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ORIGINAL RESEARCH PAPER

Optimizing *Lactiplantibacillus plantarum* JCM 1149 growth conditions for improvement of the gelation and nutritional qualities of fermented soy milk

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Abstract Exopolysaccharide (EPS) is a biopolymer produced by microorganisms. This study aimed to optimize EPS production from lactic acid bacteria (LAB) to enhance the gelation quality of fermented soy milk by varying physical growth conditions. EPS was purified using gel filtration column chromatography, and the proximate composition of the fermented soy milk was analyzed according to AOAC methods. *Lactiplantibacillus plantarum* JCM 1149 produced a crude EPS yield of 166.4 mg/100 mL under optimized conditions (i.e. 35 °C, pH 4.8, 0.5 mL inoculum) after 24 h, while the purified EPS was 158.6 mg/100 mL with a glucose equivalent of 0.209 g/100 mL. Fermented soy milk produced under these conditions (sample T) was compared to a control prepared with a commercial yogurt starter culture (sample C). Sample T had a higher water-holding capacity (69.1%) and lower syneresis (35.80%) than sample C (30.80% and 41.01%, respectively). No significant differences ($p > 0.05$) were observed between samples for protein, lipid, nitrogen-free extract, or fiber content. However, sample T showed significantly higher moisture content ($80.62 \pm 0.30\%$) and energy (46.64 ± 0.29 kcal) slightly lower ash content ($0.37 \pm 0.00\%$) compared to sample C ($0.41 \pm 0.00\%$). Total viable counts were similar between samples C (7.10×10^2 cfu/mL) and T (7.60×10^2 cfu/mL). Sensory evaluation indicated that sample T had higher overall acceptability (7.46 ± 0.08) than sample C (6.98 ± 0.10). These results demonstrate that EPS-producing LAB can improve the gelation, functional, and nutritional qualities of fermented soy milk.

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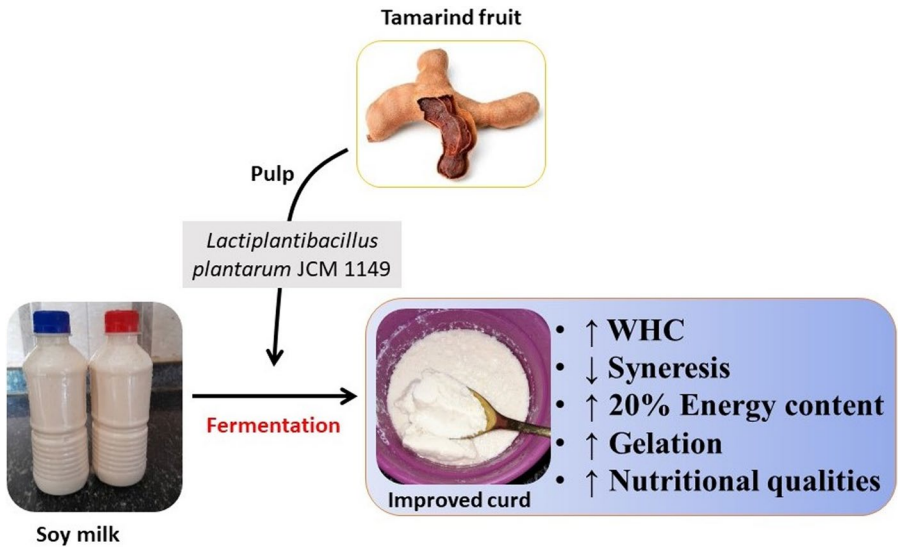
Graphical abstract



Keywords Optimization · Exopolysaccharide · Lactic acid bacteria · Fermented soy milk · *Lactobacillus plantarum* JCM 1149

Introduction

Exopolysaccharides (EPS) are biopolymers produced by microorganisms such as bacteria, yeasts, and molds. They play crucial roles in microbial adhesion, biofilm formation, and protection against environmental stress, and they can also confer functional and health-promoting properties in food and pharmaceutical applications. These biopolymers have gained the attention of researchers in recent times leading to several research breakthroughs and increased awareness of their possible industrial applications (Moradi et al. 2021). While researchers have observed the presence of exopolysaccharides in different bacterial strains, Lactic acid bacteria (LAB) stand out as an important group. LAB-synthesized polysaccharides are high-molecular-weight molecules composed of monosaccharide units linked by glycosidic bonds. These monosaccharide units exhibit biological activities, functional properties and a variety of structures (Jurášková et al. 2022). Polysaccharides help in protecting bacteria from adverse environmental conditions, and they are the main component in the formation of extracellular biofilm and matrix (Oleksy-Sobczak et al. 2020). They act as a defense mechanism by holding water around the cell to prevent dehydration (Nguyen et al. 2020). Polysaccharides also serve as food additives, enhancing texture, stability, and shelf-life in various products. Common examples include starch for thickening, pectin for gelling, and xanthan gum for emulsifying. Their natural origin and functionality make them valuable in meeting consumer demands for clean-label and sustainable food options.

The application of LAB-derived EPS in food products can enhance product optimization while meeting customer needs (Guérin et al. 2020). Product optimization involves adjusting nutrient concentrations or altering various conditions to improve organism growth, ultimately leading to higher productivity and desired outcomes. In a fermentation medium, key factors such as pH, temperature, agitation speed, and nutrient composition must be identified and optimized for efficient productivity (Guérin et al. 2020). Nutrient composition plays a

critical role in EPS synthesis, with both an excess and a deficiency of nutrients such as sugar, carbon dioxide, and nitrogen potentially affecting EPS production (Mbye et al. 2020). LAB, when exposed to high temperatures, tend to reprogram their metabolism to deal with the temperature change by increasing EPS synthesis (Liew et al. 2022). Previous optimization studies have largely focused on LAB strains isolated from dairy- or soy-based substrates, which, although well-studied, may not fully address the need for alternative, allergen-free, and culturally relevant raw materials. Soy, for instance, is associated with allergenicity and has reported adverse health implications in certain populations, prompting the search for other plant-based options. In this context, Bambara nut (*Vigna subterranea*) and tamarind (*Tamarindus indica*) offer unique nutritional profiles, underutilized potential, and cultural significance, which may influence EPS yield and functionality differently from commonly studied substrates. Therefore, this study aims to optimize EPS production by a selected LAB strain isolated from tamarind fruits, using physiological parameters under controlled fermentation conditions in soy milk as the growth substrate.

Materials and methods

Sample collection and identification of bacterial isolates

Presumptive lactic acid bacteria (LAB) were isolated from tamarind (*Tamarindus indica* L.) fruit pulp at the Nigerian Institute of Pharmaceutical Research and Development (NIPRD) Idu, Abuja, Nigeria, using standard microbiological methods for the identification and characterization of bacterial isolates, including Gram staining and biochemical analysis as outlined by Monica (Monica 2006). The presumptive isolates were further confirmed at Inqaba Biotec, Ibadan, Nigeria. The research outline is shown in Fig. 1a.

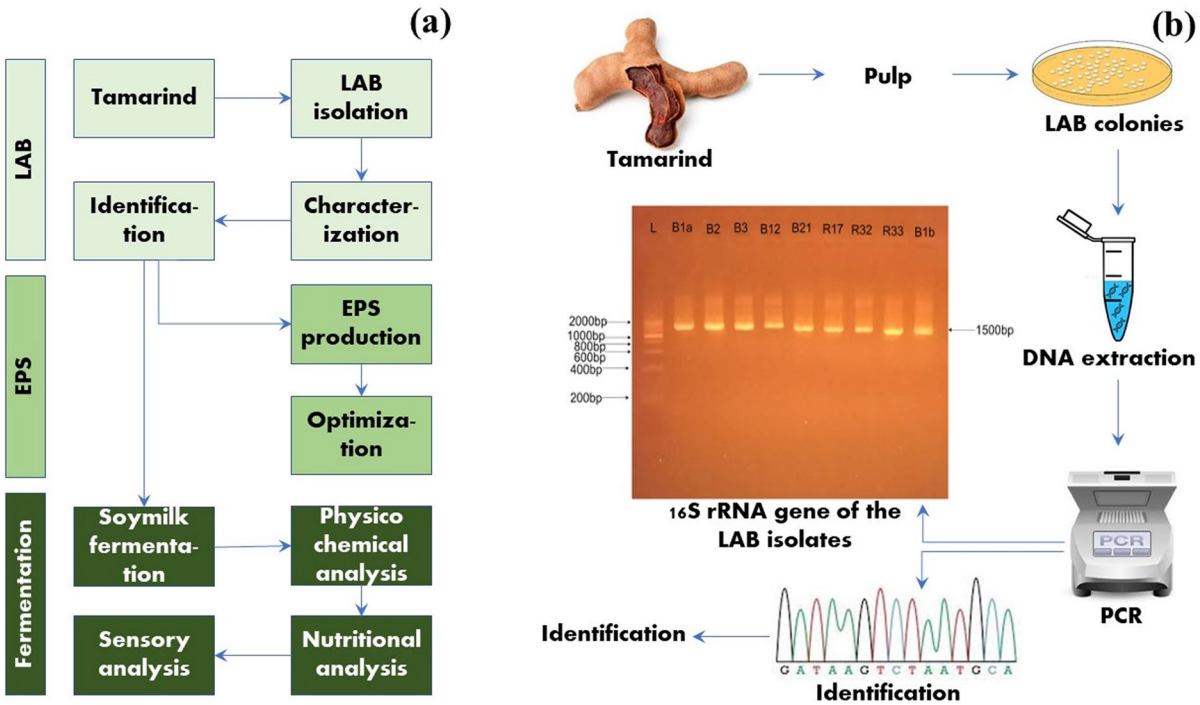


Fig. 1 Experimental workflow. (**a**) Lactic acid bacteria (LAB) isolation, expololysaccharide (EPS) production, and soy milk fermentation. (**b**) Amplified image of the 16S rRNA gene of the LAB isolates from Tamarind. **L**: Lane L represents the 1500 bp molecular ladder**; R32:** *Lactiplantibacillus plantarum* strain JCM 1149. **B1a/ B1b:** *Weissella paramesenteriodes*, **B2/ B3/ B12:** *Lactobacillus pentosus* strain 124–2, **R21/ R33:** *Lactobacillus plantarum* strain CIP 10315

Lactic acid bacteria were isolated from tamarind fruit pulp by soaking 3 g of the pulp in 5 mL of distilled water for 10 min as described by Adamu et al. (2022b). A 1 mL aliquot of the resulting solution was transferred into 9 mL of distilled water to obtain a 10^{-1} dilution, followed by serial dilutions up to 10^{-6}. Aliquots from dilutions 10^{-3}, 10^{-4}, and 10^{-6} were plated on de Man, Rogosa, and Sharpe (MRS) agar and incubated anaerobically at 37 °C for 48 h. Colony counts were performed according to Adamu et al. (2022b). The isolated strains were preserved at − 80 °C in MRS broth containing 25% (v/v) glycerol as a cryoprotectant, following the method described by Nguyen et al. (2023).

DNA extraction and amplification

Presumptive LAB isolates were subcultured on De Man Rogosa Sharpe (MRS) medium and incubated for 48 h at 30 °C, a temperature selected to promote stable growth and optimal biomass yield prior to molecular analysis as reported Stephen & Saleh. (2023). Acid production characteristics and physical morphology were then assessed. Genomic DNA was extracted and purified using the Invisorb Spin DNA Extraction Kit (version 1017, Berlin, Germany) following the method of Ohaegbu et al. (2022). Polymerase chain reaction (PCR) amplification targeting the 16S rRNA gene was performed using an Applied Biosystems 2720 Thermocycler. A molecular ladder marker (Jena Bioscience, 200 bp) was run simultaneously to determine the size of the amplicons. The PCR products were purified and sequenced using the Sanger method with an ABI 3730XL sequencer. The gene sequences were analyzed using BLAST software on the NCBI website (http://www.ncbi.nlm.nih.gov) according to the method of Adnan et al. (2021). Outlines of methodology of molecular investigation was shown in the Fig. 1b.

Production, purification and quantification of exopolysaccharide from lactic acid bacteria

The isolated strain was inoculated into a medium with soy milk containing 11% (v/v) total solids and incubated for 24 h at 37 °C. After fermentation, 100 mL of the fermented milk was mixed with 12% (v/v) trichloroacetic acid and incubated for 24 h. Bacterial cells and proteins were separated from the supernatant by centrifugation at 7000 rpm at 4 °C for 20 min. Exopolysaccharides (EPS) were precipitated from the supernatant by mixing it with three volumes of cold absolute ethanol and incubating at 4 °C for 48 h. The precipitated EPS were then separated by centrifugation (7000 rpm at 4 °C for 20 min), dissolved in double-distilled water, and concentrated at 4 °C using a centrifugal ultrafiltration device (Nanosep Pall Corporation). The concentrated EPS fraction was freeze-dried (lyophilized) for further characterization studies as described by Jurášková et al. (2022). Purification of EPS was achieved by gel filtration using a Sephadex G-100 column (2.6 × 100 cm), eluted with distilled water at a flow rate of 12 mL/hour. The major EPS fractions were pooled, dialyzed against distilled water, and freeze-dried for further study following the method of Sørensen et al. (2022). The total EPS concentration was determined using the modified phenol–sulfuric acid method, with absorbance measured at 485 nm (Nguyen et al. 2020).

Optimization of exopolysaccharide production

Lactiplantibacillus plantarum strain JCM 1149 (or *L. plantarum* JCM 1149) was inoculated into MRS broth and incubated under varying conditions of temperatures (i.e. 35, 40, and 45 °C), starter culture concentration (i.e. 0.5%, 1.0%, and 1.5%), and pH (i.e. 4.8, 5.2, and 5.6). The selected pH values were based on previous reports by Adamu et al. (2022b), indicating that EPS production in LAB can be enhanced under slightly acidic conditions, particularly within the range of 4.5–5.6, which may differ from the optimal growth pH. Similarly, while 37 °C is generally optimal for *L. plantarum* growth, 35 °C was included to investigate whether a slightly lower temperature could promote higher EPS yields, as observed in certain LAB strains in earlier studies Adamu et al. (2022b). Samples were collected at 12, 18, and 24-h intervals to determine EPS yield, as described by Almansoory et al. (Almansoory et al. 2020). This procedure aimed to identify the optimal conditions for EPS production by the LAB strain. The treatment that yielded the highest EPS production was subsequently used to ferment soy milk as the substrate, and the resulting gelation quality, assessed based on texture and consistency, was compared to that of soy milk fermented without optimization.

Production of fermented soy milk from exopolysaccharide producing lactic acid bacteria

Production of soy milk

Soy milk was produced using the traditional method described by Adamu et al. (2022a). Soybean seeds were first cleaned by removing dirt and damaged seeds, then thoroughly washed under running water. The cleaned seeds were soaked in water for 8 h, rinsed again, and ground with a Hamilton Beach Waring Blender (model 909–220). The resulting slurry was filtered through a cheesecloth with 50 µm pore size at a water-to-slurry ratio of 7:1. The filtrate was simmered for 20 min to produce soy milk.

Fermentation of soy milk

Two fermented soy milk premixes were prepared: (i) control: Soy milk inoculated with a commercial yogurt starter culture consisting of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*; (ii) test sample: Soy milk inoculated with the EPS-producing LAB isolate *L. plantarum* JCM 1149, under the optimized conditions determined in Sect. "Optimization of exopolysaccharide production": Temperature 35 °C, pH 4.8, and starter culture concentration of 0.5%. Both premixes were homogenized and pasteurized according to Collins et al. (1991). After pasteurization, the soy milk was cooled to the inoculation temperature (35 °C for the EPS-producing LAB, and 37 °C for the commercial starter) and incubated at 37 °C for 12 h under static conditions. After incubation, the premixes were stirred and cooled i at 4 °C until analysis.

Determination of water holding capacity and syneresis of the fermented soy milk

Water holding capacity (WHC) refers to the ability of fermented soy milk to retain all or part of its water content. WHC of the samples was determined using the centrifugation method described by Nguyen et al. (2020). Syneresis was assessed following the method of Ibhaze et al. (2020). For this, 30 g of each sample was placed in centrifuge tubes and centrifuged at 1535×g for 20 min at 4 °C. The resulting whey was drained for 1 min, and its weight was measured. Syneresis was calculated as the weight of the drained whey expressed as a percentage of the initial weight of the fermented soy milk.

Determination of pH and titratable acidity

The pH of the fermented soy milk samples was measured according to Abdelghani et al. (2022) using a PYE UNICAM Model 292 MK2 pH meter. The electrode was s first rinsed with sterile water, then calibrated with two buffer solutions of pH 4.0 and 7.0. After the calibration, the pH of each sample was measured and recorded.

The percentage titratable acidity (TTA) of the samples was determined using the AOAC method (AOAC 2023). Twenty grams of well-homogenized sample were placed in a beaker and titrated with 0.1N NaOH, using phenolphthalein as an indicator. Titratable acidity (°T) was expressed as a percentage of lactic acid and calculated using the formula:

$$°T = (0.009 \times \text{lacticacid}\%)$$

w here 1 mL of 0.1N NaOH corresponds to 0.0090 g of lactic acid.

Evaluation of the nutritional and sensory properties of the fermented soy milk

Proximate composition

The proximate composition of fermented soy milk produced with the EPS-producing LAB was analyzed for moisture, protein, fat, ash, fiber, energy, nitrogen-free extract, and titratable acidity, following the methods of the Association of Official Analytical Chemists (AOAC 2023).

Evaluation of sensory properties

A total of 60 trained assessors from the National Biotechnology Development Agency, Abuja, Nigeria volunteered to evaluate the sensory quality of the fermented soy drink. The assessors had prior experience in sensory evaluation of food products. Prior to this, the appropriate protocols for protecting the rights and privacy of all participants were utilized during the execution of the research, such that the privacy and rights of human subjects were considered e.g. no coercion to participate, full disclosure of study requirements

and risks, written or verbal consent of participants, no release of participant data without their knowledge, ability to withdraw from the study at any time.

Samples were served at 10 °C in plastic disposable cups, each containing 30 mL, and were coded with letters A and B. The order of sample presentation was randomized. Assessors used a test form to rate six sensory attributes—taste, color, texture, aroma, consistency, and overall acceptability—on a standard nine-point hedonic scale, where 1 corresponds to "dislike extremely" and 9 corresponds to "like extremely".

Data analysis

Data obtained were expressed as mean ± standard error of duplicate measurements. Significant differences between means were determined using a paired samples t-test at $p \leq 0.05$.

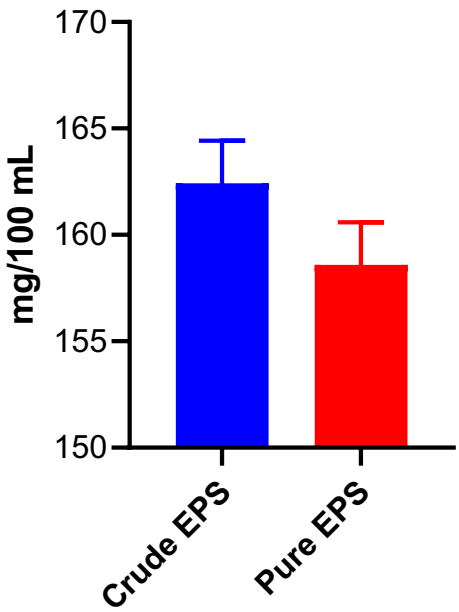


Fig. 2 Concentration of exopolysaccharides produced by *Lactiplantibacillus plantarum* JCM 1149 isolated from tamarind fruit pulp

Results

Molecular identities of bacteria isolated from Tamarind fruit pulp

The sequenced amplicons of LAB isolated from tamarind fruit pulp (*Tamarindus indica* L.) were aligned with nucleotide sequences targeting the 16S rRNA gene. Sequences with a similarity of 95% or above were considered to have substantial identity. The gel image documenting the isolated bacterial DNA after electrophoresis showed a band at 1500 bp, indicating pure isolates.

Concentration of crude and pure exopolysaccharides produced from LAB strains

The concentrated crude EPS produced by *Lactiplantibacillus plantarum* JCM 1149 was 162.43 mg/100 mL, which was slightly higher than the purified EPS (158.6 mg/100 mL) as shown in Fig. 2.

Optimal yield of the EPS

Lactiplantibacillus plantarum JCM 1149 produced the highest EPS yield of 166.4 ± 0.36 mg/100 mL after 24 h of incubation under optimized growth conditions of 35 °C, pH 4.8, and an inoculum size of 0.5 mL (i.e. 23.7×10^2 CFU/g). EPS production increased progressively over time, with 21.46 mg/100 mL at 12 h and 57.59 mg/100 mL at 18 h, demonstrating a gradual accumulation during fermentation (Fig. 3a).

Change in glucose concentration in optimized conditions

The glucose concentrations under optimum growth conditions of *L. plantarum* JCM1149 was low (0.209 ± 0.002 mg/100 mL). The optimum EPS produced was found to be inversely proportional to glucose concentration as shown in Fig. 3b.

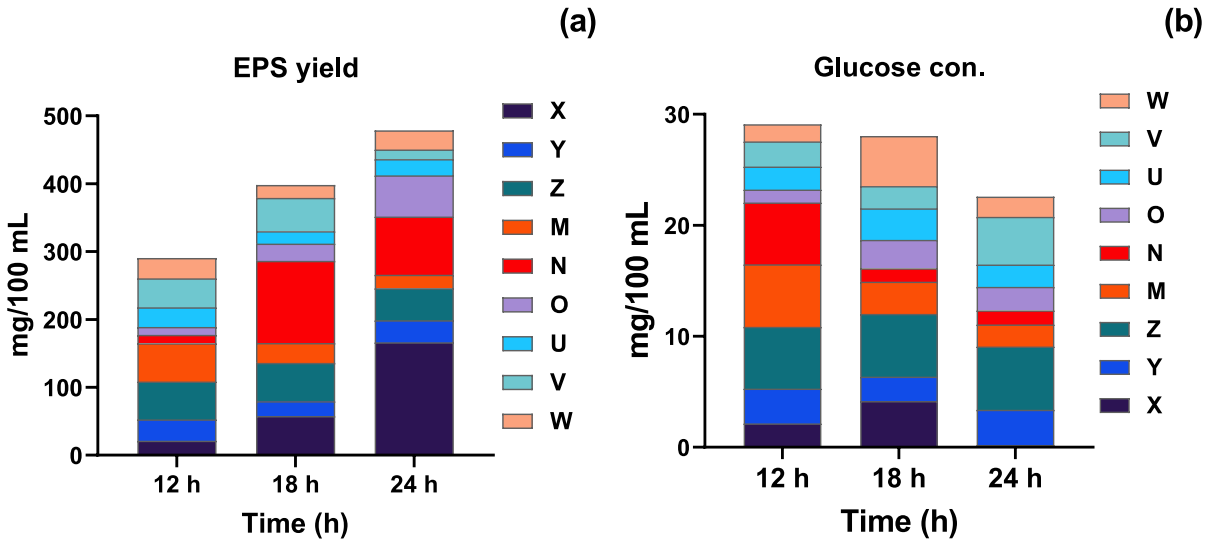


Fig. 3 Exopolysaccharide (EPS) yield potential of by *Lactiplantibacillus plantarum* JCM 1149. Optimal yield of the EPS by strain JCM 1149 (**a**) and glucose concentration for the optimized EPS production (**b**). [M: 45 °C, pH 4.8, 1.5 mL inoculum; N: 45 °C, pH 5.2, 1.5 mL inoculum; O: 45 °C, pH 5.6, 1.5 mL inoculum; U: 40 °C; pH 4.8, 1.0 mL inoculum; V: 40 °C; pH 5.2, 1.0 mL inoculum; W: 40 °C, pH 5.6, 1.0 mL inoculum; X: 35 °C, pH 4.8, 0.5 mL inoculum; Y: 35 °C, pH 5.2, 0.5 mL inoculum; Z: 35 °C; pH 5.6, 0.5 mL inoculum]

Proximate composition of the fermented soy milk

The moisture content 80.62% and energy 46.64 kcal of the treated fermented soy milk (Test) was high compared to the control (untreated fermented soy milk) 65.22% and 38.87 kcal (Fig. 4a). The Test and the control had no crude fiber (0.0). However, the control had 3.31% crude protein and 2.01% nitrogen free extract than the Test 3.13% and 1.89%. Both control and Test contained high crude lipid content of 4.69% and 4.62% and low ash content of 0.37% and 0.41 (Fig. 4b).

Microbial count of fermented soy milk

The fermented soy milk samples, control and Test had no fungal and coliform counts. Control had bacterial count 7.10×10^2 cfu/mL and the Test had 7.60×10^2 cfu/mL (Fig. 5a).

The pH value and titratable acidity of the fermented soy milk

The pH of the test was slightly high (5.6) compared to the control with pH of (5.75). The titratable acidity of control was low (0.135) than the Test (0.25), as shown in Fig. 5b.

The Water holding capacity and syneresis of the fermented soy milk

The control sample had 47.8% low water holding capacity (WHC) than the test sample with 52.2% WHC. The syneresis of 41.5% was observed in control compared to the 22.9% of the Test sample as illustrated in Fig. 5b.

The sensory properties of the fermented soy milk

The control was low in consistency (6.82) and overall acceptability (6.98) than the test sample (7.55) and (7.46). There was no difference in taste, color, texture, and aroma for the test 7.59, 7.71, 7.70, and 7.16 and control 7.45, 7.71, 7.60, and 7.21 respectively as shown in Fig. 5c.

Discussion

In this study, molecular characterization using 16S rRNA confirmed the isolate R32 as *L. plantarum*

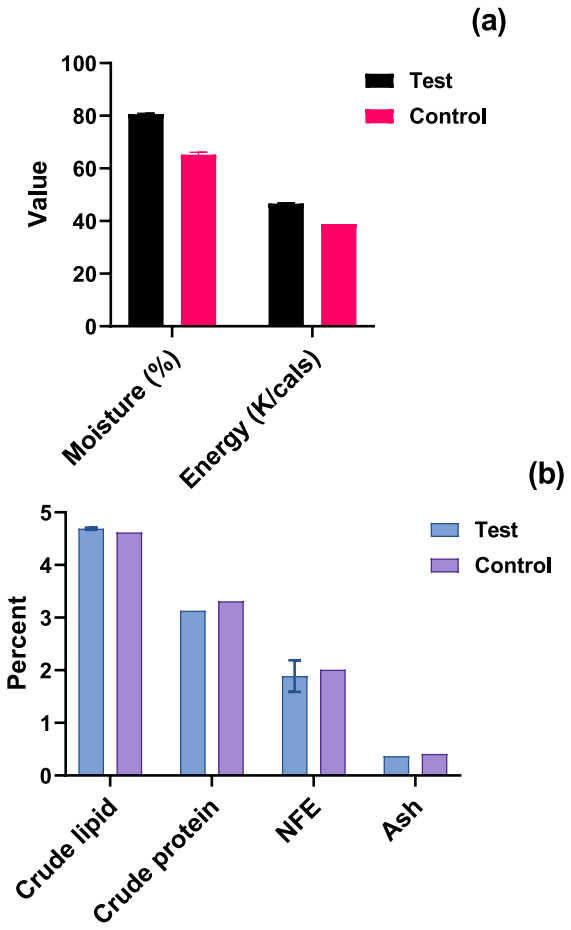


Fig. 4 Proximate composition of the fermented soy milk: (**a**) Moisture and Energy; (**b**). Crude lipid, crude protein, NFE, Ash Values are Mean ± standard error of duplicate values. [NFE: Nitrogen-free extract]

strain JCM 1149 from tamarind fruit. The results showed that the isolate's sequence aligned with the database sequence, confirming its identity and purity. The observed band migration toward the positive electrode was attributable to the inherent negative charge of the DNA. These findings are consistent with Adnan et al. (2021), who reported similar sequence alignments for LAB isolates from yogurt. Likewise, Jafari et al. (2021) noted that sequences with 90% or higher similarity are significant, supporting nucleotide sequence alignment with database entries as a reliable indicator of identity.

In this study, EPS were produced by the LAB strain, which may be attributed to strain specific metabolic capacity to utilize 11% soy milk (v/v) as a fermentation substrate. Interaction between soymilk components and bacterial metabolism may also influence EPS synthesis. These findings differ from Banwo et al. (2023), who reported EPS production using 5% skimmed milk as a substrate, and from Bachtarzi et al. (2020), who obtained EPS from *Lactobacillus plantarum* stains grown in 11% skimmed milk casein Jurášková et al. (Jurášková et al. 2022) also reported a higher EPS yield of 380 mg/L from *Streptococcus thermophilus* zlw TM11 in fermented dairy milk. These variations may be attributed to differences in fermentation substrates, particularly dairy-based media versus soy milk. Furthermore, EPS production by individual LAB strains is known to be influenced by the available carbon source in the culture medium (Nguyen et al. 2020).

The relatively low quantity of purified EPS produced by *Lactobacillus plantarum* JCM 1149 in this study may be attributed to the purification method, variations in growth conditions such as temperature and pH, and possible nutrient limitations. These findings contrast with Silva et al. (2019), who reported a higher EPS yield of 384 mg/L from *Streptococcus thermophilus* zlwTM11 in fermented cow milk. In a similar study, Sørensen et al. (2022) reported EPS yields of 159 mg/L and 107 mg/L from *L. plantarum* YO175 and *L. plantarum* OF101, respectively, when grown in MRS broth supplemented with sucrose. The quantity of purified EPS reflects the metabolic activity of the bacteria, and is important for evaluating culture viability and productivity.

The results of this study indicate increased EPS production during soy milk fermentation under optimized physiological conditions. This may be attributed to the inherent characteristics of *L. plantarum* JCM 1149 as a mesophilic, slow-growing, heterofermentative bacterium (Sugahara et al. 2022). The carbon-to-nitrogen ratio in the medium likely influenced EPS synthesis, while the temperature and pH applied were favorable for EPS production by this strain. The pH of 5.6 falls within the suitable range for soy milk fermentation, which is less acidic than yogurt or nono due to the absence or low lactose content in soy milk. The greater acidification observed in fermented soy milk reflects the lower buffering capacity of soy proteins, influenced by protein-bound acid and base groups, and organic acids. These findings agree with De et al. (2022), who reported zero lactose in soy

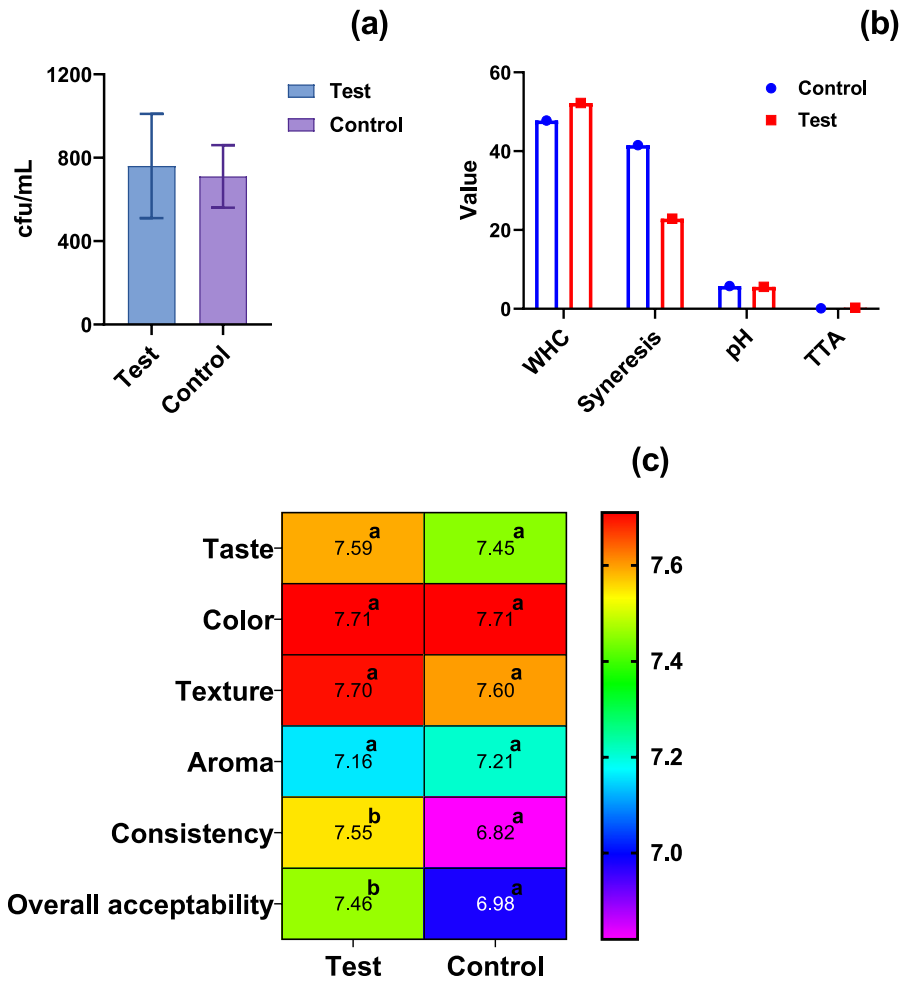


Fig. 5 Microbial characteristics of fermented soy milk. (**a**) Microbial count of fermented soy milk [Values are Mean ± Std. Error of duplicate values]. (**b**) The water holding capacity (WHC), syneresis, pH and titratable acidity of the fermented soy milk. [Test: Treated fermented soy milk; Control: Untreated fermented soy milk;TTA, Titratable acidity]. (**c**) The sensory properties of the fermented soy milk Means with dissimilar letter (s) differ significantly according to the Paired Samples t-Test at $p \leq 0.05$

milk compared with 4.8 g in cow milk. Similarly, El-Shazly et al. (2021) observed a comparable pH range (i.e. 4.92–5.28) during soy yogurt fermentation using *L. plantarum* strain at 37 °C.

In contrast, Prete et al. (2021) reported that the optimal pH for EPS production is generally around 6.0, although this varies among LAB species and strains. Temperature is also a critical factor, as LAB respond differently to specific thermal conditions. For example, Fashogbon et al. (2021) observed higher EPS production by *Lactobacillus delbrueckii* FASHADYG2 at 35 °C, which is consistent with the findings of this study. Similarly, Berthold-Pluta et al. (2019) reported that *Streptococcus thermophilus* BN1 produced significantly higher EPS at 37 C, irrespective of medium composition.

Incubation time likewise influences EPS production, reflecting differences in strain specificity, growth rate, nutrient utilization, and metabolic activity. Abdalla et al. (2021) reported maximal EPS synthesis by *L. fermentum* F6 after 32 h of incubation, which contrasts with the present findings. Extended incubation may result in EPS degradation, as some LAB strains produce glycohydrolases that catalyze polysaccharide breakdown, thereby reducing EPS yield (Berthold-Pluta et al. 2019).

Inoculum size is also an important factor in EPS production. In this study, 0.5 mL was optimal for 100 mL of soy milk, which may be related to the slow growth rate of the strain, substrate utilization, and metabolic activity at lower cell densitites. A higher inoculum may result in overcrowding and competition for nutrients, thereby reducing EPS

production, while a smaller inoculum may limit cell-to-cell interaction and prolong the lag phase. Tologana et al. (2023) reported slow growth of *L. plantarum* Dad 13 in skim milk with a 1% (v/v) inoculum after 24 h, similar to this study. In contrast,. Elmansy et al. (2022) observed rapid growth of *L. plantarum* RO30 at 25, 35, and 40 °C, with slower growth at 45 °C. Adnan et al. (2021) reported maximum EPS production using a 2% inoculum per 100 mL of medium at 30 °C after 24 h. These differences may be attributed to strain variability and distinct responses to fermentation conditions.

The moisture content of the fermented soy milk varied, which may be influenced by environmental conditions and metabolic activity during fermentation. The production of metabolites may increase moisture retention, while enhanced coagulation, could reduce moisture content (Britten and Giroux 2022). Although high moisture content can affect mouthfeel, the results obtained in this study fall within the reported standard range of 89% for fermented soy milk (Adamu et al. (2022a). Similarly, Matela et al. (2019) reported moisture contents ranging from 76.08 to 80.07%) in yogurt, while Ibhaze et al. (2020) observed a higher value of 87.8% in fermented soy milk. Higher moisture content may reduce shelf life by increasing susceptibility to microbial spoilage. Furthermore, the fermented soy milk in this study exhibited low ash content, which may reflect the inherently low mineral content of soybeans and soy milk. This agrees with Ibhaze et al. (2020), who reported a similarly low ash content (i.e. 0.52%) in fermented soy milk. In contrast, Bukar and Salami-Suleiman (2019) observed a higher ash content (0.85%) in fermented soy milk produced with *L. plantarum* isolated from nono, which decreased to 0.63% after 9 h of fermentation.

The crude protein content obtained in this study was within the standard range of 4.7% permitted by the United States Department of Agriculture (USDA 2018) for fermented soy milk. This may be attributed to the naturally high protein content of soybeans and the specific variety used. Although protein content varies with cultivar and growth conditions, soybeans generally provide higher protein levels than most legumes. The results are consistent with Adamu et al. (2022a), who reported protein contents of 3.55 to 4.41% in fermented soy milk with tamarind and nono, and with De et al. (2022), who observed the values of 3.5 to 3.75%. According to Codex standards, the protein content of yogurt and fermented soy milk should not be less than 2.7% reported by Matela et al. (2019).

The differences in crude lipid content among the fermented soy milk samples were not significant, however, the values exceeded the USDA standard of 2.7% for yogurt. This may be related to the intrinsic lipid content of soybeans and processing factors, such as whether the product is full-cream, low-fat, or non-fat. Crude lipids contribute to smooth mouthfeel and creamy texture, thereby enhancing consumer acceptability. In agreement with the present findings, Lira et al. (2023) reported crude fat levels of 2.0 to 2.5% in soy milk, while Basharat et al. (2020) observed a higher value of 3.68% Similarly, Ibhaze et al. (2020) and El-Zeiny et al. (2023) reported high crude lipid contents of 4.58% and 4.44%, respectively, in soy yogurt. In contrast, De et al. (2022) reported lower fat contents ranging from 0.47 to 0.51% in fermented soy milk. According to USDA, guidelines, products containing less than 0.5% fat are classified as non-fat, those with 0.5% to 2.0% as low-fat, and those with approximately 3.25% as full-fat yogurt.

This study recorded zero crude fiber in the fermented soy milk samples, which may be attributed to the composition of soybeans. Dietary fiber is primarily located in plant cell walls, and soybeans contain relatively low levels of structural fiber compared with other legumes. Similarly, De et al. (2022) reported low crude fiber contents ranging from 0.03 to 0.04% in soy yogurt, while Matela et al. (2019) a value of 0.08% in yogurt. In contrast, Erfanian and Rasti (2019) reported a higher crude fiber content of 1.36% in soy yogurt. Overall, soybeans are not considered a rich source of dietary fiber relative to other plant-based foods.

The nitrogen-free extract (NFE) content of the fermented soy milk samples was comparable and within the recommended range of 10 to 20%. This may be related to the use of the same soybean variety and processing method, as well as the carbohydrate composition of the fermented product, which directly influences NFE values. El-Zeiny et al. (2023) reported a higher NFE content of 6.4% in soy yogurt, while Basharat et al. (2020) reported an NFE value of 2.35% in soy milk, which is similar to the findings of this study.

This study revealed significant variation in the energy content of fermented soy milk, which may be

attributed to natural differences in nutrient composition among soybean varieties, as well as processing factors such as fermentation and mixing. Despite these variations, the energy values obtained fall within the USDA-recommended level of 59 kcal for soy yogurt. El-Zeiny et al. (2023) reported a higher energy content of 90.48 kcal, while Ibhaze et al. (2020) observed 69.22 kcal in soy yogurt, both of which are higher than the values reported in the present study.

Microbiological analysis of the fermented soy milk revealed low bacterial counts, with no detectable coliforms or fungi. This may be related to the relatively low population of *L. plantarum* JCM 1149 in the product, which contributed to the overall microbial load. In addition, the use of tamarind as the source of the inoculum may have influenced microbial growth, given its reported antibacterial and antifungal properties. The bacterial counts obtained were lower than the acceptable range of 4.0×10^3 to 5.0×10^5 CFU/g recommended by the Center for Food Safety (CFS) for food products (Otolowo et al. 2022). Otolowo et al. (2023) reported higher counts of 8.0×10^4 CFU/g in soy yogurt,, and Adamu et al. (2022b) observed 8.7×10^3 CFU/g in a fermented soy drink. In contrast, Bukar and Salami-Suleiman (2019) reported lower bacterial counts of 2.2×10^3 CFU/g in soy yogurt inoculated with *L. plantarum*, which is consistent with the present findings. Low bacterial counts can contribute to a longer shelf life of fermented soy milk. The absence of coliforms and fungi in this study may be attributed to good hygiene practices during processing and fermentation, indicating that the product is microbiologically safe for consumption. This finding is consistent with Usman and Bolade (2020).

The water-holding capacity (WHC) of fermented soy milk in this study was influenced by the type of inoculum and the formulation of the soy milk. Variation in the proportions of soybean solids, water, and other ingredients may affect the structure of the protein gel and, consequently, its ability to retain water (Ibhaze et al. 2023). In addition, the EPS produced by *L. plantarum* JCM 1149 likely enhanced WHC by interacting with the soy protein matrix to form a more cohesive gel network that binds and immobilizes water. Gel shrinkage associated with pH reduction can also influence WHC (Ziarno et al. 2022). In contrast to this study, Jurášková et al. (2022) reported a higher WHC of 92.3 5% in soy yogurt, while Ziarno et al. (2022) observed a WHC of 16% after 24 h of storage. Ramos et al. (2023) similarly reported a lower WHC of 38.7% in nonfat set yogurt.

The syneresis values obtained in this study were low for both samples. This may be attributed to the fermentation conditions, the LAB strain used, and the production of EPS, together with pH reduction and milk coagulation. The EPS produced by *L. plantarum* JCM 1149 likely contributed to reduced syneresis by strengthening the gel structure and increasing water binding within the protein-polysaccharide network, thereby limiting whey expulsion. In addition, the functional properties of soy proteins, which from a gel-like structure upon heating or acidification and trap water within the matrix, further restrict synthesis (Ibhaze et al. (2023). These findings agree with Rana et al. (2021), who reported a 43.33% syneresis in plain soy yogurt, while Ibhaze et al. (2023) reported a lower value of 29.20%. Overall, the improved WHC observed in this study explains the reduced syneresis of the fermented soy milk.

The pH of the fermented soy milk in this study was within the recommended range of 5.1 to 6.2 for soy-based fermented products. This likely reflects strain specificity, including acidification capacity and pH tolerance. Fermentation time is critical, as it affects the metabolism of soy sugars (e.g. glucose and sucrose) into organic acids. The metabolic activity of the strain directly influences the acidity of the product. These results align with Mishra et al. (2019), who reported a pH of 5.6 in soy yogurt after 24 h, and El-Shazly et al. (2021), who observed a pH of 5.31 in fermented soy milk with *Lactiplatibacillus plantarum* KU985432. Some *Lactobacillus* strains can lower pH to 3.7–4.3, despite soy milk's low lactose content (Fatima and Hekmat 2020).

The titratable acidity (TTA) varied between the two samples, potentially due to differences in fermentation temperature3 and the low lactose content of soy milk. These factors influence bacterial growth and acid production. The TTA results are consistent with Adamu et al. (2022a), who reported 0.29% in fermented soy drink, Ziarno et al. (2022), who observed 0.2% in soy yogurt. In contrast, El-Zeiny et al. (2023) reported a higher TTA of 0.99% in soy yogurt fermented with *L. plantarum* KU985432. Maintaining appropriate acidity is important for product quality and consumer acceptance.

Sensory evaluation revealed no significant differences in color, aroma, flavor, or taste between samples, likely due to similarities in the soy milk batch and inoculum. However, the treated sample (T) scored higher for consistency and overall acceptability, likely reflecting the higher EPS content, which improved gel structure and masked the beany flavor. This aligns with Ibhaze et al. (2020), who reported a consistency score of 7.68 in soy yogurt, while Adamu et al. (2022a) observed a lower score of 6.93 for fermented soy drink. The overall acceptability in this study was comparable to values reported by Adamu et al. (2022a) and Ibhaze et al. (2020) (i.e. 7.50 and 7.69, respectively), and higher than the score of 5.03 reported by Kim and Han (2019).

Conclusion

Lactobacillus plantarum JCM 1149 produced purified EPS at a concentration of 158.6 mg/100 mL, while the highest EPS yield of 166.4 mg/100 mL was obtained under optimized growth conditions of 35 °C, pH 4.8, and a 0.5 mL inoculum after 24 h of incubation. Under these optimized conditions, fermentation of soy milk by *L. plantarum* JCM 1149 resulted in a product with improved gelation properties, characterized by increased WHC and reduced syneresis. The enhanced textural stability is attributed to the ability of EPS produced by this strain to interact with soy proteins and strengthen the gel matrix, thereby improving moisture retention and structural integrity. The fermented soy milk exhibited higher moisture and energy content, while crude protein, ash, lipid, and nitrogen-free extract remained comparatively low. Despite these variations, the product demonstrated good sensory acceptability, particularly in terms of consistency and overall quality. These findings confirm that optimizing the growth conditions of an EPS-producing *L. plantarum* JCM 1149 isolated from tamarind can significantly enhance the functional and nutritional attributes of fermented soy milk. Future research should focus on elucidating the molecular interactions between EPS and soy protein networks, evaluating the stability and shelf life of the product during storage, and exploring the scalability of this fermentation process for industrial production of high-quality plant-based fermented foods.

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Declarations

Competing Interests The author(s) declare that there are no conflicts of interest regarding the publication of this manuscript.

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