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EFFECT OF REFRIGERATION STORAGE ON THE GELATION QUALITY OF FERMENTED SOYMILK USING EXOPOLYSACCHARIDE PRODUCING *LACTOBACILLUS PLANTARUM* JCM 1149 ISOLATED FROM TAMARIND FRUIT

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ABSTRACT

Lactic acid bacteria fermentation is a reliable means of prolonging shelf life. This study was aimed at assessing the effect of refrigeration storage on the gelation quality of fermented soy milk using exopolysaccharide (EPS) producing *Lactobacillus plantarum* JCM 1149 isolated from Tamarind fruit. Soybean seeds milk was produced by extraction and pasteurized at 76^{o}C for 30 minutes. Soymilk was divided into two portion, both portions were inoculated with 0.5mL (23.7×10^{2} CFU/g) inoculum and incubated for 24 hours. One portion was fermented under optimal conditions (sample T) and the other portion was fermented at 37- 38^{o}C without treatment (sample C). Fermented soy milk was stirred, packaged and refrigerated at 4^{o}C. The viscosity, water holding capacity (WHC), pH, syneresis, microbial and proximate composition were evaluated using standard methods. The viscosity of sample T increases 1468.3 mpa.s on day 3 than the increase in sample C 1351.6 mpa.s on day 15. The WHC in sample T increases and decrease on day3 and day 15. Sample T had high WHC of 79.1% than sample C 32.2%. The bacterial count of sample C was significantly ($p\leq0.05$) high on day 6 (12.70×102 CFU/mL) and decreases (9.30×10^{2} CFU/mL) on day 15. Sample T had a bacterial count (7.60×10^{2} CFU/mL) on day 0. Sample C and T had no coliform or fungal count. Sample T had high moisture content of 62.79% - 80.62%. Sample C and T had no significant difference in ash, crude protein and lipid content (3.47±0.09- 3.73±0.01), (3.02±0.00- 4.75±0.00) and (4.46±0.04- 4.51±0.01). The nitrogen free extract in sample T was high 3.47% than sample C 1.69% on day 9. Sample T had high energy content 53.46 kCal than sample C 41.31 kCal. The pH of sample C and T increases on day 6 (7.3 and 7.8) and decreases (5.6 and 6.2). However, sample C had high syneresis of 66.7% than sample T 49.04% on day 15. The EPS content of the fermented soy milk is attributed to the gelation quality of the product and assist in maintaining some yoghurt qualities. Bacteria activity is slowed down during refrigeration thus reducing spoilage and improving the shelf life of fermented soy milk.

Keyword: Exopolysaccharide, Fermented soy milk, Lactic acid bacteria, Refrigeration, storage.

INTRODUCTION

Soymilk is an excellent replacement for dairy milk to consumers with health challenges due to its high protein and phytochemical content (Abdelghani *et al*., 2022). The potential health benefits of soymilk in relation to breast and prostate cancers, cardiovascular diseases, symptoms of menopausal and osteoporosis are influenced by its quality source of bioactive phenolic compounds (Rizzo and Baroni, 2018). Despite the high quality nutrient supplement, soymilk face a drawback due to its beany flavor (Abdelghani *et al*., 2022). Fermentation is an old traditional process of improving and preservation of food and beverages. It helps in breaking down unwanted components of the food, enhances digestion, improve nutritional value and inhibits undesirable microbes (Sensoren *et al*. 2022). Fermentation plays a vital role in improving soymilk properties, it reduced soybean flavor caused by lipoxygenase activity, decreased antinutritional components and enhance bioactive components. Lactic acid bacteria (LAB) fermentation is a liable means of prolonging shelf life, improve sensory and nutrient qualities of food products.

Bacteria strains of Lactobacilli, Lactococci, Bifidobacterium and Weissella genera are commonly used in food fermentation as probiotics (Serensen *et al*., 2022). These bacteria can produce aromatic compounds, bacteriocins, carbon dioxide, exopolysaccharides, enzymes, lactic acid and other organic acids to enhance fermented food quality. Species of LAB have the potential of synthesizing and secreting extracellular polysaccharides refer to as exopolysaccharides (Silva *et al.*, 2019). These are high molecular mass extracellular carbohydrate polymers that occupy part of the outer layer of many microorganisms (Saghatelyan *et al.*, 2021). Exopolysaccharide (EPS) produced by LAB possess attractive qualities such as antioxidant activities, rheological properties in foods and hydrocolloid properties (Serensen *et al.*, 2022). Exopolysaccharides produced by bacteria offer numerous

human health benefits such as antitumor, antioxidant activity, reduced blood cholesterol, immunomodulatory and prebiotic properties (Cibelly *et al.,* 2023). Production of EPS by LAB and the amount produced is highly species- strain specific. Selection and screening for strains with highest potentials for intended use is paramount (Ramos *et al.,* 2023). *Lactobacillus plantarum* has been identified as EPS producer with several properties and activities important for commercialization by the cosmetic, food and pharmaceutical industries (Silva *et al.,* 2019; Korcz and Varga 2021). The aim of this study is to evaluate the effect of refrigeration storage on the gelation quality of fermented soy milk using exopolysaccharide producing *Lactobacillus plantarum* JCM 1149 isolated from Tamarind fruit.

MATERIALS AND METHOD

Sample collection

Exopolysaccharide producing *Lactobacillus plantarum* JCM1149 was collected from Microbiology laboratory Nigerian institute of pharmaceutical research development Idu, Abuja.

Soy milk production and fermentation

Soymilk was produced by the modified traditional method described by Adamu *et al.* (2022). Cleaned soybean seeds were soaked in water for 8 hours, the bean seeds were rinsed and ground in a warring blender Hamilton Beach (model 909-220). The slurry was filtered at ratio 7:1 of water to slurry through cheese cloth of 50µm pores size (Figure 1) and filtrate was pasteurized at 76^{o}C for 30 minutes as described by Collins *et al*. (1991) to obtain soy milk.

Two fermented soy milk premixes was formulated to contain: (i) Fifty milliliter (50mL) soy milk plus *L. plantarum* JCM 1149 (ii) Fifty milliliter soy milk plus *L. plantarum* JCM1149 at optimal conditions. The first milk premix was allowed to fermented at room temperature while the second milk premix was placed in a water bath to reduce the temperature to 35°C

for both inoculation and incubation. Samples were incubated for 24 hours. After incubation, the premixes were stirred and cooled in a refrigerator at temperature of 4°C until evaluation.

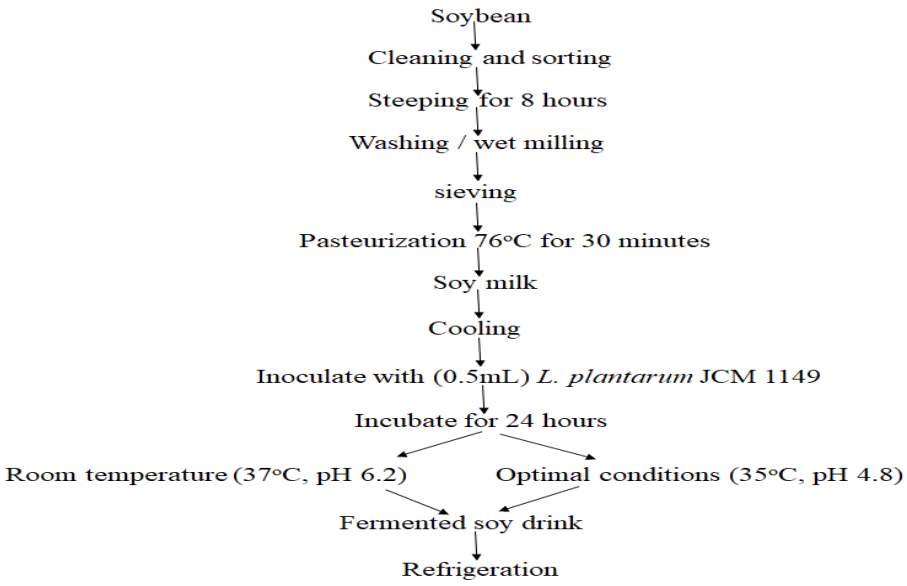


Figure 1. Fermented soy milk production
Source: Adamu *et al*. (2022).

Apparent viscosity

The apparent viscosity of the stirred fermented soy milk samples was conducted by Brookfield Viscometer (Brookfield Viscometer, DVII, USA) at 5 ° C. Measurements were performed by the spindle, No 4, and at rotational speed of 3 RPM. The results were reported as millipascal after 50 seconds of rotation (Paz-Díaz *et al.*, 2021).

Determination of water holding capacity (WHC)

Water holding capacity is the ability of fermented soy milk to hold all or part of its own water. WHC of the samples were determined by centrifugation method described by Sørensen *et al*. (2022). Fermented soymilk (30g) was centrifuged at 8000 g at 4ºC for 15

min. The supernatant was weighed and WHC was calculated according to the following Equation one:

$$WHC\ (\%) = (1- W_1/W_2) \times 100 \quad (1)$$

Where:

W_1= weight of supernatant after centrifugation (g);

W_2 = fermented soymilk weight (g).

All measurements were carried out in triplicate.

Microbial examination

One millilitre of the fermented soy milk was dispensed in 9 mL of distilled water to obtain 10^{-1} dilution. Subsequent dilution was done up to 10^{-6}. The diluents (10^{-2}, 10^{-4}, 10^{-6}) were plated in duplicate on de Mann Ragosa and Sharpe media (MRS agar) for LAB, MacConkey agar for coliform and Sabouraud Dextrose agar (SDA) for fungi. The MRS plates were incubated anaerobically at 37^{o}C for 48 hours and MacConkey agar plates were incubated aerobically at 37^{o}C for 48 hours. The SDA plates were incubated aerobically at 25^{o}C for 72 hours and the visible colonies were counted according to the method described by Cheesbrough (2000); David *et al.* (2019).

Chemical Analysis

Determination of Proximate Composition of fermented soy milk

Proximate composition of fermented soy milk samples was analyzed for moisture, protein, fat, ash, fiber, energy and nitrogen free extract by the methods of Association of Analytical Chemist (AOAC, 2012).

Moisture content

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^{o}C for 6 h 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 Equation two:

$$\% \text{ Moisture} = \frac{W_1 - W_2 \times 100}{Wt} \quad (2)$$

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

Ash

For the determination of ash content of the samples, clean empty crucible was placed in a muffle furnace at 600^{o}C for an hour, cooled in desiccator and then weight of empty crucible was noted (W_1). One gram of each of sample was taking 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^{o}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 Equation three: Difference in weight of Ash= $W_3 - W_1$ and weighed (W_3), sample weight (W_2).

$$\%\text{Ash} = \frac{\text{Difference in Wt. of Ash} \times 100}{\text{Weight of sample}} \quad (3)$$

Crude protein

Protein in the sample was determined by Kjeldahl's method. One gram (1g) of dried samples were taking in digestion flask. Fifteen milliliter (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 then 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 will be 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 (Khalil and Manan, 1990). 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 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. The distillate was then titrated against standard 0.1 N HCl solution till the appearance of pink colour. A blank was also run through all steps above. Percentage crude protein content of the sample was calculated using Equation four:

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

$$\%N = \frac{(S - B) \times N \times 0.014 \times D \times 100}{\text{Weight of the sample} \times V} \quad (4)$$

Where

S = Sample titration reading

B = Blank titration reading

N = Normality of HCl

D = Dilution of sample after digestion

V = Volume taken for distillation

Crude Lipid

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 allowed to cool in a desiccator. The percent crude fat was determined using Equation five:

$$\% \text{ Crude Fat} = \frac{\text{Weight of petroleum ether extract x 100}}{\text{Weight of sample}} \quad (5)$$

Crude fibre

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 was 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 samples were 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 was done by using Equation six:

$$\% \text{ Crude Fiber} = \frac{W_1 - W_2 \text{ x } 100}{W_0} \quad (6)$$

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

Nitrogen free extracts

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) (AOAC, 2012).

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 100gm of the sample (AOAC, 2012).

pH Determination

The pH was measured as described Olubamiwa *et al.* (2007). The fermented soy milk samples were measured directly using PYE UNICAM Model 292 MK2. The electrode of the pH meter was standardized by dipping it into sterile water after which two different buffers (4.0 and 7.0) was used. The set electrode was then used for the various samples and readings was recorded.

Titratable acidity (TTA) Determination

The percentage titatrable acid content of fermented soy milk samples were determined according to the technique AOAC (2012). Twenty grams of well homogenized sample was placed in a beaker and titrated against 0.IN NaOH with phenolphthalein as indicator. Titratable acidity was expressed as percent lactic acid and the TA (^oT, expressed as Thorner degree, ^oT 0.009 = lactic acid %) where 1mL of 0.1N NaOH is equal to 0.0090gms for lactic acid.

Evaluation of Syneresis

Syneresis was determined according to Ibhaze *et al*. (2020). Samples of 30g were centrifuged (1535 ×g, 20 minutes) and the whey was drained for 1 minute. The weight of the drained whey expressed as the percentage of the weight of fermented soy milk gives the percentage syneresis.

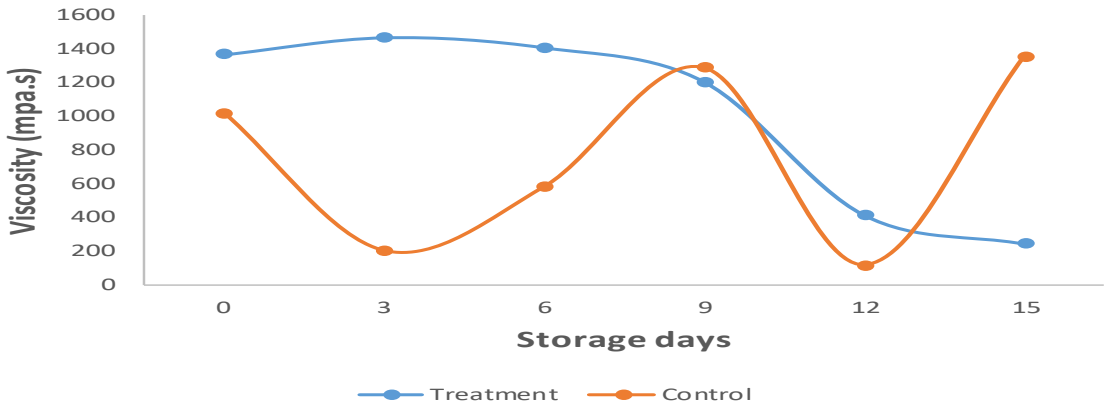
Data analysis

Data obtained were expressed as Mean± Std. Error of duplicate values. Means with dissimilar letter (s) differ significantly according to the Paired Samples t-Test at $p \leq 0.05$.

RESULTS

Viscosity of the fermented soy milk on shelf

The apparent viscosity of the fermented soy milk sample C (control) and sample T (treatment) in storage period of 15 days at 4^{O}C is shown in Figure 2. The viscosity of sample C ranged from 115.5 to 1351.6 mpa.s and sample T ranged from 243.3 to 1468.3 mpa.s. Sample C had higher viscosity 1351.6 mpa.s on day 15 than 1288.6 mpa.s day 9 and 1019.7 mpa.s. day 0. Sample C had lower viscosity 115.5 mpa.s on day 12 and 200.4 mpa.s day 3. Sample T had higher viscosity 1468.3 mpa.s on day 3 than day 0. The viscosity of sample T decreased from 1408.9 mpa.s day 6 to day 15 (243.3 mpa.s).

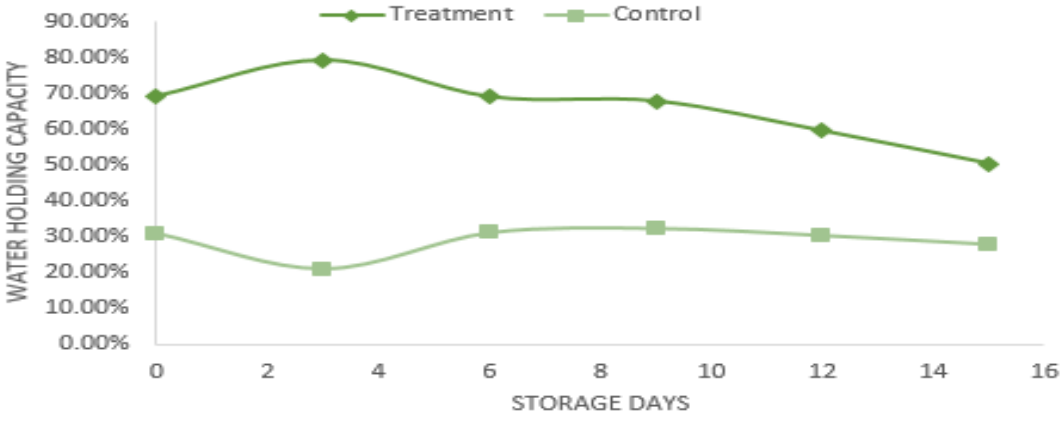


Treatment: treated fermented soy milk; **Control:** untreated fermented soy milk

Figure 2. Viscosity of fermented soy milk

The water holding capacity of the fermented soy milk on shelf

The water holding capacity (WHC) of fermented soy milk in storage is shown in Figure 3. The WHC of sample T ranged from 67.7% to 79.1% while, sample C ranged from 20.8% to 32.2%. Sample T had higher WHC of 79.1% than sample C 32.2%. There was an increase in WHC on day 3 in sample T and decreases from day 6 to day 15 during storage. Sample C had a decrease 20.8% on day 3 and increases 31.0% on day 6 to 30.2% on day 9 and decreases 30.2% on day 12 to 27.7% on day 15 respectively.

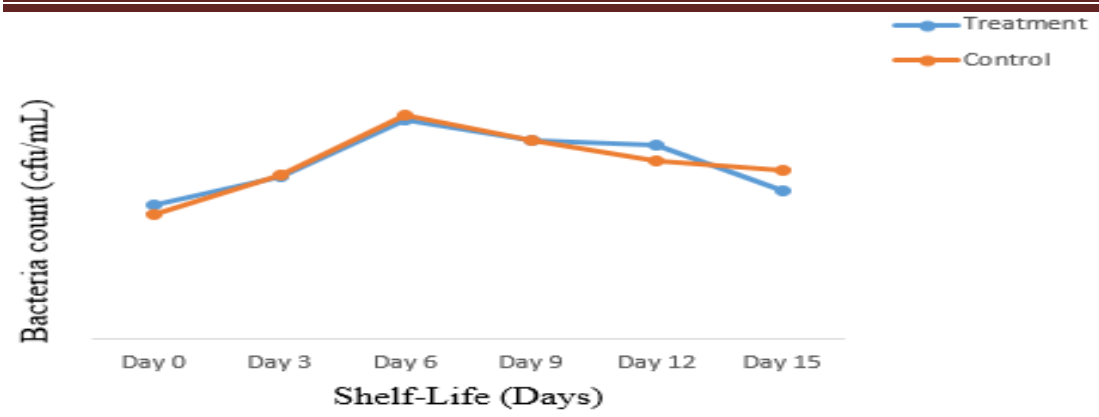


Treatment: treated fermented soy milk; **Control:** untreated fermented soy milk

Figure 3. Water holding capacity of fermented soy milk on shelf

Total bacterial counts of the fermented soy milk on shelf

The bacteria count of sample C fermented soy milk ranged from 7.10 $\times 10^2$ CFU/mL to 9.30 $\times 10^2$CFU/mL. Sample C had bacteria count of 7.10 $\times 10^2$ CFU/mL on day 0 and increases to on day 6. Decrease in bacteria count was observed in sample C from 11.30$\times 10^2$ CFU/mL day 9 to 9.60$\times 10^2$ CFU/mL on day 15. Fermented soy milk sample T had bacteria count ranged from 7.60 $\times 10^2$ CFU/mL to 12.40$\times 10^2$ CFU/mL. There was a significant increase in bacteria count of sample T 12.40$\times 10^2$ CFU/mL on day 6 and decline from 11.30 $\times 10^2$ CFU/mL on day 9 to 8.40$\times 10^2$ CFU/mL day 15. Sample C had high bacteria count 7.10±1.50 than sample T (7.60±2.50) on day 0. The significant difference was also observed on day 3,6,12 and day 15 respectively. However, there was no significant ($p \leq 0.05$) difference in sample C ($11.30^a \pm 2.50$) and sample T ($11.30^a \pm 2.00$) on day 9. There was no coliform nor fungal growth observed in the samples during the study period as shown in Figure 4.



Treatment: treated fermented soy milk; **Control:** untreated fermented soy milk

Figure 4. Bacterial counts of fermented soy milk on shelf

Proximate composition of fermented soy milk on shelf

The moisture content of fermented soy milk sample T is significantly $p \leq 0.05$ higher 80.62% than sample C 65.22%. There was no significant $p \geq 0.05$ difference in sample T 44.84±0.10 and sample C 46.93±0.79 on day 9. However, sample T had low moisture content 53.28% than sample C 66.02% on day 15. The ash content of sample C is significantly $p \leq 0.05$ higher 0.41% than sample T 0.37%. The crude protein content of sample C is significantly $p \leq 0.05$ higher 4.96±0.04 than sample T 2.02±0.00 on day 15. There was no significant difference $p \geq 0.05$ in sample T 3.13±0.00 - 4.50±0.10 and sample C 3.31a±0.00 - 5.03±0.00 from day 0 to day 12. The crude lipid content of sample C 4.26%, 4.51%, 1.52%% and sample T 4.69%, 4.46%, 0.97% had no significant $p \geq 0.05$ difference from day 0 to day3 and day 15. The nitrogen free extract (NFE) of the fermented soy milk sample C had high NFE 1.385 and 3.47% than sample T 1.025 and 0.18% on day 3 and day 6. The energy content of fermented soy milk sample T ranged from 44.72% to 53.46%. Sample T had significantly $p \leq 0.05$ high energy 46.64% than sample C 38.87%. There was an increase in energy content of sample T 50.16% on day 3, decreases 44.72% on day 6 and increases from 46.72% day 9 to 53.09% day 15 as shown in Table 1.

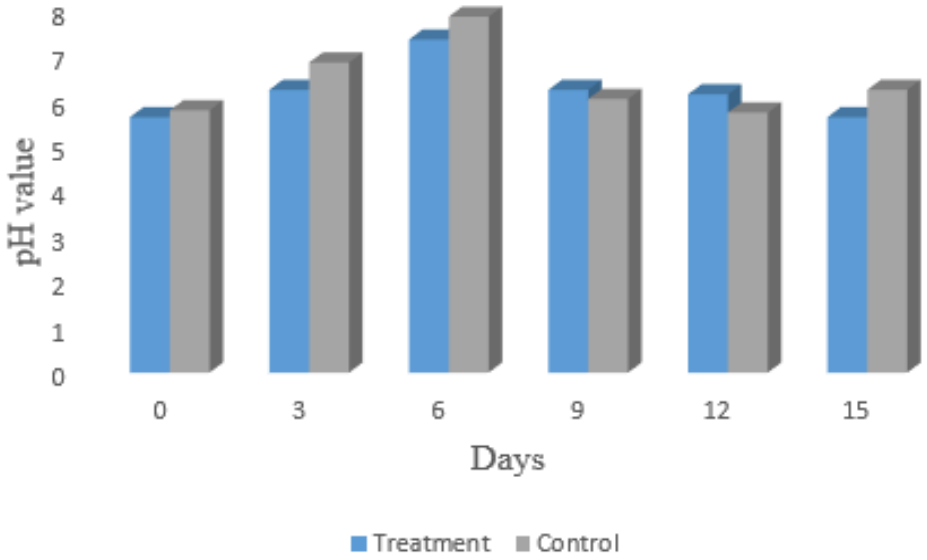
Table 1: Proximate composition of fermented soy milk on shelf

Incubation period	Sample	Moisture content (%)	Ash (%)	Crude protein (%)	Crude Lipid (%)	NFE	Energy K/cal
0 Days	T	80.62^{b}±0.30	0.37^{a}±0.00	3.13^{a}±0.00	4.69^{a}±0.02	1.89^{a}±0.30	46.64^{b}±0.29
	C	65.22^{a}±0.91	0.41^{b}±0.00	3.31^{a}±0.00	4.62^{a}±0.00	2.01^{a}±0.00	38.87^{a}±0.00
3 Days	T	69.12^{b}±0.15	3.47^{a}±0.09	3.02^{a}±0.00	4.46^{a}±0.04	1.02^{a}±0.00	50.16^{b}±0.01
	C	66.06^{a}±0.04	3.73^{a}±0.01	4.75^{a}±0.00	4.51^{a}±0.01	1.38^{b}±0.00	38.63^{a}±0.12
6 Days	T	55.62^{a}±0.01	3.28^{a}±0.02	2.63^{a}±0.00	3.26^{b}±0.06	0.18^{a}±0.00	44.72^{b}±0.01
	C	57.18^{b}±0.01	3.10^{a}±0.00	2.58^{a}±0.00	1.54^{a}±0.10	3.47^{b}±0.03	41.31^{a}±0.01
9 Days	T	44.84^{a}±0.10	2.95^{a}±0.02	3.01^{a}±0.02	3.15^{b}±0.02	1.36^{a}±0.03	46.72^{b}±0.08
	C	46.93^{a}±0.79	2.79^{a}±0.02	4.63^{a}±0.00	1.19^{a}±0.02	0.74^{a}±0.04	25.69^{a}±0.00
12 Days	T	62.79^{b}±0.00	3.61^{b}±0.02	4.50^{a}±0.10	2.66^{b}±0.01	2.87^{b}±0.07	53.46^{b}±0.01
	C	56.31^{a}±0.04	3.31^{a}±0.04	5.03^{a}±0.00	1.12^{a}±0.01	0.81^{a}±0.03	22.92^{a}±0.08
15 Days	T	53.28^{a}±0.02	3.50^{b}±0.00	2.02^{a}±0.00	0.97^{a}±0.09	2.60^{a}±0.27	53.09^{b}±0.02
	C	66.02^{b}±0.01	3.27^{a}±0.02	4.96^{b}±0.04	1.52^{a}±0.00	1.10^{a}±0.00	21.89^{a}±0.90

T: Treatment; **C**: Control. Values are Mean± Std. Error of duplicate values. Means with dissimilar letter (s) differ significantly according to the Paired Samples t-Test at $p \leq 0.05$.

pH value

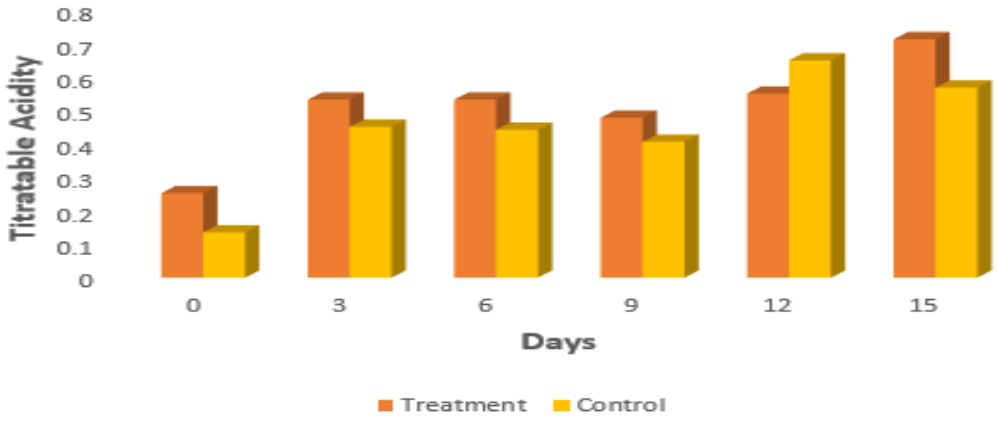
The pH value of fermented soy milk samples in storage are shown in Figure 5. The pH of sample T increases from 5.6 on day 0 to 7.3 on day 6 and decreases from day 9 (6.2) to 5.6 on day 15. While sample C had pH value 5.75 increasing to 7.8 and decreases to 6.2 on day 15.



Treatment: treated fermented soy milk; **Control:** untreated fermented soy milk
Figure 5. pH value of fermented soy milk at storage

Titratable acidity of fermented soy milk at storage

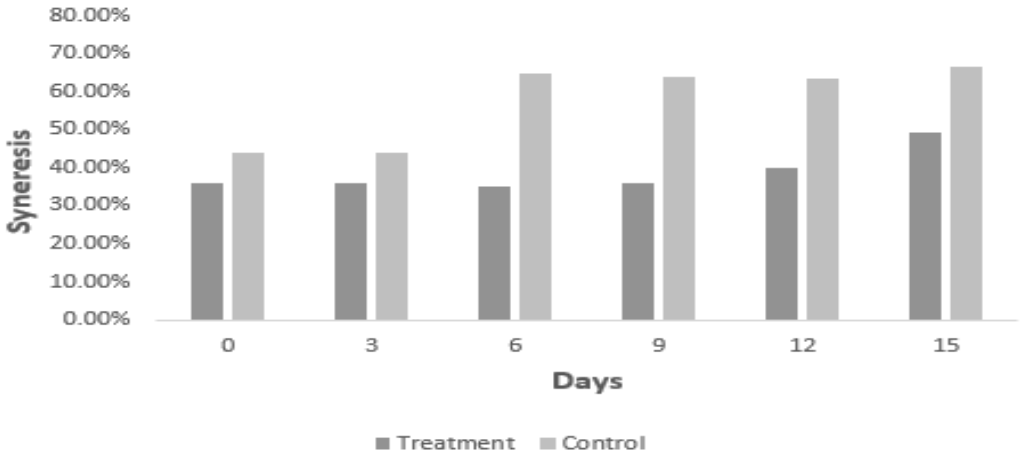
The titratable acidity of fermented soy milk on storage is shown on Figure 6. Sample T had higher titratable acidity (0.252) than sample C (0.135). The titratable acidity of sample T ranged from 0.252 to 0.711 during the study period of 15 days. While sample C ranged from 0.135 to 0.567. The titratable acidity of the samples increases during the storage period.



Treatment: treated fermented soy milk; **Control:** untreated fermented soy milk
Figure 6. Titratable acidity of fermented soy milk at storage

Syneresis of fermented soy milk on shelf

The percentage syneresis of fermented soy milk sample C (control) ranged from 44.01% to 66.70% while sample T (treatment) ranged from 35.80% to 49.04%. However, sample C had higher syneresis 44.01% on day 0 than sample T 35.8%. Sample C increases to 66.70% on day 15 while sample T increases 49.04% as shown in Figure 7.



Treatment: treated fermented soy milk; **Control:** untreated fermented soy milk
Figure 7. Syneresis of fermented soy milk on shelf

DISCUSSION

In this study, the viscosity of fermented soy milk was relatively high during the period of storage. This could be due to the present of exopolysaccharide (EPS) produced by the starter culture, the EPS production might continue slowly as fermentation does not seize during refrigeration. Overtime, soybean protein molecules undergoes changes during storage to form protein gelation that contribute to an increase in viscosity (Ibhaze *et al.,* 2023). Similar to this study, Naklong *et al.* (2023) reported an increase in apparent viscosity of concentrated yogurt during cold storage. Similarly, Paz-Díaz *et al.* (2021) reported rapid increase in viscosity of fruit- flavored yoghurt to day 7 and increases slowly up to day 14 of storage. In agreement to this study, Yekta and Ansari (2019) reported an increase in viscosity of green soybean yoghurt up to day 20 during storage, which is 28% higher than the viscosity of day1. This increase in viscosity during storage could be due to changes in protein-protein binding in a three-dimensional protein network of yogurt and their rearrangement (Naklong *et al.* 2023).

The percentage water holding capacity (WHC) in this study during storage was high. This might be due to water binding ability of the EPS produced by the sample to form a gel- like medium that entangles the water molecules, which give the fermented soy milk a cream and thicker texture. The result of this study agrees with the report by Ibhaze *et al*. (2023) that observed an increase in WHC and whey drainage obtained during 14days storage. Obiora *et al.* (2020) reported similar result of high WHC of 75.33% in yoghurt from cow milk during storage. Similarly, Kong *et al*. (2022) reported high WHC in soy yoghurt that increases from 90.75% to 96.13% with time during storage, this suggest that the electrostatic interaction of protein network and polysaccharides strengthens slowly with low pH which forms an averagely stable compound, that improve the features of protein gel and enhance WHC.

The bacterial counts of fermented milk samples during refrigeration storage was low and there were no significant changes after the first six days. This could be due to the low initial

inoculum size used in the fermentation, the strain used and the slow fermentation cause by refrigeration storage. Previous study by Adamu *et al.* (2021) reported similar findings in fermented soy drink during storage with an increase on day6 16×10^3CFU/mL and decreases to 11×10^3CFU/ mL on day12. El-Attar *et al.* (2022) reported similar result, which observed an increase on day5 of 31×107 CFU/mL in soy yoghurt and decreases to 7.7×106 CFU/mL on day10 during refrigeration storage. In contrast to this study, Obiora *et al.* (2020) reported high bacteria count in yoghurt produced with blend of power milk, corn starch and soy milk in ratio 50:20:30. Such result was expected due to the presence of starter culture which are mainly LAB. The absence of coliform in this study imply that the samples are free from fecal contamination. However, aside the good hygiene during fermentation, the samples pH might not be suitable for fungal growth.

The proximate composition of fermented soy milk samples was observed in this study. There were no significant changes in moisture, ash, crude protein, crude lipid, Nitrogen free extract and energy content of the samples after the first nine days compared to the last six days of refrigeration. This could be due to the long time storage in which the starter culture might have been exhausted. The change in pH or any physicochemical properties of the fermented soy milk may reduce some nutrient quality. In agreement to this study, Ibhaze *et al*. (2023) reported a decrease in proximate composition in yoghurt during fourteen days' refrigeration storage. Similarly, Adamu *et al.* (2021) reported that proximate composition of fermented soy drink did not change significantly after 6days of refrigeration storage.

In this study, the pH value of fermented soy milk on shelf decreases after day six of storage. This result could be due to the effect of soy milk pH and starter culture on the fermented soy milk. The shock undergo by the inoculum strain at refrigeration can initiate lag phase again there by increasing pH as a result of the chemical changes cause by environmental change. The pH decreases as fermentation continues slowly during storage. Similar result was reported by Ibhaze *et al*. (2020) that observed an increase and decrease in pH during eight

(8) days refrigeration storage at 6^{o}C. Previous study by El-Attar *et al.* (2022) reported similar result that observed an increase 4.99 and decrease 4.82 in pH value of soy yoghurt during ten days of refrigeration storage. In agreement to this study, Paz-Díaz *et al.* (2021) observed an increase 4.38 and a decrease 3.90 in pH value of soy yoghurt during refrigeration. Soybean is naturally poor in lactose that could be the cause of the relatively high pH in fermented soy milk.

The titratable acidity of fermented soy milk in this research was low. However, increases overtime on shelf. This result might be due to complete fermentation process in which the bacteria utilized available sugars and acid production slow down leading to decrease in titratable acidity. On shelf the acid in fermented soy milk may undergo chemical reactions that reduce acidity and the bacteria strain may be dominant. In contrast to this study, Okafor and Anyalogbu (2021) reported high titratable acidity of soy yoghurt using *S. boulardii* from 8.55 on day one to 10.08 on day twenty-one of storage. The selection of microbial strain with high metabolic activity and a suitable substrate might lead to such result. Similar to this study, Paz-Díaz *et al*. (2021) observed low titratable acidity in soy yoghurt during refrigeration storage. Previous study by Kim and Han (2019) reported low titratable acidity in soy yoghurt containing D- allulose and sucrose range from 0.56–1.00% and increases to 1.02–1.17% after 92 hours. Adequate fermentation time may enhance thorough assimilation and utilization of soymilk sugar that can improve quality of the end product.

In this study, the percentage syneresis of fermented soy milk sample with treatment was low in comparison to the control during refrigeration storage. This could be due to the high content of EPS produced by *L. planetarium* JCM 1149 in the fermented soy milk that might improve the gelation of the product. Fermentation temperature and time can influence the consistency of the fermented soy milk. Similar to this study, Lemnyuy *et al.* (2023) reported low syneresis of 37.75 to 54.09% on the first two days and 38.13 to 56.21% on the twenty-one day in soy yoghurt during storage. Similarly, Erfanian and Rasti (2019) observed high

syneresis in yoghurt produced by sonicated milk-soymilk yoghurt and increases with increase in sonication time. Moreso, Okafor and Anyalogbu (2021) reported low syneresis of 34% to 27% in soymilk yoghurt and increases after 7, 14 and 21 days of storage. Syneresis cause undesirable appearance, it limits acceptability and shelf life of soy yoghurt. High level of syneresis in fermented soy milk condemned the product in the eye of the consumers.

CONCLUSION

The shelf life of Fermented soy milk produced from EPS producing *L. plantarum* JCM 1149 with treatment stored by refrigeration remains satisfactory for the period of fifteen days and still maintained it acceptability. The fermented soy milk produced with same strain, without treatment was rejected by consumer due to poor consistency at the same period. The EPS content of the fermented soy milk attributed to the gelation quality of the product and assist in maintaining some yoghurt qualities. Syneresis was a challenge without thickener or stabilizer in fermented soy milk for long time storage. Bacteria activity is slowed down during refrigeration thus reducing spoilage and improving the shelf life of fermented soy milk.

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