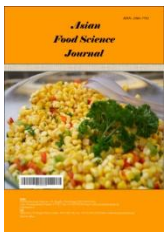

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Effect of Cold Storage on the Nutritive and Microbiological Quality of Fermented Soy Drink from Tamarind and Nono

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The effect of cold storage on fermented soy drink from tamarind and nono was assessed. Soymilk was produced by milk extraction from whole soybean seeds and pasteurized at 76°C for 30 minutes. The soymilk was divided into two portions. One portion inoculated with tamarind pulp containing 5.3×10^3 cfu/mL and the other with *nono* containing 11.6×10^3 cfu/mL. They were incubated at 42°C for 12 hours, fermentation was harvested by stirring, packaged, refrigerated at 5°C and subjected to microbial analysis using standard method. Preservation of drink by refrigeration method increased the microbial load of sample A from day 0 (8.7×10^3 cfu/mL) to day 9 (15.0×10^3 cfu/mL) but decreased on day 12 (11×10^3 cfu/mL). Similar results were recorded for samples B and C. However, sample A had neither coliform nor fungal growth. Sample A and B had no significant ($p>0.05$) difference in energy value (41.91±0.89 and 42.50±1.14) but sample C had the highest energy (96.69±2.03- 77.80±1.17), ash (4.10±0.13- 96.69±2.03), crude protein (0.51±0.01- 0.55±0.03), oil extract (3.44±0.17- 3.65±0.15) and NFE (7.61±0.14- 11.16±0.17) but lowest in moisture (79.84±1.07- 80.27±1.30) contents on day 6– 12. However, sample B had high moisture content ranged (84.43±1.17- 87.15±2.3) but lower in other parameters. Statistical analysis

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for the vitamin C, potassium and calcium of sample's A, B and C were carried to determine their significant differences. Refrigeration slows down the bacterial activity hence reducing spoilage thus making fermented soy drink a good source of desired protein in Nigeria.

Keywords: Cold storage; soy drink; fermentation; proximate composition.

1. INTRODUCTION

In the production of fermented milk, adding thermophilic bacteria to milk originates the drop in pH, which provokes changes in the protein casein [1,2] the fundamental structure of fermented milk is its protein network. Fermented milk (yoghurt) has texture, which is mainly determined by how the milk base is heated, by the starter culture and by yogurt shearing after fermentation [3]. Depending on numerous internal and external factors, the growth of microorganisms during the storage of dairy products results in their sensory changes, that is spoilage. The consumer's primary requirement is that during the estimated time of storage the food remains safe, without adverse changes [4]. The shelf life of milk products is primarily influenced by the number and type of microorganisms present in the raw milk, state of packaging material, hygienic and sanitary conditions during the production process as well as the storage temperature of the final product [5].

Main advances in functional foods area include the selection and use of beneficial probiotic microorganisms. They are defined as "microbial cells preparations or components of microbial cells that have a beneficial effect on health and well-being of the host" [6]. In recent times, the food industry wants to expand the range of probiotic fermented drink but each probiotic bacteria offers different and specific health benefits. Insignificant information exists on the influence of probiotic strains on physicochemical properties and sensory characteristics of fermented milks [7]. The behavior of probiotic cultures differ from yoghurt cultures in fermentation time, although they grow 2 log cycles during this time. On the other hand the probiotic fermented milk shown similar textural properties to fermented milk by yoghurt cultures. *Lactobacillus acidophilus* decreased during cold storage until 28 days to a level that doesn't fulfill the minimum viable counts to reach health beneficial effects. *Bifidobacterium lactis* and yoghurt bacteria remained stable [8]. Health associated benefits related with the consumption of probiotic microorganisms could be concise as: enhancement of immune modulation and prevention of certain diseases and ailments in humans [9,8]. The aim of this study was therefore to determine the effect of refrigeration on the nutritive and microbiological quality of fermented soy drink from tamarind and nono.

2. MATERIALS AND METHODS

2.1 Collection of Raw Materials

Soybean (*Glycine max*) seeds and tamarind (*Tamarindus indica*) fruits were obtained from the field after harvest while the commercially available starter cultures (*nono*) was obtained from Bosso market a local government in Minna, Niger State, Nigeria. Soybean seed and Tamarind were transported in sterile sampling bags and *nono* was collected in sterile sampling bottles and immediately transported in ice packed box to the laboratory of Department of Microbiology, Federal University of Technology, Minna, Nigeria for analysis.

2.2 Preparation of Tamarind Pulp

The tamarind fruits were spread on a foil paper and sundried in a one square metre (1sqm) wood frame and a 70µm pore size net covered tent dryer to prevent the samples from insectinfestation and dust. The fruits were placed in a desiccator in the evening on daily basis for the period of five days. *Tamarindus indica* fruit pulp powder was obtained by aseptic technique.

2.3 Fermented Soy Drink Production

Two fermented soy drink premixes were formulated to contain: (a) soy milk plus tamarind (b) soy milk plus (*nono*) starter culture. Each of the two soymilk premixes were homogenized and pasteurized at 76°C for 30 minutes as described by Collins *et al*. [10] and Abd- El Gawad *et al.* [11]. The milk was subsequently placed in a water bath to reduce the temperature to 45°C prior to inoculation with 11.6×10^3 cfu/mL *nono* and 5.3×10^3 cfu/mL tamarind pulp juice respectively. All the milk premixes were poured into plastic cups before inoculation and incubated

at 42°C and allowed to ferment for 12 hours. After incubation, the premixes were stirred and cooled in a refrigerator at temperature of 5°C until evaluation within 12 hours.

2.4 Microbiological Examination of Fermented Soy Drink

Tamarind pulp and fermented soy drink samples were examined for viable count of bacteria, enteric bacteria, possible lactic acid bacteria, and fungi using Nutrient agar, MacConkey agar, Lactic acid bacteria agar, De Mann Rogosa Sharpe agar, M17 agar, and Sabouraud Dextrose agar, respectively.The pour plate method of Cheesbrough [12] was used for microbial count. Serial dilution to 10^{-3} was carried out on tamarind pulp and fermented soy drink using normal saline and 1 ml of 10^{-3} diluent was transferred unto petri dishes. Nutrient agar and MacConkey agar were added into separate plates, swirled gently and allowed to solidify and incubated at 37°C for 48 hours. The numbers of colonies were counted on the plates taking into consideration the dilution factor to obtain the total viable count. While yeasts and moulds were determined by inoculating aliquot of 1ml of the sample on Sabouraud Dextrose agar, the plates were incubated at 25°C for 72 hours. The number of colonies were counted and expressed as colony forming units per gram (cfu/g) for tamarind pulp and colony forming unit per milliliter (cfu/mL) for fermented soy drink.

2.5 Characterization and Identification of Microbial Isolates

The bacteria isolates were characterized using colonial morphology, Gram staining and biochemical tests. The biochemical tests conducted include catalase, citrate, spore forming and sugar fermentation profiles. The isolates were identified by comparing their characteristics with those of known taxa using Bergey's Manual of Determinative Bacteriology [13].

2.6 Determination of Proximate Composition of Fermented Soy Drink in Storage

Proximate composition of tamarind, fermented soy drink from tamarind, *nono* and commercial yoghurt samples were determined. Five commercial samples were used. This was to ensure choice of best commercial sample to use as control. The seeds were removed from the pulp, the tamarind pulp was ground to powdered form by using a blender. The powdered samples was sieved to obtain uniform size that were analyzed for moisture, protein, fat, ash, fiber and nitrogen free extract by the methods of Association of Analytical Chemist AOAC [14].

2.7 Determination of Moisture Content of Fermented Soy Drink in Storage

The moisture was determined by oven drying method. One point five gram (1.5g) of well-mixed sample was accurately weighed into a clean dried crucible (W_1). The crucible was transferred to an oven at 105°C for 6 hours until a constant weight was obtained. Then the crucible was placed in the desiccator for 30 minutes to cool. After cooling, it was weighed again (W_2).The percentage moisture content was calculated using the standard equation below:

$$\% \text{ Moisture} = \frac{W_1 - W_2}{Wt} \times \frac{100}{1}$$

Where

W_1 = Initial weight of crucible + Sample
W_2 = Final weight of crucible + Sample
Wt = weight of the sample

Note: Moisture free samples were used for further analysis

2.8 Determination of Ash Content of Fermented Soy Drink in Storage

For the determination of ash content of the samples, clean empty crucible was placed in a muffle furnace at 600°C for an hour using Kjeldahl's method, cooled in desiccator and then weight of empty crucible was noted (W_1). One gram of each of sample was taken in crucible (W_2). The sample was ignited over a burner with the help of blowpipe, until it charred. Then the crucible was placed in muffle furnace at 550°C for 2 hours. The appearance of gray white ash indicated complete oxidation of all organic matter in the sample and thereafter the ashing furnace was switch off. The crucible was cooled, percentage ash content was calculated using the relation below: Difference in weight of Ash= W_3 - W_1 and weighed (W_3),

$$\%\text{Ash} = \frac{W_3 - W_1}{Wt} \times \frac{100}{1}$$

Where

W_1= crucible weight, W_2= sample weight, W_3= final weight

2.9 Determination of Crude Protein Content of Fermented Soy Drink in Storage

Protein in the sample was determined by Kjeldahl's method for protein content. One gram (1g) of dried samples was transferred to digestion flask. Fifteen millilitre (15mL) of concentrated H_2SO_4 and eight gram (8g) of digestion mixture i.e. K_2SO_4: $CuSO_4$ (8:1) was added. The flask was then swirled in order to mix the contents thoroughly. It was thereafter placed on heater to start digestion till the mixture become clear (blue green in colour). It was left to stand for 2 hours. The digest was cooled and transferred to 100 mL volumetric flask and volume was made up to mark by the addition of distilled water. Distillation of the digest was performed in Markam Still Distillation Apparatus [15]. Ten millilitres (10mL) of digest was introduced in the distillation tube then 10 mL of 0.5 N NaOH was gradually added through the same way. Distillation was continued for at least 10 minutes and NH_3 produced was collected as NH_4OH in a conical flask containing 20 mL of 4% boric acid solution with few drops of modified methyl red indicator. During distillation, yellowish colour appeared due to NH_4OH formation. The distillate was then titrated against standard 0.1 N HCl solution till the appearance of pink colour. A blank was also ran through all steps above. Percentage crude protein content of the sample was calculated using the relation below:

% Crude Protein = 6.25* x %N (*. Correction factor)

$$\%N = \frac{(S - B) \times N \times 0.014 \times D \times 100}{\text{Weight of the sample} \times V}$$

Where

S = Sample titration reading
B = Blank titration reading
N = Normality of HCl
D = Dilution of sample after digestion
V = Volume taken for distillation
0.014 = Milli equivalent weight of Nitrogen

2.10 Determination of Crude fat Content of Fermented Soy Drink in Storage

One gram (1g) of moisture free sample was wrapped in filter paper, placed in fat free thimble and then introduced in the extraction tube. Weighed, cleaned and dried receiving beaker was filled with petroleum ether and fitted into the apparatus. Water and heater were turned on to start the extraction. After six siphoning, petroleum ether was allowed to evaporate and then beaker was disconnected. The extract was transferred into clean glass dish with petroleum ether washed and evaporated on water bath. The dish was placed in an oven at 105^o C for 2 hours and was cooled in a desiccator. The percent crude fat was determined using the formula below:

$$\% \text{ Crude Fat} = \frac{\text{Weight of petroleum ether extract} \times 100}{\text{Weight of sample}}$$

2.11 Determination of Crude Fibre Content of Fermented Soy Drink in Storage

Aliquots of 0.15g of the sample was weighed (W_0) and transferred to porous crucible and the crucible was placed into the Dosi-fiber unit and the valve was kept in "OFF" position. Thereafter, 150 mL of preheated H_2SO_4 solution and some drops of foam-suppresser were added to each column. The cooling circuit was opened and the heating elements (power at 90%) turned on. On boiling, the power was reduced to 30% and left for 30 minutes. Valves were opened for drainage of acid and rinsed with distilled water thrice to ensure the complete removal of acid from the sample. The same procedure was used for alkali digestion by using KOH instead of H_2SO_4. The sample was dried in an oven at 150°C for 1 hour. Then it was allowed to cool in a desiccator and weighed (W_1). The samples in crucibles were then kept in muffle furnace at 55°C for 4 hours. The samples were then cooled in a desiccator and weighed again (W_2). Calculations were done by using the formula:

$$\% \text{ Crude Fiber} = \frac{W_1 - W_2}{W_0} \times \frac{100}{1}$$

Where: W_1 = initialweight, W_2 =final weight, W_0 = weight of sample

2.12 Determination of Nitrogen Free Extracts of Fermented Soy Drink in Storage

Nitrogen Free Extract (NFE) was calculated by difference after analysis of all the other items.

NFE = (100-% moisture + % crude protein + % crude fat + % crude fiber + % ash) [14].

Energy calculation: The percent calories in selected samples were calculated by multiplying the percentage of crude protein and carbohydrate by four and crude fat by nine. The values were then converted to calories per 100g of the sample [14].

2.13 Determination of Mineral Content of Fermented Soy Drink in Storage

One gram (1g) of the samples was measured and added into a digesting glass tube. Twelve millilitres (12mL) of HNO_3 was added to the tamarind pulp powder and the fermented soy drink samples respectively and the mixtures were kept for overnight at room temperature. Four millilitres (4.0mL) perchloric acid (HC_lO_4) was added to the mixture and was kept in the fume block for digestion. The temperature was increased gradually, starting from 50^{o}C to 250^{o}C. The digestion was completed after 70 minutes as indicated by the appearance of white fumes. The mixture was left to cool and the contents of each tube was transferred to 100 mL volumetric flask and the volume of the contents were made to 100 mL with distilled water. The wet digested solution was transferred to labelled plastic bottles [14].

2.14 Determination of Calcium

One millilitre (1 mL) of lithium oxide solution was added to samples to unmask calcium (Ca) from magnesium (Mg). The concentration of minerals recorded in terms of "ppm" were converted to milligrams (mg) of the minerals by multiplying the ppm with dilution factor and dividing by 1000, as follows:

$$\text{Mass} = \frac{\text{absorbency (ppm) x dry weight x D}}{\text{Weight of sample x 1000}}$$

Where D = dilution factor

Note: Dilution factor for phosphorus is 2500, for magnesium is 10000, while for other minerals including calcium, iron, potassium (K), sodium (Na), manganese and chromium is 100.

2.15 Determination of Sodium and Potassium Content of Fermented Soy Drink in Storage

Flame photometry was used in determining the Na and K content of the samples. The same wet digested tamarind pulp powder and the fermented soy drink solution as used inatomic absorption spectroscopy (AAS) were used for the determination of Na and K contents. Standard solutions of 20, 40, 60, 80 and 100 milli equivalent/L were used both for Na and K. The calculations for the total mineral intake involved the same procedure as given in AAS.

2.16 Determination of Vitamin C Content of Fermented Soy Drink in Storage

Five grams (5g) of the sample was extracted in 4% oxalic acid and centrifuged. Five millilitre of the supernant was pipetted and added to 10ml of oxalic acid and it was titrated against the Dye (42mg sodium hydrogen trioxocarbonate IV, 52mg 2, 6-dichloro phenol indophenol dissolve and made up to 200ml with distilled water). End point is the appearance of pink colour which persisted for a few minutes. The amount of Dye consumed is equivalent to the amount of ascorbic acid present.

$$\text{Ascorbic acid mg/100g} = \frac{0.5\text{mg}}{V_2\ \text{mL}} \times \frac{V_2}{5\text{mL}} \times \frac{100\text{mL}}{\text{wt}} \times 100$$

Given that

Sample weight= 1g. Volume of extract =100mL; Volume of extract used =5mL; Dye titre value against standard V_2 = 5.0mL; Dye titre value V_2 = 1.5mL; Wt = weight of sample

2.17 pH Determination of Fermented Soy Drink in Storage

The pH of the tamarind and fermented soy drink samples were measured directly using PYE UNICAM Model 292 MK2. The pH meter was standardized with pH 4.0, 7.0 and 9.0 buffer solutions. The electrode of the pH meter was standardized by dipping it into sterile water after which two different buffers (4.0 and 7.0) were used. The set electrode was then used for the various samples and readings were recorded [16].

2.18 Determination of Titrable Acidity of Fermented Soy Drink in Storage

The percentage lactic, acetic, citric, and malic and tartaric acids content of Tamarind and fermented soy drink samples were determined according to the technique AOAC [17]. Twenty grams of well homogenized sample were placed in a beaker and titrated against O.IN NaOH with phenolphthalein as indicator. Titratable acidity was expressed as percent lactic acid where 1mL of 0.1N NaOH is equal to 0.0090g for lactic acid, acetic 0.0060g, malic 0.0067g, citric 0.0070g and tartaric 0.0075g.

$$\text{Titrable acidity (\%)} \times \text{Vol} \times \frac{100}{\text{wt}}$$

Where:

Vol = volume of NaOH used
Wt = weight of sample

3. RESULTS

3.1 Total Microbial Count for Fermented Soy Drinking Storage

The microbial load of the fermented soy drink in storage is shown on Table 1. On day 0 there were no coliform and fungal counts in sample A and sample C, moreso, throughout the refrigeration time neither coliform nor fungi were present in sample A. However, the bacterial count in sample A increased progressively from day 0 (8.7×10^3 cfu/mL) to day 9 (15.0×10^3 cfu/mL) then decreased on day 12 (11×10^3 cfu/mL). The same observation was made with sample B and sample C. However, the bacterial and fungal counts for sample B were (4.3×10^3cfu/mL to 22.3×10^3 cfu/mL) and (3.7×10^3 cfu/mL to 269.3×10^3 cfu/mL) while sample C (2.7×10^3 cfu/mL to 122×10^3 cfu/mL) and (3.7×10^3 cfu/mL to 271.7×10^3 cfu/mL). Hence, sample B and C had a decline on bacterial and fungal counts of (13×10^3 cfu/mL), (97×10^3 cfu/mL) and (28×10^3 cfu/mL), (122×10^3 cfu/mL) respectively.

3.2 Proximate Composition of Fermented Soy Drink in Storage

Proximate composition of fermented soy drink in storage is shown in Table 2. Sample A and B had no significant ($p>0.05$) difference in moisture content while sample C had the lowest moisture content. But sample B and C had no significant ($p>0.05$) difference in their ash contents more so, sample C had the highest value for crude protein (3.74±0.03), oil extract (4.44±0.12), NFE (12.29±0.12) and energy (104.08±0.12) followed by sample A (0.38±0.03), (1.54±0.13), energy (36.46±0.23) but lower in NFE content (2.05±0.03). However, sample B had the lowest content of crude protein (3.55±0.07), oil extract (1.35±0.06), energy (35.87±0.15) but high content of NFE (2.38±0.07) on day 0. Sample C had low moisture content but significantly ($p<0.05$) higher in other parameters. There were no significant ($p>0.05$) difference for sample A and B in crude protein and NFE contents, although, sample A had the lowest moisture and ash contents of 81.79±1.71 and 3.66±0.12 on day 3. Sample A and B had no significant ($p>0.05$) difference in energy value (41.91±0.89 and 42.50±1.14) but sample C had the highest energy (96.69±2.03), ash (96.69±2.03), crude protein (0.55±0.03), oil extract (3.65±0.15) and NFE (11.16±0.17) but lowest in moisture (79.84±1.07) contents on day 6. While, sample A had significantly ($p<0.05$) higher moisture content of (82.82±1.31), whereas, sample B had the lowest ash 3.30±0.08, crude protein 0.40±0.01 and oil extract 0.40±0.01. Day 9 – 12, sample C had significantly ($p<0.05$) lower moisture contents ranging from 80.27±1.30 - 84.31±1.70 but significantly ($p<0.05$) higher range of ash (4.40±0.20- 4.10±0.13), crude protein (0.51±0.01-0.54±0.03), oil extract (3.62±0.14- 3.44±0.17), NFE (11.20±0.23-7.61±0.14) and energy (94.98±2.56-77.80±1.17). However, sample B had high moisture content ranged (84.43±1.17-87.15±2.3). Samples A and B had no significant ($p>0.05$) difference in ash contents, while, sample B had the lowest contents of crude protein ranging from (0.40±0.03- 0.42±0.01), oil extract (1.18±0.09- 1.03±0.03), NFE (0.84±0.02-0.60±0.01) and energy (26.58±0.57- 25.67±0.19) respectively.

3.3 Mineral and Vitamin C Contents of Fermented Soy Drink in Storage

Table 3 shows the result of mineral and vitamin C contents of fermented soy drink in storage, on day 0, sample C had significantly ($p<0.05$) high mineral contents, calcium (168.0±2.15), potassium (823.6±2.12) and sodium (504.4±2.34) but low in vitamin C content (3.75±0.06) while sample B had significantly high vitamin C content of (4.86±0.13). However, on day 3, 6 and day12 there were no significant ($p<0.05$) difference in calcium and potassium

contents for sample A and C but differ significantly ($p<0.05$) on day 9 with sample A high in Calcium (138.10±1.64) but had the low potassium (463.00±2.40) although, sample B had higher significant ($p<0.05$) amount of calcium and potassium ranging from (144.80±2.57-142.45±2.42) and (556.00±3.98- 583.00±3.10) respectively. However, the vitamin C contents of sample A and C were not significantly different from each other on day three while sample B had the low Vitamin C content. Moreso, from day3-12 the sodium contents of sample A, B and C were significantly different from each other and sample C had the highest value while sample A had the lowest ranging from (188.84±1.12-188.40±1.23), (204.12±1.47 -204.90±1.53) and (216.35±1.83-214.00±1.19) respectively.

3.4 The pH of Fermented Soy Drink in Storage

The pH values of fermented soy drink in storage are shown in Fig. 1. The pH of sample A decreases from 5.28 to 4.07 during the study period of 12 days while sample B had the pH value of 4.63 decreasing to 3.68. However, sample C had the lowest reduction in pH from 4.33 to 3.87.

4. DISCUSSION

The fermented soy drink in storage was examined for microbial load in the present research. There was relatively high growth within nine days and a decrease on day 12 of storage. Similar result was obtained by Shori [18] that reported increase and decrease of microbial load on day seven and 14 for fermented soy drink blended with cow and camel milk. Falade et al. [19] also obtained result of high growth on day nine for plain yoghurt. In the present study, fungal species were identified as contaminants. However, increase in the population of fungi may be attributed to an increase in acidity which possibly might have provided suitable conditions for fungal growth [19].

The result for this study indicated that low temperature of refrigeration inhibited the growth of the lactic acid bacteria which grew well at temperatures between 20 and 40°C with an optimum temperature range of 30–32°C [20].

Table 1. Total microbial count for fermented soy drinkin storage

Duration	Bacteria	Microbial count (cfu/ml) coliform	fungal
Day 0			
A	8.7×10^3	0.0×10^3	0.0×10^3
B	4.3×10^3	1.0×10^3	3.7×10^3
C	2.7×10^3	0.0×10^3	0.0×10^3
Day3			
A	10×10^3	0.0×10^3	0.0×10^3
B	8.0×10^3	0.0×10^3	6.0×10^3
C	6.0×10^3	0.0×10^3	5.0×10^3
Day6			
A	16×10^3	0.0×10^3	0.0×10^3
B	10×10^3	0.0×10^3	60×10^3
C	30×10^3	0.0×10^3	87×10^3
Day9			
A	15.0×10^3	0.0×10^3	0.0×10^3
B	22.3×10^3	0.0×10^3	269.3×10^3
C	42.0×10^3	0.0×10^3	271.7×10^3
Day12			
A	11×10^3	0.0×10^3	0.0×10^3
B	13×10^3	0.0×10^3	97×10^3
C	28×10^3	0.0×10^3	122×10^3

A: fermented soy drink with tamarind, B: fermented soy drink with nono C: commercial yoghurt

Table 2. Proximate composition of fermented soy drinkin storage

Incubation period	Sample	Moisture content (%)	Ash (%)	Crude protein (%)	Oil extract (%)	NFE (%)	Energy (K/cal)
0 days	A	88.43±0.37^{a}	0.38±0.03^{b}	0.38±0.03^{b}	1.54±0.13^{b}	2.05±0.03^{c}	36.46±0.23^{b}
	B	88.31±0.23^{a}	0.41±0.06^{a}	3.55±0.07^{c}	1.35±0.06^{c}	2.38±0.07^{b}	35.87±0.15^{c}
	C	79.13±0.12^{b}	0.40±0.03^{a}	3.74±0.03^{a}	4.44±0.12^{a}	12.29±0.12^{a}	104.08±0.12^{a}
3 days	A	81.79±1.71^{c}	3.66±0.12^{c}	0.47±0.03^{b}	3.23±0.10^{b}	1.85±0.09^{b}	51.11±1.37^{b}
	B	88.30±2.88^{a}	3.90±0.17^{b}	0.45±0.01^{b}	1.78±0.06^{c}	1.57±0.02^{b}	38.94±1.08^{c}
	C	84.86±1.64^{b}	4.16±0.23^{a}	0.53±0.01^{a}	3.62±0.13^{a}	6.83±0.15^{a}	76.54±1.42^{a}
6 days	A	82.82±1.31^{a}	3.48±0.03^{b}	0.49±0.02^{b}	3.03±0.09^{b}	0.18±0.01^{c}	41.91±0.89^{b}
	B	81.05±1.10^{b}	3.30±0.08^{c}	0.40±0.01^{c}	1.66±0.02^{c}	3.59±0.03^{b}	42.50±1.14^{b}
	C	79.84±1.07^{c}	96.69±2.03^{a}	0.55±0.03^{a}	3.65±0.15^{a}	11.16±0.17^{a}	96.69±2.03^{a}
9 days	A	81.48±1.41^{b}	3.45±0.06^{b}	0.47±0.02^{b}	3.12±0.10^{b}	1.48±0.06^{b}	47.80±1.23^{b}
	B	84.43±1.17^{a}	3.15±0.15^{b}	0.40±0.03^{c}	1.18±0.09^{c}	0.84±0.02^{c}	26.58±0.57^{c}
	C	80.27±1.30^{c}	4.40±0.20^{a}	0.51±0.01^{a}	3.62±0.14^{a}	11.20±0.23^{a}	94.98±2.56^{a}
12 days	A	85.04±1.68^{b}	3.70±0.10^{b}	0.47±0.02^{b}	2.88±0.10^{b}	2.91±0.06^{b}	52.36±2.10^{b}
	B	87.15±2.34^{a}	3.50±0.09^{c}	0.42±0.01^{c}	1.03±0.03^{c}	0.60±0.01^{c}	25.67±0.19^{c}
	C	84.31±1.70^{c}	4.10±0.13^{a}	0.54±0.03^{a}	3.44±0.17^{a}	7.61±0.14^{a}	77.80±1.17^{a}

Values are means ± Standard error of mean for n= 2. Values with different superscripts across the rows are significantly different at p<0.05
A: fermented soy drink with tamarind B: fermented soy drink with nono, C: control (commercial yoghurt), NFE: nitrogen free extra

Table 3. Mineral and vitamin C contents of fermented soy drink in storage

Incubation period	Sample	Calcium (mg/L)	Potassium (mg/L)	Sodium (mg/L)	Vitamin C (mg/100g)
0 days	A	48.60±0.14^{c}	693.3±1.90^{c}	171.2±1.58^{b}	4.13±0.09^{b}
	B	65.28±0.94^{b}	788.0±2.35^{b}	168.8±2.10^{c}	4.86±0.13^{a}
	C	168.0±2.15^{a}	823.6±2.12^{a}	504.4±2.34^{a}	3.75±0.06^{c}
3 days	A	138.40±2.30^{b}	472.00±2.67^{b}	188.84±1.12^{c}	6.25±0.75^{a}
	B	144.80±2.57^{a}	556.00±3.98^{a}	204.12±1.47^{b}	4.60±0.28^{b}
	C	136.20±1.16^{b}	490.00±2.80^{b}	216.35±1.83^{a}	6.40±0.59^{a}
6 days	A	138.09±2.25^{b}	487.00±2.43^{b}	188.07±1.43^{c}	5.48±0.37^{b}
	B	144.75±2.43^{a}	533.00±3.14^{a}	209.91±2.13^{b}	4.20±0.28^{c}
	C	136.00±1.98^{b}	498.00±2.71^{b}	212.30±2.05^{a}	6.06±0.57^{a}
9 days	A	138.10±1.64^{b}	463.00±2.40^{c}	189.20±1.46^{c}	5.15±0.61^{b}
	B	144.80±2.10^{a}	581.00±2.97^{a}	204.84±1.94^{b}	4.20±0.38^{b}
	C	134.00±1.63^{c}	493.00±2.13^{b}	215.55±1.87^{a}	5.80±0.68^{a}
12 days	A	138.60±2.11^{b}	470.00±2.09^{b}	188.40±1.23^{c}	3.88±0.21^{b}
	B	142.45±2.42^{a}	583.00±3.10^{a}	204.90±1.53^{b}	3.35±0.27^{b}
	C	136.10±1.35^{b}	495.00±2.85^{b}	214.00±1.19^{a}	5.12±0.43^{a}

Values are means ± Standard error of mean for n= 2. Values with different superscripts across the row are significantly different at p<0.05.
A: fermented soy drink with tamarind B: fermented soy drink with nono, C: control (commercial yoghurt)

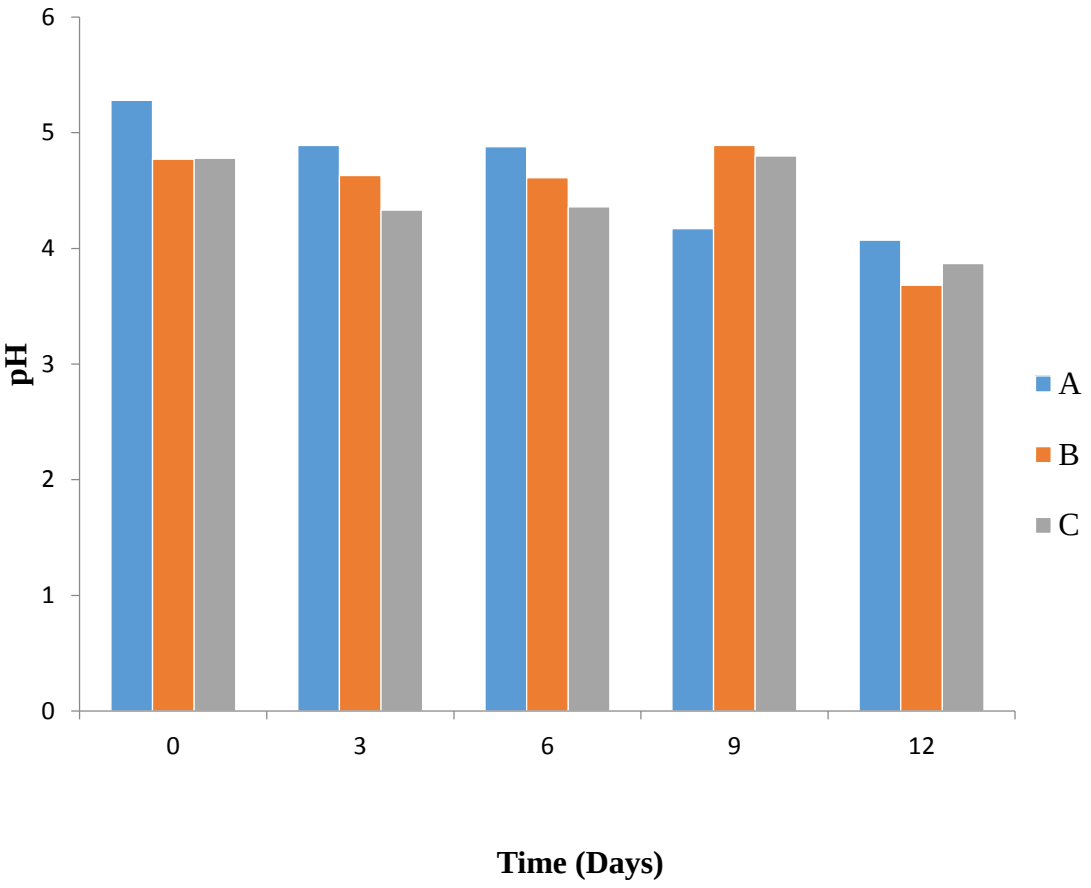

Fig. 1. The pH of fermented soy drink in storage
A: fermented soy drink with tamarind, B: fermented soy drink with nono: C: commercial yoghurt

Furthermore, the reduction of viable cell count during storage for both yogurt and fermented soy drink could be associated with the post acidification which caused further reduction in pH. In addition, the increased hydrogen peroxide produced by lactic bacteria may affect the survival of Lactobacillus spp [21].

The pH of fermented soy drink in storage was obtained in the present study, the pH value of fermented soy drink and the commercial yoghurt decreased from day zero to day twelve. Similarly, Osundahunsi et al. [20] reported a decrease in pH of plain fermented soy drink refrigerated and stored at 6 °C for 8 days, pH value of 4.7 and 4.3 was reported for day one and day eight, respectively. According to Falade et al. [19] pH of fermented soy drink stored at 7 °C decreased over the storage period from 4.97 (day 0) to 4.20 (day 9).

The result of this study revealed that the decrease in pH over storage time may be attributed to the starter culture's activity such as post acidification due to formation of lactic acid or growth of the bacteria used during fermentation [16,20].

The proximate composition of fermented soy drink in shelf was examined in this study. There was no significant changes for energy, ash, crude protein, oil extract, moisture and nitrogen free extract during storage but the slight occurrence of physicochemical changes was more rapid at the first six days of refrigeration in comparison to last six days. Farnworth et al. [22] reported similar result that the probiotics utilized the substrate to give nutritious and easy to digest end products for yoghurt. Also, according to Cardarelli et al. [23] and Opara et al. [24], during storage, hydrolysis of the milk protein continued, the pH dropped, the viscosity increased and bacterial metabolites are produced that contribute to the taste and possibly to the health promoting properties of yoghurt.

The result of this study indicated that samples were not found unsuitable for consumption which implied that storage at lower temperature might retard the deterioration process. This implies that nutrient content of the fermented soy drink is maintained during refrigeration at 5 °C for 12 days. Rezvan et al. [25] reported that fermented soy drink retained its nutrient content during cold storage at 4oC for 28 days.

The mineral content of fermented soy drink in shelf was examined in the present study, the calcium content for fermented soy drink with tamarind 48.60mg/100g and nono 65.28mg/100g increased to 138mg/100g and 142mg/100g during storage. Farnworth et al. [22] reported similar result that the probiotics utilized the substrate to give nutritious, easy to digest and improved mineral content of end products for yoghurt. Similar result was reported by Huth et al. [26] for vitamin and mineral benefits of stored yoghurt. The result of this study suggests that fermentation might have continued slowly during refrigeration at 5oC for 12 days that might improve the nutrient content and increased calcium content of fermented soy drink.

There was a decrease in Vitamin C content of fermented soy drink in storage obtained in this research which is similar with the result reported by Zulueta et al. [27] that prolonged storage of fermented soy drink may reduce the Vitamin C content drastically on exposure to sunlight. In this study, Vitamin C content of fermented soy drink tend to decrease over time. Therefore, storage beyond two weeks may require fortification due to the increased in demand on antioxidant nutrient such as Vitamins which have an effective role in the optimization of human health [28,29].

5. CONCLUSION

The storage life of the tamarind fermented soy drink remained satisfactory for a period of twelve days, while maintaining its acceptability whereas the nono fermented soy drink lost its consistency and acceptability within the same period. Refrigeration slows down the bacterial activity hence reducing spoilage thus making fermented soy drink a good source of desired protein in Nigeria.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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