelf life of the product.

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