Nutritional and Sensory Evaluation of Soy-Yoghurt Produced with *Lactobacillus plantarum* Isolated from Locally Fermented Milk (*Nono*)

***Bukar, A. and Salami-Suleiman, F.A.**
Department of Microbiology, Faculty of Life Sciences, College of Natural and Pharmaceutical Sciences, Bayero University, PMB 3011, Kano state, Nigeria

Abstract: This study was aimed at determining the nutritional and sensory evaluation of soy yoghurt produced by fermentation of soymilk with *Lactobacillus plantarum* isolated from fermented skimmed cow milk. *Lactobacillus platarum* which was randomly isolated, and identified with biochemical and molecular processes underwent fermentation for 9 hours, together with commercial yoghurt starter culture containing *Streptococcus thermophilus* and *Lactobacillus delbreuckii* subsp. *bulgaricus*, which served as control to produce two types of fermented soy yoghurts labeled A and B respectively. Samples of the soy yoghurts were collected at 3 hours interval, analysed for proximate and physicochemical parameters, and at the end of 9 hours fermentation period, sensory evaluation was carried out. Also there was an overall drop in pH from 7.13 to 4.3 in soy yoghurt A and from 7.1 to 4.2 in B, while the titratable acidity (Lactic acid equivalent) showed an overall increase from 0.51 to 0.92% in A and 0.74- 0.90% in B. However, total solids and viscosity increased in sample A from 9.02- 13.25%, 0.3-0.5PaS and in B from 3.66-5.86%, 9.56- 13.62%, 0.24 to 0.40 PaS between 0 – 9 hours respectively. The ash, moisture and crude fat content had an overall decrease in sample A from 0.85 - 0.61% , 92.4- 92.28% and 1.92 - 0.79%, respectively and Sample B from 0.85- 0.63%, 92.87- 92.06% and 1.87- 0.85%, respectively. The protein content increased from 3.72-5.54% in sample A, and 3.66-5.86% in sample B respectively. The carbohydrate content of the soy yoghurt samples decreased after 3-9 hours, from 1.20-0.78% in sample A and 0.80- 0.60% in sample B respectively. The microbiological analysis indicated that there was an increase in cell growth of sample A containing *Lactobacillus plantarum,* from 3.2 log cfu/ml to 4.6 logcfu/ml and from 3.7logcfu/ml to 5.0logcfu/ml in sample B. Sample B was most preferred in terms of aroma, taste, texture, colour and overall acceptability (70% acceptability), while sample A had a 64% acceptability. The result revealed that the use of isolates from fermented skimmed cow milk in the fermentation of soy milk produced soy yoghurt of acceptability and sensory attributes similar to that produced using commercially sold starter culture.
Key words: Soy yoghurt, Starter culture, Milk, Sensory, Acceptability

INTRODUCTION

Milk is a dairy product and an essential dietary component for over 6 billion people worldwide. Its annual world production is about 730 million tons. (Food and Agricultural Organization, FAO, 2012). Dairy products are foods or drinks that derived from milk of cows, buffaloes, goats, or sheep (United Kingdom Agency, 2009). Most dairy products contain large amount of saturated fat, usually fermented and sour in taste. They are high in calcium and vitamins and are a good source of energy. Fermented milk products are made by either natural fermentation of the milk or by the use of starter culture. Lactic acid bacteria such as *Lactobacillus*, *Streptococcus*, *Enterococcus, Pediococcus, Leuconostoc* and *Aerococcus* are used as starter culture and they include species (Bladino *et al*, 2003).

[1]*Corresponding Author:
abukar.bio@buk.edu.ng +2348023567064

Nono is the Hausa name for naturally fermented skimmed milk product sold by mostly Fulani women in Northern Nigeria. It is usually prepared from cow's milk but occasionally from goat's milk. It is prepared by allowing fresh milk in a covered calabash to ferment at room temperature for 24 hours. The fermentation of *nono* may be accomplished by a number of different bacterial species from various sources that naturally contaminate the fresh milk. Microorganisms that have been reported to be implicated in the fermentation of *nono*, which include *Streptococcus lactics, Lactobacillus acidophilus*, and *Streptococcus cremoris* (Wouters *et al*., 2002).

Soymilk is a water extract of soybean, a grain legume and one of the oldest known food sources of human being. It contains good quality ingredients for food, feed and pharmaceuticals and other industrial applications (Tripathi and Mangaraj, 2011).

The edible portion of soybean contains about 40% protein, 27% complex carbohydrates, 20% oil, 8% moisture and 5% minerals. Special features of soymilk are low cost, good nutrient and its suitability to lactose-intolerance people. Soymilk can be used for feeding infants, and as supplements to diets of the pre-school children, young adult and old people (Tripathi and Mangaraj, 2013). Soy yoghurt is the yoghurt prepared using soymilk, yoghurt bacteria and sometimes additional sugar, like fructose, glucose, honey or raw sugar (Le-Ngoc and Cao, 2000). Soy yoghurt is a fermented product obtained through anaerobic fermentation of lactose in soymilk by yoghurt bacteria, most of which are known as Lactic acid bacteria. There has been a recent increase in the consumption of soymilk due to a greater consciousness of health as well as the need to cure diseases. However, soymilk has a characteristic beany flavour due to presence of lipoxygenase enzymes in soybeans, which may not be accepted by all consumers. Traditionally, some studies have showed that the beany flavour in soy beans has been controlled/ minimized by activating the enzymes by heating and fermentation of soy milk into soy yoghurt., although soy yoghurt is still at the indigenous level of fermentation with no standard starter culture reported for its fermentation as in Yoghurt, therefore there is still the need for research to exploit the use of different LAB either singly or in combination to produce the desired product, which is the objective of this research.

This goal can be achieved by randomly selecting effective strains of LAB isolated from *nono* capable of fermenting soy milk into soy yoghurt of good nutritional quality and consumer acceptability. The readily availability and affordability of soy bean makes it a cheap protein source for nursing mothers and their babies and the results from this study may be employed in the food industry to solve the problem of objectionable beany flavour in soy milk.

MATERIALS AND METHODS

Sample collection and processing

The samples of soy beans, yoghurt culture, gelatin and sucrose used in this study were purchased at Tarauni and Bata markets, Kano state. Three samples of freshly prepared *nono* were purchased from different Fulani women at Dambare, Ungogo local government, and Rigar Fulani, opposite Bayero University, Kano New campus.

It was aseptically collected into sterile McCartney bottles in triplicates and immediately transported to the Department of Microbiology laboratory situated in Bayero University Kano, old campus in an iced packed container maintained at a temperature of 4^{o}C. The samples of soy bean were processed sorted, soaked in water and grinded with a sterile food blender. The fresh *nono* samples were left covered in a refrigerator until used. Serial dilution was carried out by aseptically introducing 10ml of the sample into 90ml of sterile normal saline solution. It was homogenized by shaking followed by further decimal dilutions to up to 10^{-9} concentrations.

Isolation and morphological characterization of Lactic Acid Bacteria

A mililitre of 10^{-4}-10^{-6} of appropriately diluted sample was added into sterile petri dishes (in triplicates), and already sterilized De Mann Rogosa Sharpe (MRS) agar was added intosterile petri dishes and agitated. It was allowed to solidify and incubated anaerobically at 37^{o}C for 48 hours. The isolates were purified by repeated sub-culture by streak plate method on fresh medium of MRS agar. The morphological characteristics such as cell shape, colour, and arrangement were noted.

Identification of the Isolate

Gram staining, catalase test and Endospore staining were carried out on the isolate A to identify the *Lactobacillus* species (Pelczer, 2007). Subsequently, the species of *Lactobacillus* were further confirmed for production of acids from carbohydrates and related compound by use of the API 50 CHL system.

All LAB identification procedures were conducted in accordance with manufacturer's instructions.

Molecular characterization of *Lactobacillus* species

Molecular characterization of the *Lactobacillus plantarum* was first identified by sequencing 16S rDNA gene of the bacteria using the primer pair 27F- 5´-AGAGTTTGATCCTGGCTCAG -3´. and 1492R 5´-GGTTACCTTGTTACGACTT -3´. The PCR reaction was carried out using the Solis Biodyne 5X HOT FIREPol blend master mix. PCR was performed in 25ul of a reaction mixture, and the reaction concentration was brought down from 5x concentration to 1X concentration containing 1X blend master mix buffer (Solis Biodyne), 1.5 mM $MgC_{l2,}$ 200uM of each deoxynucleoside triphosphate (dNTP) (Solis Biodyne), 25pMol of each primer (Gena Bioscience, Germany), 2 units of Hot FIREPol DNA polymerase (Solis Biodyne), proofreading enzyme, 5ul of the extracted DNA, and sterile distilled water was used to make up the reaction mixture. Thermal cycling was conducted in a Pelthier thermal cycler (PTC100). (MJ Research series) for an initial denaturation of 95^{o}C for 15 minutes followed by 35 amplification cycles of 30 seconds at 95^{o}C: 1 minute 30 seconds at 72^{o}C. The amplification products was separated on a 1.5% agarose gel and electrophoresis was carried out at 80V for 1 hour 30 minutes. After electrophoresis, DNA brands were visualised by ethidium bromide staining. 100bp DNA ladder was used as DNA molecular weight maker. All PCR products were purified and sent to Inqaba (South Africa) for Sanger sequencing. The corresponding sequences were identified using the online blast search at http://blast.ncbi.nlm.nih.Gov/Blast.cgi.

Processing of Soybean to produce Soymilk

One (1) Kg of disease-free Soy beans were carefully selected. The Soy beans were soaked for 14 hours in 1 litre of distilled water at a room temperature of $\pm$28^{o}C. The soaked water was drained from the soy beans and the beans was blanched at 98^{o}C in boiling distilled water for 20 minutes, in order to remove some of its lipoxygynase enzymes present in the beans. The drained beans were hand washed thoroughly in order to remove their testa, and this was followed by grinding in a sterile food blender. The resulting slurry was filtered through two layers of cheese cloth with tiny pore size (0.5mm), in order to separate out soymilk from residue. The soy milk in a flask obtained was capped with cotton plugs, covered with aluminium foil and was pasteurized in clean water bath at 90^{o}C for 15 minutes. The pasteurized soy milk produced was held at 5^{o}C until used (FAO, 2012)

Production of Soy yoghurt

To the prepared soymilk, 3% (w/v) sugar and 0.5%(w/v) gelatin were added and mixed thoroughly. This was pasteurized at 60^{0}C for 30 minutes and then cooled to 43^{o}C. A 50ml quantity of soymilk equivalent was put in 2 different sterilized conical flasks. Flask labeled A containing soymilk was inoculated with 1ml 0.5 McFarland standard of 24 hours old culture inocula 10^{8} cells/ml *Lactobacillus plantarum* M1 while flask labeled B was used as control and it contained soymilk and 1ml industrial culture, named Yo-Ci03-F containing *Streptococcus thermophilus and Lactobacillus delbreuckii* subsp *bulgaricus* (Lee *et al.,* 1990). The mixture was stirred for two minutes followed by incubation of the inoculated soymilk at 44^{o}C. Sampling was carried out every 3 hours during soy milk 9 hours of fermentation. The soy yoghurt formed was stored at 4^{o}C until sensory analysis was conducted.

Physicochemical analyses of Soy yoghurt samples

The pH was measured using pH meter after standardization with pH 4, 10 and 7 buffers (BDH England). Titratable acidity was determined by method described by Association of Official Analytical Chemists, while the total solids was determined by method described by Association of Official Analytical Chemists (AOAC, 2010).

All measurement of the physicochemical parameters was carried out in triplicate at intervals of 3 hours within 9 hours of fermentation.

Determination of viscosity

The apparent viscosity was determined using a viscometer on the different soy yoghurt samples produced. The determination was done according to the manufacturers' instructions (Brookfield, USA).

Proximate analyses of samples

The ash, moisture and fat content of the prepared soy yoghurt were determined by method described by Association of Official Analytical Chemists (AOAC, 2010). The crude protein content was determined by the macro Kjeldah method as described by AOAC (2005). The Carbohydrate content was determined by difference as described by AOAC (2000).

Determination of Lactic acid bacterial count

This was achieved by using the serial dilutions and pour plate technique procedures described by Cheesbrough (2006) and Willey *et al*. (2011).

Sensory Evaluation

The sensory evaluation was carried out by using a 10 man semi-trained panelist. The panelists were instructed to indicate their preferences of the sample. A nine point Hedonic scale was used, where 9 is the highest score and 1 being the lowest score for each characteristics such as taste, flavour, colour, appearance and overall acceptability were determined (Hashim *et al.,* 2009).

Statistical analysis

Mean values of the results with standard deviations (mean± standard deviation) were recorded. The statistical significance of differences observed among treatment means was evaluated using One- way Analysis of Variance (ANOVA)(IBM SPSS version 23 2018), followed by post hoc Duncan test. The statistical significance was accepted at $p < 0.05$.

RESULTS AND DISCUSSION

Table 1 presents the morphological characteristics of *Lactobacillus plantarum* isolated, and they were mostly creamish in colour, flat, smooth, roundish shape, gram positive, catalase negative and endospore negative and was identified as *Lactobaccillus* specie.

Figure 1 presents the sugar fermentation of *Lacobacillus* specie produced using API CHL, it was able to ferment L-arabinose, ribose, galactose, D-glucose, D-fructose, D-mannose, rhamnose, α- methyl D mannoside, α- methyl D glycoside, N-acetyl glucosamine, amygdaline, arbutine, esculine, alicine, cellibiose, maltose, lactose, mallibiose, saccharose, trehalose, inuline, melezitose, D-raffinose, B- gentiobiose, D-turanose, D-arabitol, and glyconate, and were not able to ferment control sugar, glycerol, erythritol, D- arabinose, D-xylose, L- xylose, adonitol, xyloside, L- sorbose, dulcitol, inositol, mannitol, S-orbitol, amidon, glycogen, xylitol, D-lyxose, D-tagatose, D-fucose, L -fucose, L-arabitol, 2-ceto-gluconate, and 5- ceto-gluconate.

Figure 1 shows the PCR amplification of the *Lactobacillus plantarum*, which was carried out based on 16S rRNA sequence analysis. The partial sequence of 16S rRNA obtained from *L. plantarum* were aligned with all the presently available 16S rRNA sequences in the Gen Bank Database.

The presence of *L. plantarum* in raw milk could be attributed to the contamination of raw milk mostly by strains of lactic acid bacteria genera during milking, from various sources such as the exterior of the udder, dairy utensils, dust, grass, feedstuffs (FAO, 2008). *Lactobacillus plantarum* have also been found to be associated with fermented milk (Maged *et al* 2018).

Table 2 shows changes in physicochemical properties of Soy yoghurt samples A and B during fermentation period of 0-9 hours.

The mean initial values of pH at time 0 for sample A and B were 7.13±0.03 and 7.1±0.00 respectively, while a significant decrease in pH for both samples were observed from time 3-9 hours interval, with a final pH of 4.3±0.03 and 4.2±0.00 respectively. The mean titratable acidity of sample A and B however shows an insignificant increase within the 9 hour of fermentation with an initial value of 0.51± 0.05 and 0.74±0.03 respectively ($p>0.05$). At 9 hours, the titrability acidity increased to 0.92±0.01 respectively.

This result is similar to the acidic pH values of 4.81 reported by Oluwabamiwa and Kolapo (2007), in which soymilk underwent fermentation using commercial starter culture. The decrease in pH value of soy yoghurt with increase in fermentation time might be due to the yoghurt bacterial ability to grow in the samples and ferment the carbohydrate that was present. The action of these Lactic acid Bacteria by converting the sucrose, stachyose and raffinose in soy milk to simpler sugar and organic acids, as well as the accumulation of organic acids, thereby lowering the pH of the soy yoghurt produced (Zourari *et al.,* 1992; Almeida *et al*., 2007). An increase in titratable acidity supports earlier reports of some researchers (Gesinde *et al*., 2008, Almeida *et al*., 2007), and this could be due to the accumulation of some organic acids such as lactic acid and acetic acid resulting from the activities of the Lactic acid bacteria in the fermenting foods. The role of these acids in fermented milk is for natural preservation, in order to increase their quality, as well as extending their shelf life (Brown *et al.,* 2005).

The total solids of samples A and B shows a significant increase($p>0.05$) in their initial mean values from 9.02% and 9.56% respectively, with a final increase at 9 hours from 13.25% and 13.62% respectively. This could be as a result of the loss in moisture of the soy yoghurt samples within the fermentation period. This result is similar with the report of Osudahunsi *et al*. (2007), where an increase in total solids of between 9 to 12% was reported. Reduction in free water in yoghurt increases the proportion of their solid contents and these are the two factors that help in decreasing the wheying off in yoghurts with high total solid contents (Mahdian and Mostafa, 2007).

The mean apparent viscosity of samples A and B shows a significant difference ($p>0.05$) of the soy yoghurt samples ($p<0.05$) with an increase in their initial values from 0.30paS and 0.24paS respectively, with a final increase in the mean viscosity after 9 hours of fermentation at 0.50 and 0.40paS respectively. This result is similar to lower apparent viscosity (0.4-1PaS) obtained by Sodini *et al*. (2005) during his study on the fermentation of yoghurt samples. Yoghurt viscosity is directly related to the protein content (Sodini *et al*., 2005). After the increase in protein content, the enhancement in the viscosity is evident. Also, the use of fortificant (gelatin) led to the increase in viscosity of the soy yoghurt samples produced.

Table 3 shows changes in proximate composition of Soy yoghurt samples A and B during fermentation period. There was no significant difference in the ash contents ($p>0.05$) of the soy yoghurt samples A and B, and the mean initial value were the same at 0.85%. There was a decrease in the values at 9 hours from 0.61% and 0.63% respectively. This result is similar to earlier reports of Obadina *et al.* (2013), with ash content values of 0.23- 0.74%, during soymilk fermentation. This reduction in ash content with change in fermentation time might be due to reduction of carbohydrate, moisture and fat contents of the soy yoghurt samples during fermentation (Obadina *et al.,* 2013).

The mean values of moisture content of soy yoghurt samples A and B are significantly different from one another ($p>0.05$) with the mean initial values ranging from 92.44% and 92.8% respectively to Similar decrease in moisture content was observed in the study of Obadina *et al*. (2013), which recorded the moisture content of between 93.45-93.30% from 0- 12 hours of natural fermentation of soymilk.

The decrease in moisture content could be due to the increase in dry matter content as a result of microbial cell proliferation. Morris *et al.* (2004) reported that decrease in moisture generally causes an increase in the concentration of nutrients.

There was an increase in protein contents among all soy yoghurt samples produced, from 3.72 – 5.54% between 0- 9 hours for sample A and for sample B, 3.66 – 5.86% from 0- 9 hours interval. Statistical analysis results in the significant difference ($p<0.05$) between protein contents among all the treatments within the fermentation period was observed. The increase in protein content is similar to values of flavoured soy yoghurt that was reported by Osundahunsi *et al*. (2007). This increase in protein content might be as a result of yoghurt starter cultures bringing about increase in peptides and amino acids during proteolysis in the process of fermentation, which results in changes in the nitrogenous compounds in yoghurt by splitting urea, thereby increasing the level of ammonia nitrogen in cultured milk (Liu *et al*., 2010).

The fat content among all soy yoghurt samples A and B reduced from 0.30- 0.50% at 0 hour to a range of 0.24- 0.40% after 9 hours respectively. Statistical analysis showed a significant ($p<0.05$) difference between the fat content among all the treatments within the fermentation period. This reduction in fat content has been reported by Obadina *et al*. (2013), and this may be due to increased activity of the lipolytic enzymes during fermentation, which lead to the hydrolysis of fat components (Triglycerides) into fatty acid and glycerides.

The mean values of carbohydrate increased from 0.98- 1.20% between 0 to 3 hours, and there was a subsequent decrease in carbohydrate content from 1.20- 0.78% from 3 to 9 hours of fermentation. Statistical analysis results showed a significant ($p<0.05$) difference between in carbohydrate content among all the treatments within the fermentation period. . The initial increase may be as a result of the initial breakdown of sucrose, stachyose and raffinose in soy milk to glucose, fructose and galactose, while the final decrease in carbohydrate content from 3 hours to 9 hours of fermentation may be due to the action of *Lactobacillus* species by completely breaking down the monosaccharides in soy yoghurt, which is essential as energy source for growth and other cellular activities (Vondrasek, 2014). The final reduction in carbohydrate content of soy yoghurt in this study is similar to the findings of Obadina *et al.* (2013), who reported a reduced carbohydrate content of yoghurt from 1.52 to1.37% at 6 hours of fermentation.

Table 1: Preliminary identification results of the isolate

Growth on MRS agar	Specie Morphology	Gram reaction	Catalase reaction	Endospore reaction	Inference
+	Creamish, mostly flat Smooth, mostly round growth	Gram positive	Catalase negative	Endospore negative	*Lactobacillus* specie

Table 2: Changes in physicochemical properties of Soy yoghurt samples A and B during fermentation period of 0-9 hours

		Treatments	
Parameters	Time (hr)	A	B
pH	0	7.13±0.03[a]	7.10±0.00[c]
	3	4.90±0.00[a]	5.40±0.00[d]
	6	4.60±0.00[a]	4.80±0.10[b]
	9	4.30±0.03[a]	4.20±0.00[a]
Titratable acidity (%)	0	0.51±0.05[a]	0.74±0.03[b]
	3	0.81±0.02[a]	0.83±0.02[a]
	6	0.86±0.15[a]	0.86±0.01[a]
	9	0.92±0.02[a]	0.90±0.01[a]
Total solids (%)	0	9.02±0.04[a]	9.56±0.06[d]
	3	9.32±0.04[a]	9.82±0.03[d]
	6	11.7±0.21[a]	11.19±0.07[c]
	9	13.25±0.04[a]	13.62±0.07[d]
Viscosity (cp)	0	0.30±0.00[a]	0.24±0.02[b]
	3	0.30±0.00[a]	0.29±0.00a
	6	0.30±0.01[a]	0.31±0.00[a]
	9	0.50±0.00[a]	0.40±0.00[a]

A= soymilk and *L. plantarum* B= soymilk and commercial starter culture (control). The values are Mean ±SD of triplicate determination. Mean with different superscripts on the same row are significantly different (P<0.05).

Plate I: Identification of *Lactobacilli* species with API CHL

Where 0= Control , 1= glycerol 2= erythritol,3= D- arabinose,4= L-arabinose,5=ribose, 6= D-xylose, 7=L- xylose, 8= adonitol, 9= xyloside, 10=galactose,11= D-glucose, 12=D-fructose, 13=D-mannose,14= L- sorbose, 15= rhamnose,16= dulcitol, 17= inositol, 18= mannitol, 19= S-orbitol, 20= α- methyl D mannoside, 21=α- methyl D glycoside, 22= N-acetyl glucosamine, 23=amygdaline, 24=arbutine, 25=esculine, 26=alicine,27= cellibiose, 28=maltose, 29=lactose, 30=mallibiose, 31=saccharose, 32=trehalose, 33=inuline, 34=melezitose, 35=D-raffinose, 36= amidon, 37= glycogen, 38= xylitol, 39=B- gentiobiose,40= D-turanose 41=D-lyxose,42=D-tagatose,43=D-fucose, 44= L -fucose, 45=D-arabitol, 46= L-arabitol, 47= glyconatel, 48= 2-ceto-gluconate, 49=5- ceto-gluconate.

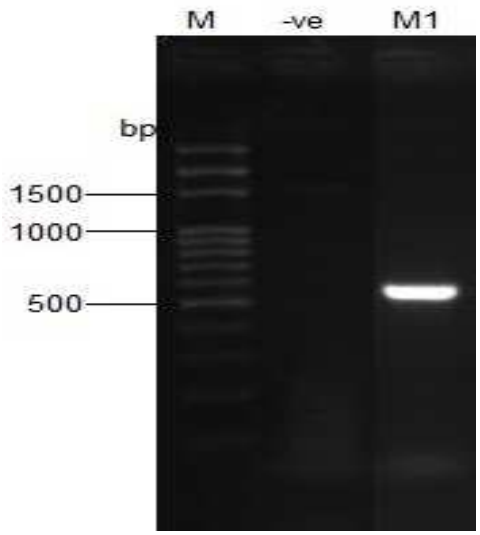

Figure 1: PCR amplification of 16S rDNA gene (500bp), M1 : Isolate

Table 3: Change in proximate composition of Soy yoghurt samples A and B during fermentation period of 0-9 hours

Parameters (%)	Time (hr)	A	B
Ash content	0	0.85±0.03[a]	0.85±0.03 [a]
	3	0.72±0.04 [a]	0.71±0.91 [a]
	6	0.66±0.32 [b]	0.65±0.02 [b]
	9	0.61±0.03 [a]	0.63±0.02 [a]
Moisture Content	0	92.44±0.02[a]	92.87±0.03 [ab]
	3	92.35±0.02 [a]	92.12±0.02 [c]
	6	92.32±0.01 [a]	92.10±0.05 [b]
	9	92.28±0.02 [a]	92.06±0.02[c]
Protein content	0	3.72±0.01[a]	3.66±0.01[d]
	3	4.62±0.02[a]	4.72±0.01 [c]
	6	5.20 ±0.15[a]	5.40±0.03[a]
	9	5.54±0.02 [a]	5.86±0.02[b]
Crude fat content	0	1.92±0.02 [a]	1.87±0.01 [c]
	3	1.12±0.01 [a]	1.66±0.01 [c]
	6	0.92±0.01 [a]	1.10±0.00 [a]
	9	0.79±0.02 [a]	0.80±0.01[b]
Carbohydrate content	0	0.98±0.30 [a]	0.75±0.03 [b]
	3	1.20±0.01[a]	0.80±0.01[b]
	6	0.90±0.08 [a]	0.75±0.03[b]
	9	0.78±0.02 [a]	0.60 0.01[c]

A= soymilk and *L. plantarum* B= soymilk and commercial starter culture (control)
The values are Mean ±SD of triplicate determination. Means with different superscripts on the same row are significantly different (P<0.05).

Table 4: Log (cfu/ml) lactic acid bacteria count of different soy yoghurt samples

Time (Hr)	A	B
0	3.27±0.01 [a]	3.70±0.06 [b]
3	3.80±0.06 [a]	4.30±0.15 [b]
6	4.30±0.15 [a]	4.80±0.06 [b]
9	4.60±0.06[a]	5.00±0.10 [b]

Mean± SD of triplicate readings

Values with different superscript on the same row are significantly different, ($p < 0.05$). Sample A= soy yoghurt + *L. plantarum,* Sample B = soy yoghurt + commercial yoghurt culture

Table 5: Sensory attributes of soy yoghurt samples

Samples	Sensory attributes				
	Aroma	Texture	Colour	Taste	Overall acceptability
A	6.50±0.40[a]	6.20±0.55[ab]	7.5±0.40[abcd]	6.9±0.35[d]	7.4±0.31 [c]
B	8.10±0.23 [a]	7.7±0.34 [ac]	7.9±0.31[a]	8.5±0.22 [b]	8.6±0.16 [da]

Mean± SD of triplicate readings.

Values with different superscript on the same row are significantly different, ($p < 0.05$). Sample A= soy yoghurt + *L. plantarum,* Sample B = soy yoghurt + commercial yoghurt culture

Table 6: Sensory Evaluation of Respondents to soy yoghurt Servings A and B after 9 Hours of Fermentation

S/No.	A	B
1	8	9
2	8	8
3	8	8
4	7	8
5	7	7
6	7	7
7	6	7
8	6	6
9	4	5
10	3	5
	*6.4±0.52	*7.0±0.41
	% Dislike = 20%	% Dislike = 0

***Mean± SD of ten readings.**

There was an increase in LAB count during the fermentation time from 3.2 to 4.6 *log*cfu/ml Sample A) and 3.7 to 5.0*log*cfu/ml Sample, after the 9 hours fermentation period (Table 4). Statistical analysis results showed a significant ($p<0.05$) difference in LAB count among all the treatments A and B within the fermentation period. This may be due to proliferation of the bacteria as the fermentation time increases. The original bacterial in yoghurt are beneficial to human health, although the quantitative standard for yoghurt bacteria differs, it is generally accepted that yoghurt should contain 10^7cfu of viable LAB bacteria per ml of yoghurt (Chougrani *et al*., 2009).

There was no significant difference among all the soy yoghurt samples in term of aroma and colour, although sample A was significantly different among other samples in terms of taste (Table 5).

In terms of texture, soy yoghurt sample B was significantly different (p>0.05). from sample A produced. The sensory attributes of aroma of soy yoghurt samples produced showed that yoghurt D was rated highest in aroma, this might be due to the good flavor perceived by the panelists.

Soy yoghurt sample B had the highest mean score value of 7.0, with 0% dislike, and was thus, most preferred and best rated with respect to all quality attributes. This might be due to the effect of the combined *Lactobacillus* species used in manufacturing the industrial starter culture (Table 6).

CONCLUSION

The results obtained from this work indicated that soy yoghurt with *Lactobacillus plantarum* compared favourably with one produced with standard yoghurt starter culture in terms of physicochemical, proximate and sensory properties. This implies that if the mixture of *nono* with soy milk is commercialized, production of soy yoghurt with good consumer acceptability will be a cheaper alternative to their cow milk counterpart.

RECOMMENDATIONS

Further research is recommended in order to deodourize soybeans thereby removing its antinutritive contents, as a way of increasing its consumption by consumers as well as ascertaining the shelf-life stability of soy beans thereby increasing soybeans as a food value in the food industry.

REFERENCES

Almeida, E.G., Rachid, C.C., Schwan, R.F. (2007): Microbial population present in fermented beverage "caiun" produced by Brazilian Amerindians. *International Journal of Food Microbiol*ogy. 146-151.

AOAC (2000): Official methods of analysis of *Association of official Analytical Chemistry* international, 17th edition. Gaithersburg, MD, USA, Official methods, 920.124, 926.08, 955.30, 2001.14.

AOAC (2005): Official method of analysis. *Association of official Analytical Chemistry.* 17th edition, Washington D.C.

AOAC (2010): Official methods of analysis. *Association of Official Analytical Chemists.* (18th Edition). Gaithersburg, USA.

Bladino, A., Alaseeri M. E., Pandiella S.S, Cantero D. and Webb, C. (2003): Cereal-based fermented food and beverages. *Food Research International*. 36: 527-543.

Brown, A.C., A. Shovic, S.A, Ibrahim, P., Holck and A. (2005). A non-diary probiotic influence on changing the gastrointestinal tracts microflora enrironment. Altern. Ther. Health Med. 11: 58-64.

Cheesebrough, M. (2002): *District Laboratory Practice in Tropical Countries*. ECBS edition. Cambridge University Press. 2: 97-182.

Cheesebrough, M. (2006): *District Laboratory Practice in Tropical Countries.* ECBS edition. Cambridge University Press. 2: 124-134.

Chougrani, F., Cheriguene, A. and Bensoltane, A. (2009): Physicochemical and rheological properties of yoghurt manufactured with ewe's milk and skim milk. *African Journal of Biotechnology.* 8(9): 1938-1942.

Damir, A.A., Salama, A.A., Saftwat, M.M. (1992): Acidity, microbial, organic and free amino acids development during fermentation of skimmed milk. *Food chemistry*. 43: 256-269.

Food and Agricultural Organization, FAO. (2008): FAOSTAT. Food and agriculture Organization of the United Nations. *http://faostat.fao.org/default.aspx.n.*

Food and Agricultural Organization, FAO. (2012): FAOSTAT. Food and agriculture Organization of the United Nations. *http://faostat.fao.org/default.aspx.n.*

Gesinde, A.F., Oyewoye O.M., Adebisi A. (2008): Comparative studies on the qualityand quantity of soymilk from different varieties of soybean. *Pakistan Journal of Nutrition*. 7(1): 157-160.

Haelein, G.F.W. (1996): Nutritional value of dairy products of ewe and goat milk.In production and utilization of ewe and goat milk. *Proceedings on the IDF/Greek National committee of IDF/CIRVAL Seminar, Brussels, Belgium*.

Hashim I.B., Khalil, A.H., Afifi H.S. (2009): Quality characteristics and consumer acceptance of yoghurt fortified with dietary fiber. *Journal of Dairy Science*. 92: 5403-5404.

Isono, Y, Shingu I, Shimizu S. (2001): Identification and characteristics of lactic acid bacteria isolated from Maasai fermented milk in northern Tanzania. *Bioscience, Biotechnology, and Biochemistry*. 58: 660-664.

Le-Ngoc, T. and Cao, N. D. (2000): Fermented soy milk using Lactic acid bacteria. *Biotechnology.* 1:33-36.

Liu, J.E., Zhang, Y., Zhang J., Dong, P.L., Chen, M., and Duan, Z,P.,(2010). Probiotic yoghurt effects on intestinal flora of patient with chronic liver disease . *Nursing research*. 59;426-432.

Mahdian, E. and Mostafa, M.T., (2007): Evaluation of the effects of milk on total solids on the relationship between growth and activity of starter cultures and quality of concentrated yoghurt. *Journal of Agriculture and Environment Science*. 2(5): 587-592.

Maged S. Bin Masalam, Ahmed Bahieldin, Mona G. Alharbi, Saad Al-Masaudi, Soad K.,Al-Jaouni, Steve M. Harakey, and Rashad R. Al-Hindi.(2018). Isolation, Molecular characterization and probiotic potential of Lactic Acid Bacteria in Saudi raw and fermented milk. 1-12

Morris, A.,Barnett, A. and Burrows, O.(2004): Effect of processing on nutrient content of foods. *Cajartocles*. 37:160-164.

Obadina, A,O., Akinola, O.J., Shittu T.A., Bakare, H.A. (2013): Effect of natural fermentation on the chemical and nutritional composition of fermented soymilk *nono. Nigerian food Journal.* 31(2): 91-97.

Olubamiwa, A.O., Kolapo A.L. and Odetoyinbo, B.B. (2007): Effect of different starter culture on the chemical composition and acceptability of soy yoghurt. *International Journal of Food and Agricultural Research.* 4: 1-8.

Opara, C.C., Ahiazunwo, N.J. and Okorie, O. (2013), "Production of soy yoghurt by fermentation of soy milk with *Lactobacillus* isolated from *Nono", International Journal of Science and Engineering Investigation*. 2(12): 1 – 5.

Osundahunsi, O.F., Amisun D., Ifesan, B.O.T. (2007): Quality evaluation and acceptability of soy yoghurt with different colours and fruit flavours. *American Journal of Food and Technology*. 2(4): 273-280

Pelczer, M.J. (2007): *Fundamentals of Microbiology*. Pelczer Microbiology International new Edition. (Jakarta: UI Press). www.downloadgeek.com.

Sodini, I., Montella, J. and Tong, P.S. (2005): Physical properties of yoghurt fortified with various commercial whey protein concentrates. *Journal of the Science of Food and Agriculture.* 853-859.

Tripathi, M.K. and Mangaraj, S. (2013): Effect of coagulants on nutrient and antinutrient parameters of soy tofu. *Trends Biosci*ence 6(2):156-160.

Tripathi, M.K. and Mangaraj, S. (2011): *Soy food in demand: a present and future perspective*. In soybean: cultivation, uses, and nutrition (ed. Maxwell, J .E), Nova Publishers, Inc, New York.

United Kingdom Agency (2009): Online: Total milk production in the United Kingdom from milk year 2001. *www.statista.com*.

Vondrasek, J.R. (2014): Yoghurt making – How carbohydrate type affect yoghurt chemistry. *Proceedings of the Association for Biology Laboratory education.* 35:314-326.

Willey, J.M., Sherwood L.M., Wolverton C.J. (2011). *Microbiology of Food*, Prescott's Microbiology (8th edition).Mc Graw Hall International.

Wouters, J.T.M, Ayad, E. H.E. Hugenholtz, J. and Smith, G. (2002): Microbes from raw milk for fermented dairy products*. International Dairy Journal.* 91-109.

Yoon, M.Y. and Hwang, H.J. (2008): Reduction of soybean oligosaccharides and properties of a-galactosidase from *Lactobacillus curvatus* R08 and *Leuconostoc mesentroides* JK. *Food Microbial.* 53-57.