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STO conceptualised the study; AG participated in data collection and the conduct of the experiments. AG and STO contributed to writing the manuscript. All authors read and approved the final version of the manuscript.

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Enhancement of Nutritional Profiles in Plant-Based Yoghurt through Lactic Acid Bacteria Fermentation

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Abstract:

Yoghurt is widely consumed as a healthy, nutrient-rich food across many cultures. However, dairy intolerance and the growing interest in sustainable diets have driven a significant increase in demand for plant-based alternatives. Fermentation with lactic acid bacteria (LAB) presents a promising approach to enhancing the nutritional and sensory qualities of non-dairy yoghurts. This study aimed to identify suitable LAB strains with desirable fermentative properties to improve the nutritional profile of soy-based yoghurt. LAB strains were isolated from traditional fermented foods, including ogi, wara, and yoghurt, using standard microbiological methods. A total of forty isolates were identified as *Lactiplantibacillus plantarum* (11), *Lactococcus lactis* (3), *Lactobacillus acidophilus* (16), and *Limosilactobacillus fermentum* (10). Based on probiotic potential, *Lb. fermentum* and *Lb. plantarum* were selected as starters, individually and in combination, for soymilk fermentation, with spontaneous fermentation as the control. Fermentation was carried out in heat-treated soybean extract, producing yoghurt-like products with characteristic acidity, creamy aroma, and mustard-like texture. Nutritional analysis revealed that starter-produced soymilk yoghurt contained higher protein and carbohydrate levels, lower fat, and increased fibre compared to spontaneously fermented soymilk. The protein content was slightly lower than that of cow milk yoghurt, while the fat content was significantly reduced. Fibre levels in starter-produced and spontaneously fermented soymilk were comparable. The starter-based product exhibited superior carbohydrate content and the highest overall sensory acceptability. These findings highlight the potential of selected LAB strains to produce nutritionally improved and more acceptable plant-based yoghurts. This study demonstrates that LAB fermentation can enhance the quality of soy-based yoghurt, providing a viable non-dairy alternative for consumers.

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INTRODUCTION

Plant-based milk alternatives, also known as milk analogues, are water-based extracts of plants that have gained increasing popularity in the field of human nutrition. Plant-based yoghurts are gaining popularity as consumers seek alternatives to dairy products due to health concerns, lactose intolerance, and environmental considerations (Tangyu *et al.,* 2019). However, plant-based milk alternatives often fall short of the complete nutritional profile found in dairy milk, especially regarding protein quality and essential micronutrients, and their flavour and appearance limit their acceptance (Moshtaghian *et al.,* 2024). To produce more acceptable and flavourful products, fermentation can enhance the sensory profiles, nutritional properties, texture, and microbial safety of plant-based milk alternatives, leading to more valuable and flavourful products (Tangyu *et al.,* 2019).

Lactic acid bacteria (LAB) are renowned for their role in dairy fermentation, contributing to the texture, flavour, and nutritional value of dairy products. To increase the availability of milk-like products, especially in regions where milk is scarce, various milk and milk products derived from leguminous plants have been developed to mitigate this problem. Since legumes are valuable sources of affordable, high-quality protein, incorporating imitation milk products produced from legumes may help alleviate protein malnutrition (Rao *et al*., 2008).

Fermentation by Lactic acid Bacteria of non-dairy alternatives, such as legume-based milks, has been used to prolong the shelf life of the products, introduce variety, increase consumer acceptability, and improve nutritional value (Terna & Musa, 1998). Additionally, specific plant-based yoghurt alternatives offer unique health benefits. For instance, flax and hemp-based yoghurts are rich in omega-3 fatty acids and fibre (Craig & Brothers, 2021). Fermented soymilk, gaining popularity as a biotherapeutic, boasts high protein content and is associated with various positive effects on human health, including antihypertensive, allergy alleviation, antioxidant, antidiabetic, anticancer, and hypocholesterolemic effects (Kumari *et al.,* 2022). This non-dairy milk alternative, renowned for its numerous health benefits and nutraceutical potential, has gained popularity as a healthful beverage. Its high content of mono- and polyunsaturated fatty acids and oils, high-quality protein, phosphatidylcholine, B vitamins, calcium, amino acids, and potent natural antioxidants, such as isoflavones and phytoestrogens, contribute to its appeal (De *et al*., 2022).

As a dairy milk substitute, soy milk is low-calorie, cholesterol-free, and particularly preferred by consumers with lactose intolerance. Plant-based milk fermentation primarily employs single cultures of microbes, such as lactic acid bacteria, bacilli, and yeasts. Recently, new concepts have been proposed for mixed-culture fermentations involving two or more microbial species. These methods promise synergistic effects that enhance the fermentation process and improve the quality of the final products. *Limosilactobacillus fermentum* and *Lactoplantibacillus plantarum* are exceptional probiotic and bio-therapeutic Lactic Acid Bacteria (LAB) that can survive the conditions of the gastrointestinal tracts of humans, survive the low pH and bile salts exposure (Kongsinkaew *et al*., 2024). While existing literature has extensively explored the production of soymilk yoghurt, this study uniquely discusses the role of LAB in significantly enhancing the nutritional profile of soymilk yoghurt.

MATERIALS AND METHODS

Sample collection

Fermented food samples (*ogi, yoghurt,* spontaneous fermented soymilk*)* and *Wara* were purchased randomly from Bodija and Ojoo markets in Ibadan, Oyo State. Raw milk was purchased from an abattoir in the Akinyele Local Government area of Oyo State.

Isolation and characterisation of Lactic Acid Bacteria (LAB)

Using the serial dilution method introduced by Robert Koch in 1883, samples were serially diluted by transferring 1ml of each sample into 9 ml of sterilised water in different test tubes, and from each test tube containing the various samples, 1 ml each was transferred from dilution 10^{-1} to 10^{-9}, making a 10-fold dilution factor for each sample. The appropriate diluents were plated on De Man, Rogosa, and Sharpe (MRS) media using the pour plate method, appropriately labelled, and incubated at 37 °C for 24 hours. After 24 hours of incubation, colonies were picked randomly and re-streaked on sterilised solid MRS agar. Purified strains were stored in glycerol stock at -18°C till further use.

Phenotypic characterisation of LAB

Colony morphology, cell morphology, colony arrangement, Gram staining, and endospore staining were performed for phenotypic characterisation**.** Colonies' appearance on agar plates was macroscopically described based on the following characteristics: size, colour, elevation, margin/edges, shape, and texture. Biochemical characterisation, including the catalase test, potassium hydroxide test, citrate utilisation, and sugar fermentation test, was also performed (Rahayu & Setiadi, 2023).

Environmental stress tolerance assay

Growth at 2.5%, 4.5%, and 6.5% NaCl

The resistance of LAB to osmotic stress was carried out by inoculating LAB strains into test tubes containing modified MRS broth with varying concentrations of NaCl (2.5 g, 4.5 g, and 6.5 g per 100 ml). The inoculated test tubes were incubated at 37°C for 24 to 48 hours. The results were determined by observing the level of turbidity, which indicates bacterial growth (Ma *et al*., 2022).

Effect of temperature

The effect of different temperature ranges on LAB strains was examined by suspending the strains in MRS broth and incubating them at various temperatures (25°C, 35°C, and 45°C). Each test tube containing different isolates was labelled correctly and incubated. Growth was evaluated by measuring the optical density (OD) after 24 hours of incubation (Ma *et al*., 2022).

Screening of LAB for virulent properties

Antibiotic susceptibility test

The LAB strains were subjected to antibiotic susceptibility screening using the disc diffusion method with the Kirby-Bauer technique. Diluted strains (a loopful of LAB colony in 5ml of sterile normal saline) were spread on the surface of pre-prepared Mueller-Hinton agar plates using a sterile swab stick. Antibiotic discs (Gentamicin, Erythromycin, Imipenem, and Ampicillin) were placed on the plates in quadrants. The plates were then incubated at 37°C for 24 to 48 hours. After incubation, the antibiotic inhibition zones were measured with a ruler, and susceptibility was determined according to CLSI (2006) standards (Aernan *et al.,* 2024; Iqbal *et al.,* 2015).

Hemolytic activity

The hemolytic activity of the LAB strains was determined by inoculating them on blood agar plates. After preparation, 5ml of blood was added to the cooled media and mixed thoroughly. The mixture was then poured into sterile plastic Petri dishes aseptically and allowed to solidify. The LAB strains were streaked onto the agar, and the plates were incubated at 37°C for 24 hours. Hemolytic activity was observed after the incubation period (Padmavathi *et al*., 2018).

Screening of LAB for potential probiotic characteristics

Bile salt tolerance

Selected LAB isolates were assayed for bile salt tolerance using various concentrations of bile salts (0.8% and 0.6%). The prepared MRS broth

with different bile salt concentrations was dispensed into test tubes. The selected LAB strains were inoculated into each test tube, properly labelled, and incubated at 37°C for 24 hours. After the incubation period, growth was recorded by measuring the optical density (OD) using a spectrophotometer at a wavelength of 600 nm (Padmavathi *et al.,* 2018).

Tolerance of acidity (pH 2, 3, 4, and 5)

LAB strains were subjected to different pH conditions to determine their ability to grow in acidic environments. Acidity tolerance was assessed by preparing MRS broth with varying pH levels (2, 3, 4, and 5). Concentrated HCl was used to achieve a more acidic pH, and higher pH values were achieved using 0.1 M NaOH (Padmavathi *et al.,* 2018).

Cell Surface Hydrophobicity of LAB strains

The 24-hour LAB culture was centrifuged to obtain pellets, and the pellets were resuspended in phosphate buffer solution and adjusted to an absorbance of 0.7 at 600 nm. Next, 3.0 mL of KNO_3 at pH 6.2 was added, and the mixture was incubated at 37°C for 10 minutes. After proper shaking, the mixture's absorbance was read at 600 nm and recorded. The percentage of surface hydrophobicity was calculated using the formula proposed by Huligere et al. (2023).

Surface hydrophobicity (%) = $[OD_{initial} – OD_{final})/OD_{initial} \times 100$

Auto-aggregation of LAB strains

The 24-hour LAB culture was centrifuged, and the supernatant was discarded to obtain the pellets. The pellets were washed with phosphate buffer solution and adjusted to an absorbance of 0.3 at 600 nm. The mixture was then incubated at 37°C for 2 hours. After incubation, the final absorbance was read at 600 nm and recorded (Huligere *et al*., 2023). Cell aggregation was calculated using the following equation:

Cell aggregation% % = $[OD_{initial} – OD_{final})/OD_{initial} \times 100$

Determination of Lactic Acid Production

A loopful of 24-hour-old cultures was resuspended in 20 mL of MRS broth and incubated for 24, 48, and 72 hours, respectively. After the incubation periods, the production of lactic acid was determined by titrating 5 mL of MRS broth containing LAB isolates at 24 hours with 0.1 M NaOH and three drops of phenolphthalein indicator (0.5% in 50% alcohol) until a persistent pink colouration was observed for 2 minutes. The titratable value was calculated as lactic acid (% v/v). The lactic acid was calculated according to AOAC (2000).

Total titratable acidity of lactic acid (mg/ml) = ml NaOH x N NaOH x M.E

The volume of the sample used

Where, ml NaOH = Volume of NaOH used

N NaOH = Molarity of NaOH used,

M.E = Equivalent factor = 90.08mg.

Determination of Diacetyl Production

A loopful of 24-hour-old cultures was resuspended in 20 ml of MRS broth and incubated for 24, 48, and 72 hours, respectively. After the periods of incubation, diacetyl production was determined by titrating 5ml of MRS broth containing LAB isolates at 24 hours with 0.1N HCl to a greenish yellow endpoint using bromophenol blue as an indicator (0.3% in 97% alcohol. The titratable value was calculated as lactic acid (% v/v). The lactic acid was calculated according to AOAC (2000).

Total titratable acidity of lactic acid (mg/ml) = ml NaOH x N NaOH x M.E

The volume of the sample used

Where: ml NaOH = Volume of NaOH used,

N NaOH = Molarity of NaOH used,

M.E = Equivalent factor = 90.08mg.

Selection of starter culture

The LAB strains were selected based on their safety, potential probiotic properties, and predominant isolates for molecular characterisation and yoghurt production.

Molecular identification of LAB isolates

The 16S rRNA gene of selected isolates was sequenced. Genomic extraction kit (Promega, USA) was used as a template to amplify the 16S universal primer 27F (5'-AGA GTT TGA TCM TGG CTC AG-3' and - 1525R, 5′-AAGGAGGTGATCCAGCC-3′). After purifying the amplicons, the amplified fragments were sequenced using a Genetic Analyser 3130xl sequencer from Applied Biosystems using the manufacturer's manual. The sequencing kit used was the BigDye Terminator v3.1 Cycle Sequencing Kit. Bio-Edit software and MEGA 6 were used for all genetic analyses. The 16S rRNA gene sequences were analysed using BLAST searches of the NCBI database.

Fermentation of soy milk for yoghurt production

Preparation and extraction of soy milk

Soybean seeds were hand-picked, De-stoned, and washed properly. After washing, the bean seeds were soaked in sterilised water for approximately 8-10 hours. The soaked beans were washed and dehulled until no hulls were present. Dehulled bean seeds were then boiled for 20-30 minutes on medium heat. The boiled bean seeds were then blended in a blender with 2 litres of sterilised water until smooth, followed by sieving with a fine muslin cloth. The extract was then pasteurised by boiling for about 15 minutes and cooled to 45 °C.

Inoculum preparation

The inoculum size of 10^6 CFU/mL of the selected starter culture was obtained using a 0.5 McFarland standard. This was done by centrifuging a 24-hour-old culture grown in MRS broth at 5000 rpm for 15 minutes; the supernatant was discarded, and the cells were washed three times with sterile water. The washed cells were diluted with sterile distilled water to obtain 10^6 CFU/mL.

Inoculation of soy milk with LAB starters

Soymilk yoghurt was made according to the method of Obi et al. (2023) with some modifications using various starter combinations (Table 1). A 100 mL of freshly prepared soymilk was transferred to glass containers, heat-treated for 30 minutes, and cooled to 40 ^oC. The heat-treated soymilk was then aseptically inoculated with the prepared suspension (10^6 CFU/mL) of the selected starters in single and mixed cultures (1:1, 1:2, 2:1, and 2:2). The inoculated soymilk was incubated at 37-40 ^oC for 8 hours using a thermostatically controlled water bath to allow for fermentation. At the end of the fermentation period, the fermented soymilk samples (yoghurt) were transferred to a refrigerator for storage at 4 ^oC. Uninoculated soy milk served as a control for spontaneous fermentation (Obi *et al*., 2023).

Table 1. Combinations of selected starters for the fermentation of soymilk.

S/N	Strains	Ratio
1	*Lactoplantibacillus plantarum* (IS11) and *Limosilactobacillus fermentum* (IS26)	1:1
2	*Lactoplantibacillus plantarum* (IS11) and *Limosilactobacillus fermentum* (IS26)	1:2
3	*Lactoplantibacillus plantarum* (IS11) and *Limosilactobacillus fermentum* (IS26)	2:1
4	*Lactoplantibacillus plantarum* (IS11) and *Limosilactobacillus fermentum* (IS26)	2:2

Analysis of fermented soy milk (yoghurt)

Microbiological analysis

The pour plate method was used to determine the cell population of the selected starters. 1 ml each from the varying ratios of fermented soy milk was taken into 9 ml of water to give a stock solution from which other dilutions were made up to 10^{-8}. 1 mL each from dilutions 10^{-3} and 10^{-8} was poured and plated on sterilised MRS agar. The plates were labelled correctly and incubated

at 37 °C for 24 hours. Viable cells were counted and reported as CFU/mL (Ramos *et al*., 2023).

Physiochemical Determinations

Change in pH during fermentation.

Changes in physical parameters, such as pH, were determined using a digital pH meter at 0, 2, 4, 6, and 8-hour intervals (Ramos *et al.,* 2023).

Water holding capacity of the produced yoghurt

The water-holding capacity (WHC) of the produced yoghurt was determined according to the method described in Ramos et al. (2023). For this purpose, 20g (20mL) of the sample was weighed into a centrifuge tube and centrifuged at 5,000 rpm at 4 °C for 20 minutes. After centrifugation, the whey was weighed and recorded. The following is used for the calculation of the WHC of the sample:

$$\text{WHC} = \frac{W}{Wo} x100$$

Where W is the weight of the residue after centrifugation, and W_o is the weight of the sample.

Syneresis

The level of syneresis of the produced yoghurt was determined by centrifugal acceleration. For this purpose, 10g of the sample was weighed, placed in a 15 mL centrifuge tube, and centrifuged at 1200 rpm for 15 minutes at room temperature. The volume of whey separated from the sample was measured to estimate the rate of syneresis and expressed as the weight percentage of free whey in the total yoghurt sample after fermentation (Ramos *et al*., 2023).

Viscosity determination

A 100mL sample of cooled fermented soy was manually stirred for 1 minute before measurement using a rotational digital viscometer with spindle four at a rotational speed of 30rpm. The apparent viscosity reading of the sample, expressed in mega pascals (MPa), was taken at the 30th second (Ramos *et al*., 2023).

Moisture determination

The method involved drying aluminium dishes at 103 ± 2°C for at least 2 hours, cooling them in a desiccator, and weighing them (W1). Approximately 5g of the homogenised sample was added to each dish, and the combined weight was recorded (W2). The dishes were then dried in an oven at 103 ± 2°C for at least 2 hours until a constant weight was achieved. After drying, the dishes were cooled in a desiccator and weighed again with the dried sample (W3) (AOAC, 2006).

Calculation:

Per cent Dry Matter (% DM):

% DM = (W3 – W1) x 100 / (W2 – W1)

Where;

W1 = weight of empty dish (g),

W2 = weight of dish and sample (g), and

W3 = weight of dish and sample after drying (g).

Per cent Moisture: % Moisture = 100 – % DM

Protein determination

The method involved weighing 1g of the sample (or 2 mL for liquids) into a 250 mL digestion tube, adding Kjeldahl Cu 3.5 and 12 mL of concentrated H_2SO_4, and digesting the mixture for 1 hour at 420 °C. After cooling for 10-20 minutes, the tubes were inserted into a distillation unit. Deionised water was added to the tubes, and 30 mL of the receiver solution was placed in a conical flask. The contents were then treated with 50 mL of 40% NaOH and distilled for approximately 5 minutes. The distillate was titrated with 0.2 N HCl to a blue-grey endpoint, and the volume of acid consumed was recorded (AOAC, 2006).

Calculation:

$$\% \text{ protein} = \frac{(T-B) \times N \times 14{\cdot}007 \times 100 \times F}{W_1}$$

W_1 = sample weight (mg)

T = titration volume of the sample (ml)

B = titration volume of blank (ml)

N = normality of acid to 4 decimal places

F = conversion factor for nitrogen to protein

Fat determination

The Rose-Gotish gravimetric method, as described by AOAC (2000), was employed. This method processed five grams of the sample using various petroleum-based fat solvents in a Rose-Gotish apparatus until the oil fat was extracted entirely. The weight of the extracted fat was then calculated.

Calculation:

% fat = (W3 – W2) ÷ W1 × 100

W1 = Weight of the sample (g)

W2 = Empty extraction cup weight (g)

W3 = Extraction cup + residue weight (g)

Ash determination

The process involved drying empty crucibles in an oven at 130 ± 15ºC, cooling them in a desiccator, and recording their weight as W0. Next, 5.00g of the sample was weighed into each crucible (W1). The samples were ashed in a furnace at 500 ± 15ºC for 3 hours. After ashing, the crucibles were cooled in the furnace for 30 minutes and then transferred to a desiccator, where they were allowed to cool at room temperature for an additional 45 minutes. Finally, the weight of each crucible with its content was recorded as W2 to determine the sample's ash content (AOAC, 2006).

Calculation:

% Ash content = (W2 – W0) ÷ W1 × 100

Fiber determination

The AOAC (2006) method was used. The crude fibre content was calculated as follows;

Acid detergent fiber (ADF) % = 100 × (B + A – B) ÷ C

Where:

A = weight of residue

B = weight of pan

C = weight of fermented sample

Determination of anti-nutrients

The presence of anti-nutritive compounds was determined for unfermented soymilk and laboratory-fermented soy milk.

Phytic acid

To determine phytate content, 500-700 mL of the starter-produced soymilk yoghurt and spontaneously fermented soymilk was extracted in 50 mL of 3% TCA. The suspension was centrifuged, and a 10-mL aliquot of the supernatant was heated with $FeCl_3$. The resulting precipitate was washed and then treated with NaOH, followed by heating and filtration. The precipitate was dissolved in HNO_3 and diluted to 100 mL. A 5-mL aliquot was mixed with KSCN, and the colour was measured at 480 nm using a spectrophotometer (AOAC, 2000).

Calculation:

Curve the micrograms of iron present in the test from the calibration

curve, and calculate the phytate P as per the following equation:

Phytate P mg/100 g sample = Fe (µg) × 15 / Weight of sample in g

Tannin

To analyse phenol content, 250 mg of the sample was mixed with 25 mL of 70% acetone and sonicated for 20 minutes. The mixture was centrifuged, and the supernatant was collected. The pellet was treated again with acetone and sonicated; the supernatant was then collected.

For phenol analysis, 500 µL of the extract was mixed with Folin-Ciocalteu reagent and sodium carbonate, diluted with water, vortexed, and the absorbance was measured at 725 nm after 40 minutes. Total phenol content was calculated using a standard curve created with known concentrations of phenol standard solutions (Makkar *et al*., 1993).

Alkaloids

To extract alkaloids, 5g of the sample was mixed with 200 mL of 10% acetic acid in ethanol and left for 4 hours. The mixture was then filtered, and the filtrate was evaporated to one-quarter of its volume. Concentrated NH_4OH was added to precipitate the alkaloids, which were filtered using pre-weighed filter paper (w1). The filter paper with the precipitate was dried at 60°C until a constant weight (w2) was reached. The difference in weight (w2-w1) represented the alkaloid content (Harborne, 1984).

Sensory evaluation

The organoleptic properties of the laboratory-produced soy yoghurt were tested to test product acceptability. The organoleptic properties of the produced soy yoghurt were assessed by a ten-member panel familiar with consuming commercially produced yoghurt, employing a 9-point hedonic scale method that varied from 9, signifying 'Like Extremely', to 1, signifying 'Dislike Extremely'. Individuals were asked to examine and assess the Starter-produced soy yoghurt sample singly, indicating the extent of preference for the samples provided on the survey form. The sample was evaluated for parameters, such as appearance, texture, flavour, aroma, pungency, and general acceptability (Obi *et al*., 2023).

RESULTS

Forty LAB isolates were identified as *Lactiplantibacillus plantarum* (11), *Lactococcus lactis* (3), *Lactobacillus acidophilus* (16), and *Limosilactobacillus fermentum* (10) from the food samples such as *wara, ogi,* and yoghurt. *Lactobacillus acidophilus* had the highest occurrence, while *Lactococcus lactis* had the lowest, as shown in Figure 1.

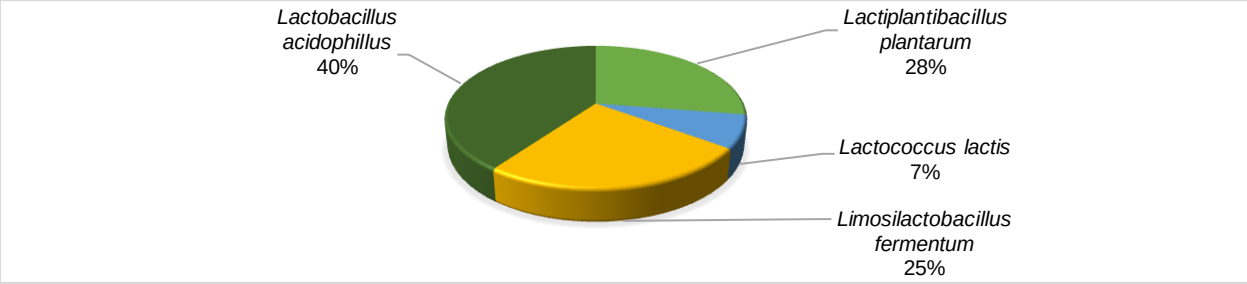

Fig. 1. Percentage occurrence of LAB isolated from *Ogi*, *Wara*, and Yoghurt

All LAB isolates were Gram-positive, rod and coccoid, creamy-white colonies, catalase-negative, non-sporing, citrate-negative, KOH-negative, non-hemolytic, and amylase-negative. Most isolates were heterofermentative, with a few homofermentative. They tolerated salinity levels of 2.5% and 4.5%, with minimal tolerance at 6.5% NaCl. Few strains could tolerate pH levels of 2 and 3, with maximum growth at pH 4 and 5 (Table 2).

Table 2. Morphological, Biochemical, and Physiological Characteristics of LAB isolated from Ogi, Wara, and Yoghurt.

Isolate code	Colony morphology	Cell morphology	Gram stain	Catalase	KOH	Spore staining	Motility	Citrate	Starch Hydrolysis	Hemolysis	2.5% NaCl	4.5%	6.5%	pH 2	pH 3	pH 4
IS01	Medium, creamy-white	Short rods in a chain	+	_	_	_	_	_	_	_	++	+	W	+	+	+
IS02	Small, tiny, creamy-white	Cocci in cluster	+	_	_	_	_	_	_	_	+	+	+	+	+	+
IS03	Medium, white, smooth-rough	Short rods	+	_	_	_	_	_	_	_	+	+	+	+	+	+
IS04	Small, round, creamy-white	Cocci in cluster	+	_	_	_	_	_	_	_	+	+	+	+	+	+
IS05	Small, tiny, creamy-white	Cocci in cluster	+	_	_	_	_	_	_	_	+	+	+	+	+	+
IS06	Moderate, round, creamy-white	Long rods	+	_	_	_	_	_	_	_	+	+	+	W	+	+
IS07	Medium, round, creamy-white	Short rods in a chain	+	_	_	_	_	_	_	_	+	+	W	+	+	+
IS08	Medium, white, smooth-rough	Short rods	+	_	_	_	_	_	_	_	+	+	+	+	+	+
IS09	Small, tiny, creamy-white	Cocci in cluster	+	_	_	_	_	_	_	_	+	+	+	+	+	+
IS10	Medium, white, smooth-rough	Short rods	+	_	_	_	_	_	_	_	+	+	+	+	+	+
IS11	Medium, round, creamy-white	Long rods, in chains or singly	+	_	_	_	_	_	_	_	+	+	+	W	+	+
1S12	Moderate round, creamy-white	Long rods	+	_	_	_	_	_	_	_	+	+	+	+	+	+
IS13	Medium, round, creamy-white	Short rods in a chain or single	+	_	_	_	_	_	_	_	+	+	+	+	+	+
IS14	Medium, white, smooth-rough	Short rods	+	_	_	_	_	_	_	_	+	+	+	+	+	+
IS15	Medium, white, smooth-rough	Short rods	+	_	_	_	_	_	_	_	+	+	+	+	+	+
IS16	Small, tiny, creamy-white	Cocci in cluster	+	_	_	_	_	_	_	_	+	+	+	+	W	+
IS17	Moderate, round, creamy-white	Long rods	+	_	_	_	_	_	_	_	+	+	+	W	+	+
IS18	Medium, round, creamy-white	Short rods in a chain	+	_	_	_	_	_	_	_	+	+	+	W	+	+
IS19	Medium, white, smooth-rough	Short rods	+	_	_	_	_	_	_	_	+	+	+		+	+
IS20	Medium, white, smooth-rough	Cocci in cluster	+	_	_	_	_	_	_	_	+	+	+	+	+	+
IS21	Medium-sized, round, creamy-white	Short rods in a chain or singly	+	_	_	_	_	_	_	_	+	+	+	+	+	+
IS22	Medium-sized, round, creamy-white	Short rods in a chain or singly	+	_	_	_	_	_	_	_	+	+	+	+	+	+
IS23	Medium-sized, white, smooth-rough	Short rods	+	_	_	_	_	_	_	_	+	+	+	W	W	+
IS24	Medium-sized, white, smooth-rough	Short rods	+	_	_	_	_	_	_	_	+	+	+	+	+	+
IS25	Medium-sized, round, creamy-white	Short rods in a chain or singly	+	_	_	_	_	_	_	_	+	+	+	+	+	+
IS26	Small-tiny, creamy-white	Short rods in a chain or singly	+	_	_	_	_	_	_	_	+	+	+	+	++	++
IS27	Medium-sized, round, creamy-white	Short rods in a chain or singly	+	_	_	_	_	_	_	_	+	+	+	+	+	+
IS28	Small-tiny, creamy-white	Cocci in cluster	+	_	_	_	_	_	_	_	+	+	+	+	+	+
IS29	Medium-sized, round, creamy-white	Short rods in a chain or singly	+	_	_	_	_	_	_	_	+	+	+	+	+	+
IS30	Moderate, round, creamy-white	Long rods	+	_	_	_	_	_	_	_	+	+	+	+	+	+
IS31	Medium-sized, round, creamy-white	Short rods in a chain or singly	+	_	_	_	_	_	_	_	+	+	+	W	+	+
IS32	Medium-sized, white, smooth-rough	Short rods	+	_	_	_	_	_	_	_	+	+	+	+	+	+
IS33	Moderate, round, creamy-white	Long rods	+	_	_	_	_	_	_	_	+	+	+	+	+	+
IS34	Medium-sized, round, creamy-white	Short rods in a chain or singly	+	_	_	_	_	_	_	_	+	+	+	+	+	+
IS35	Medium-sized, round, creamy-white	Short rods in a chain or singly	+	_	_	_	_	_	_	_	+	+	+	+	+	+
IS36	Small, tiny, creamy-white	Cocci in cluster	+	_	_	_	_	_	_	_	+	+	+	+	+	+
IS37	Medium-sized, round, creamy-white	Short rods in a chain or singly	+	_	_	_	_	_	_	_	+	+	+	W	W	+
IS38	Medium, creamy-white	Short rods in a chain	+	_	_	_	_	_	_	_	+	+	+	+	+	+
IS39	Small, tiny, creamy-white	Cocci in cluster	+	_	_	_	_	_	_	_	+	+	+	+	+	+
IS40	Medium, white, smooth-rough	Short rods	+	_	_	_	_	_	_	_	+	+	+	W	+	+

A sugar fermentation test was conducted to differentiate species, showing that all LAB moderately utilised glucose, mannitol, sucrose, lactose, and fructose. However, there were variations in the utilisation of sorbose, sorbitol, mannose, arabinose, and xylose, as detailed in Table 3.

Table 3. Sugar fermentation profile of LAB isolated from Ogi, Wara, and Yoghurt.

Isolate code	Sucr	Fruc	Mal	Mann	Lact	Xyl	Glu	Sorb	Man	Ara	CO_2 production	Probable organism
IS01	+	+	+	+	+	-	+	-	+	+	+	*Lactobacillus fermentum*
IS02	+	+	+	+	+	-	+	-	+	+	-	*Lactobacillus acidophilus*
IS03	+	+	+	+	-	+	+	-	-	+	-	*Lactobacillus plantarum*
IS04	+	+	+	+	-	+	+	-	+	+	+	*Lactobacillus lactis*
IS05	+	+	+	+	-	-	+	-	-	+	+	*Lactococcus lactis*
IS06	+	+	+	+	+	+	+	-	+	+	+	*Lactobacillus fermentum*
IS07	+	+	+	+	-	+	+	-	+	+	+	*Lactobacillus plantarum*
IS08	+	+	+	+	+	-	+	-	+	+	-	*Lactobacillus acidophilus*
IS09	+	+	+	+	+	-	+	-	+	+	-	*Lactobacillus acidophilus*
IS10	+	+	+	+	-	+	+	-	+	+	+	*Lactobacillus fermentum*
IS11	+	+	+	-	+	+	+	-	+	-	+	*Lactobacillus plantarum*
IS12	+	+	+	+	-	+	+	-	-	+	+	*Lactobacillus fermentum*
IS13	+	+	+	-	+	+	+	-	-	+	-	*Lactobacillus acidophilus*
IS14	+	+	+	-	+	+	+	-	-	-	-	*Lactobacillus acidophilus*
IS15	+	+	+	-	-	+	+	-	-	-	+	*Lactobacillus acidophilus*
IS16	+	+	+	-	-	+	+	-	-	-	+	*Lactobacillus plantarum*
IS17	+	+	+	-	+	+	+	-	+	+	-	*Lactobacillus acidophilus*
IS18	+	+	+	-	+	+	+	-	-	+	-	*Lactobacillus acidophilus*
IS19	+	+	+	-	-	+	+	-	-	+	+	*Lactobacillus plantarum*
IS20	+	+	+	+	-	+	+	-	+	+	+	*Lactococcus lactis*
IS21	+	+	+	-	-	+	+	-	-	+	+	*Lactobacillus plantarum*
IS22	+	+	+	-	-	-	+	-	-	+	+	*Lactobacillus plantarum*
IS23	+	+	+	+	+	+	+	-	+	+	+	*Lactobacillus fermentum*
IS24	+	+	+	-	-	+	+	-	-	+	+	*Lactobacillus plantarum*
IS25	+	+	+	+	+	-	+	-	+	+	+	*Lactobacillus fermentum*
IS26	+	+	+	+	-	+	+	-	+	+	+	*Lactobacillus fermentum*
IS27	+	+	+	+	+	+	+	-	-	+	+	*Lactobacillus plantarum*
IS28	+	+	+	-	+	+	+	-	+	-	-	*Lactobacillus acidophilus*
IS29	+	+	+	-	-	+	+	-	-	-	-	*Lactobacillus acidophilus*
IS30	+	+	+	+	-	+	+	-	-	-	+	*Lactobacillus fermentum*
IS31	+	+	+	+	-	+	+	-	-	-	+	*Lactobacillus plantarum*
IS32	+	+	+	-	-	+	+	-	-	+	+	*Lactobacillus fermentum*
IS33	+	+	+	-	+	+	+	-	-	+	-	*Lactobacillus acidophilus*
IS34	+	+	+	-	+	-	+	-	+	+	-	*Lactobacillus acidophilus*
IS35	+	+	+	-	+	+	+	-	-	+	-	*Lactobacillus acidophilus*
IS36	+	+	+	+	+	+	+	-	-	+	+	*Lactobacillus fermentum*
IS37	+	+	+	-	+	+	+	-	+	+	-	*Lactobacillus acidophilus*
IS38	+	+	+	-	+	+	+	-	-	+	-	*Lactobacillus acidophilus*
IS39	+	+	+	+	+	+	+	-	-	+	+	*Lactobacillus plantarum*
IS40	+	+	+	-	+	+	+	-	+	+	-	*Lactobacillus acidophilus*

Figure 2 shows that optimal growth for *Lb. fermentum* and *Lb. plantarum* was at 35°C, *Lb. fermentum* showed minimal growth at 25°C *and maximum growth* at 45°C. *Lb. plantarum* had maximum growth at 35°C and minimal at 45°C. *Lb. acidophilus* (IS37) and (IS40) both showed maximum growth at 35°C, moderate growth at 45°C, and minimal growth at 25°C.

Lb. fermentum showed maximum growth at pH 3 and 5 and minimal growth at pH 2 and 4. *Lb. plantarum* exhibited weak growth at pH 2 and 3 and maximum growth at pH 4 and 5. *Lb. acidophilus* (IS40) showed maximum growth at pH four and moderate growth at pH 3, and minimal growth at pH 2. *Lb. acidophilus* (IS37) grew best at pH 5, moderately at pH 4, and minimally at pH 2 and 3 (Figure 3).

The LAB strains showed maximum growth at 0.8% bile salt concentration, with *Lb. plantarum* exhibiting higher growth than *Lb. fermentum*. Control samples without bile salts showed the highest growth rates, as represented in Figure 4.

The percentage of lactic acid produced by LAB strains over 24, 48, and 72 hours is shown in Figure 5.

Figure 6 shows the diacetyl production by LAB strains at 24, 48, and 72 hours. *Lb. plantarum* had the lowest percentage at 24 hours, while *Lb. fermentum* had the highest at 72 hours. *Lb. acidophilus* (IS37) and *Lb. acidophilus* (IS40) followed a similar trend. Diacetyl production decreased in all strains after 72 hours.

The isolated LAB strains, *Lb. plantarum* (IS11), *Lb*. *fermentum* (IS26), *Lb*. *acidophilus* (IS37) and *Lb*. *acidophilus* (IS40) exhibited auto-aggregation and hydrophobic properties. The adhesion capacity varied among the species. The highest auto-aggregation percentage was observed in *Lb. plantarum,* and the lowest in *Lb. fermentum* (IS26). The maximum hydrophobicity was recorded in *Lb*. *plantarum* and the minimum percentage in *Lb. fermentum*. *Lb*. acidophilus revealed a slightly low percentage of auto-aggregation properties; while moderate auto-aggregation and hydrophobicity were exhibited by *Lb*. *acidophilus* (IS40) (Figure 7).

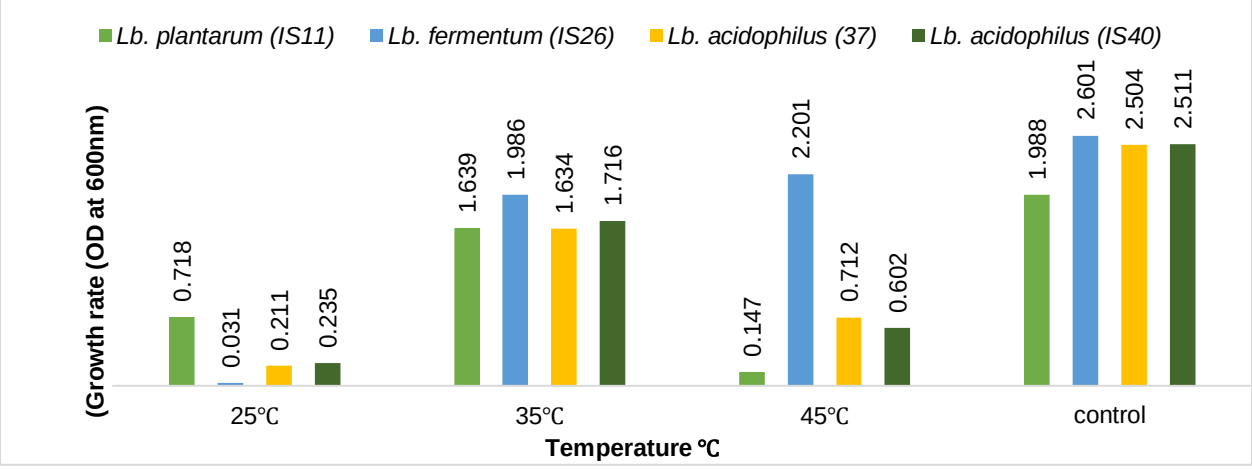

Fig. 2. Effect of temperature on the growth of *Lb. plantarum, Lb. fermentum*, and *Lb*. *acidophilus* isolated from *Ogi*, *Wara*, and Yoghurt.

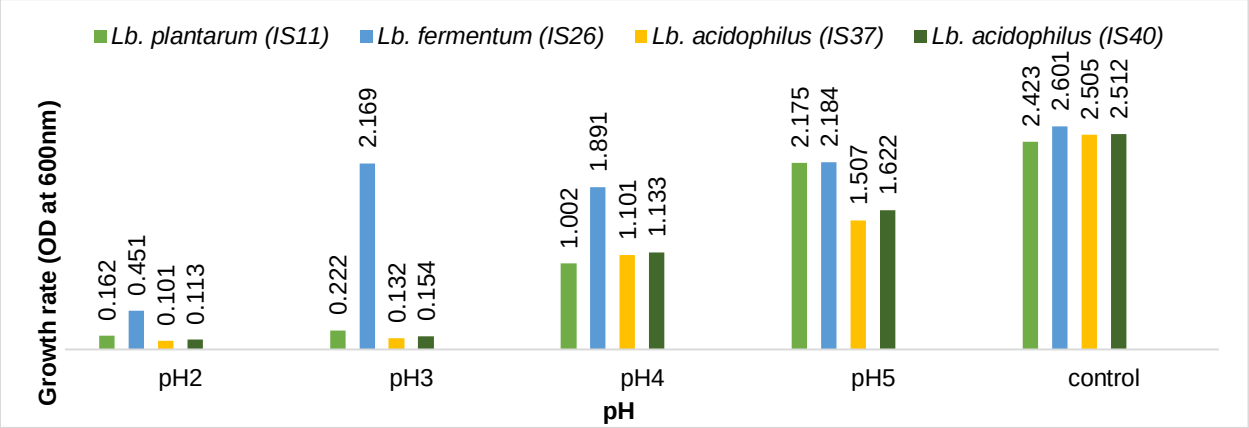

Fig. 3. Growth rate of *Lb. plantarum*, *Lb. fermentum*, and *Lb. acidophilus* isolated from *Ogi*, *Wara*, and Yoghurt at different pH levels.

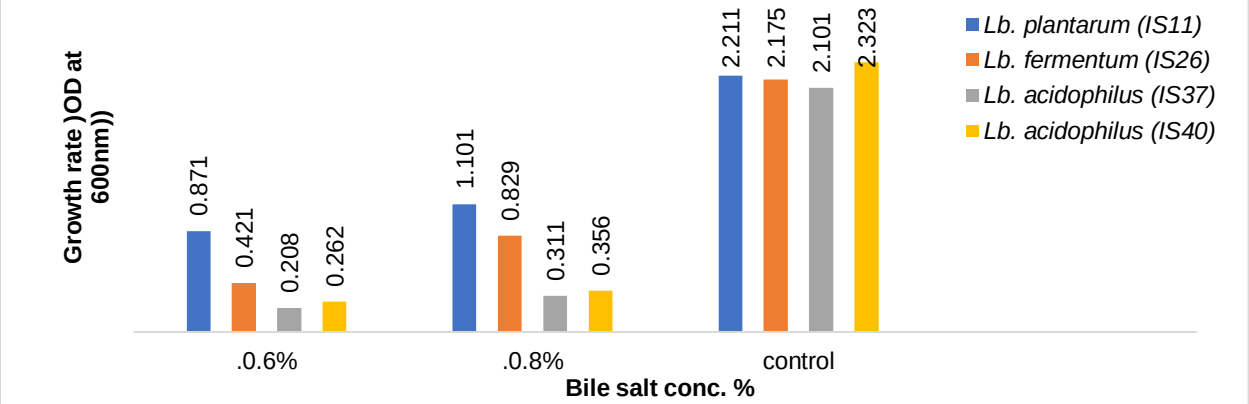

Fig. 4. Bile salt tolerance of *Lb. plantarum*, *Lb. fermentum*, and *Lb. acidophilus* isolated from *Ogi*, *Wara*, and Yoghurt.

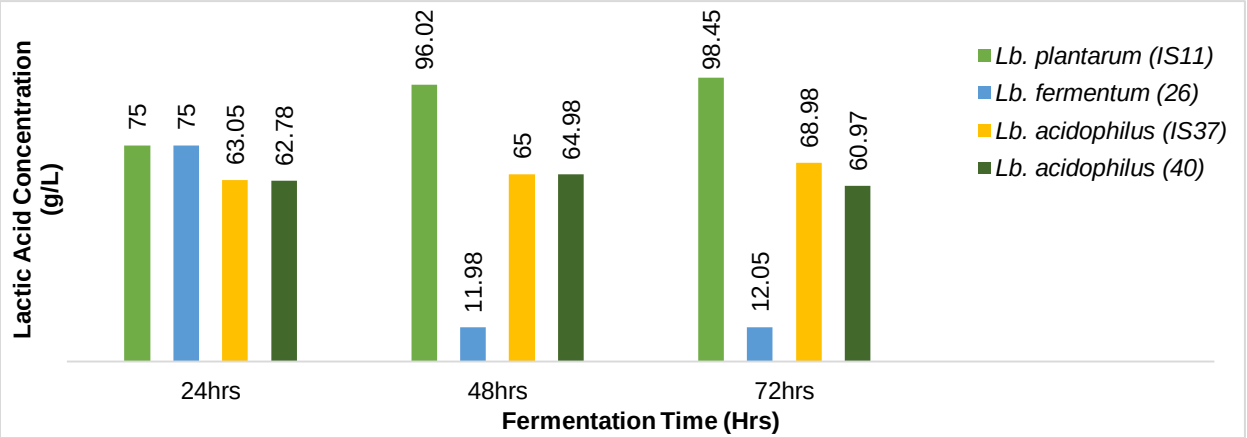

Fig. 5. Concentration of lactic acid production by *Lb. plantarum*, *Lb. fermentum*, and *Lb. acidophilus* isolated from *Ogi*, *Wara*, and Yoghurt.

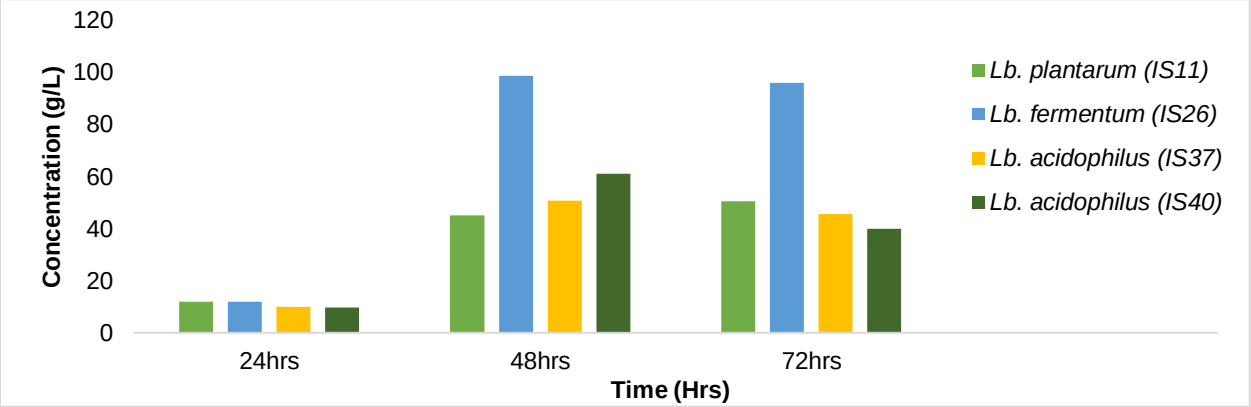

Fig. 6. Quantity of Diacetyl produced by *Lb. plantarum*, *Lb. fermentum*, and *Lb. acidophilus* isolated from *Ogi*, *Wara*, and Yoghurt.

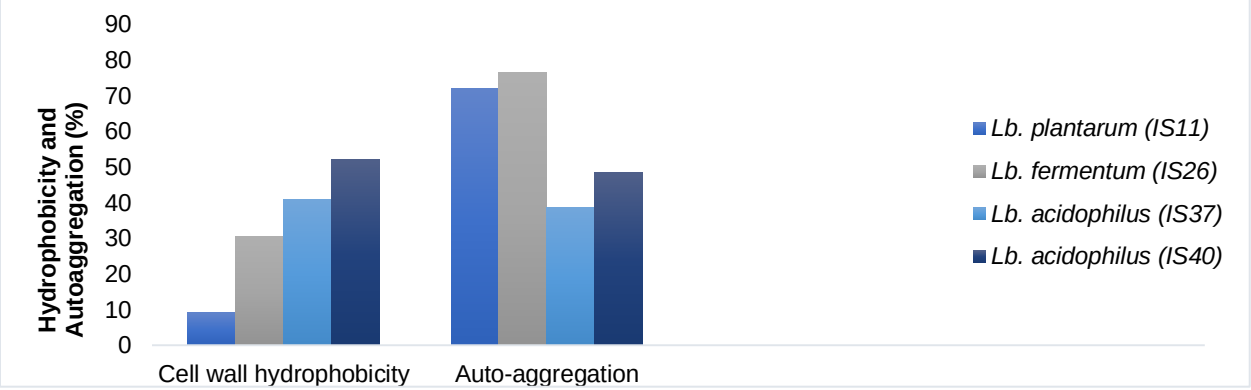

Fig. 7. Auto-aggregation and cell wall hydrophobicity properties of *Lb. fermentum, Lb. plantarum*, and *Lb. acidophilus* isolated from *Ogi*, *Wara*, and Yoghurt.

Table 4 represents the antibiotic susceptibility patterns of the LAB isolates (*Lb. fermentum* (IS26), *Lb. plantarum* (IS11), *Lb. acidophilus* (IS37), and *Lb. acidophilus* (IS40)) subjected to different concentrations of antibiotics and their zone of clearance recorded in (mm). All tested LAB isolates were susceptible to the antibiotics used (erythromycin, ampicillin, imipenem, and gentamicin) with varying zones of inhibition measured in mm.

Table 5 represents the data from NCBI Blast showing the sequence identity of the isolates' edited sequences.

During the fermentation of soy milk with varying ratios and volumes of *Lb. plantarum* and *Lb. fermentum*, pH was recorded at 0, 2, 4, 6, and 8 hours. The initial pH was 6.8, dropping to 4.4 after 8 hours due to the metabolic activities of the LAB isolates (Figure 8).

Table 6 shows significant differences ($p < 0.05$) in water-holding capacity between the starter-produced and the spontaneously fermented soymilk yoghurt. The highest water-holding capacity was in starter-produced yoghurt with a 2:2 combination. The lowest was in a 1:1 combination.

Table 4. Antibiotic susceptibility patterns of *Lb. plantarum*, *Lb. acidophilus* and *Lb. fermentum* isolated from Ogi, Wara, and Yoghurt.

Isolate name	Antibiotic disks	Paper content (µg/piece)	Diameter of the zone of inhibition	Antimicrobial susceptibility type
Lb. plantarum (IS11)	Ampicillin	10	35	S
	Imipenem	10	40	S
	Gentamicin	10	39	S
	Erythromycin	15	38	S
Lb. fermentum (IS26)	Ampicillin	10	21	S
	Imipenem	10	35	S
	Gentamicin	10	34	S
	Erythromycin	15	38	S
Lb. acidophilus (IS37)	Ampicillin	10	13	S
	Imipenem	10	43	S
	Gentamicin	10	40	S
	Erythromycin	15	34	S
Lb. acidophilus (IS40)	Ampicillin	10	36	S
	Gentamicin	10	43	S
	Imipenem	10	38	S
	Erythromycin	15	32	S

Key: S= sensitive, µg= microgram

Table 5. Sequencing confirmed the identity of isolates as Lb. fermentum and Lb. plantarum from Ogi, Wara, and Yoghurt samples.

Sample ID	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Sample 26	*Limosilactobacillus fermentum*	2329	2329	100%	0	99.76%	PP417818
Sample 11	*Lactiplantibacillus plantarum*	2274	2274	99%	0	99.84%	PP417819

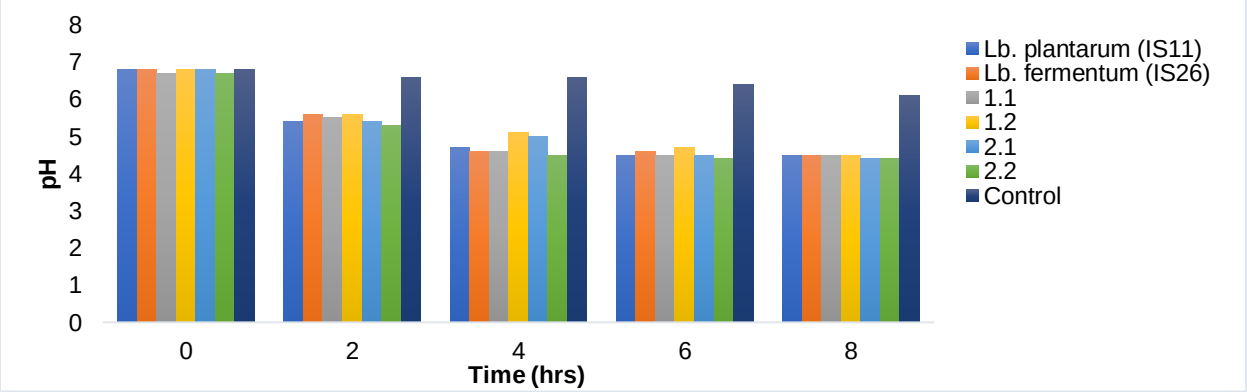

Fig. 8. Changes in pH during the fermentation of soymilk with *Lb. plantarum* and *Lb. fermentum* isolated from *Ogi*, *Wara*, and Yoghurt.

Table 6. Water-holding capacity of starter-produced Soymilk-Yoghurt using the selected starters (*Lb. plantarum* and *Lb. fermentum*) isolated from Ogi, Wara, and Yoghurt.

	Storage time (hrs)		
Ratio	**24**	**48**	**72**
Lb. plantarum	85.85±0.07^{a}	83.56±0.07^{b}	81.50±56.43^{a}
Lb. fermentum	89.25±0.70^{a}	86.45±007^{a}	84.55±0.07^{a}
1:1	81.30±10.18^{a}	72.10±0.14^{d}	70.65±0.07^{a}
1:2	84.70±0.14^{a}	83.20±0.28^{b}	80.55±0.07^{a}
2:1	83.65±0.21^{a}	82.15±0.07^{c}	81.70±0.14^{a}
2:2	89.65±0.21^{a}	86.40±0.28^{a}	84.90±0.14^{a}
Control	70.75±0.35^{b}	70.05±0.07^{e}	68.45±0.35^{a}

Means along the rows with distinct superscripts substantially differ from each other at α = 0.05

Key: *Lb. plantarum*: *Lactiplantibacillus plantarum*, *Lb. fermentum*: *Limosilactobacillus fermentum*, Control: (spontaneously fermented soymilk)

Ratio 1:1, 1:2, 2:1, and 2:2: combinations of *Lactiplantibacillus plantarum* and *Limosilactobacillus fermentum*

Figure 9 shows that syneresis increases with more extended storage periods. Significant differences ($p < 0.05$) were observed in the whey separation process. The spontaneously fermented soymilk displayed the highest whey separation. The lowest whey separation was in starter-produced soymilk yoghurt with a 2:2 combination.

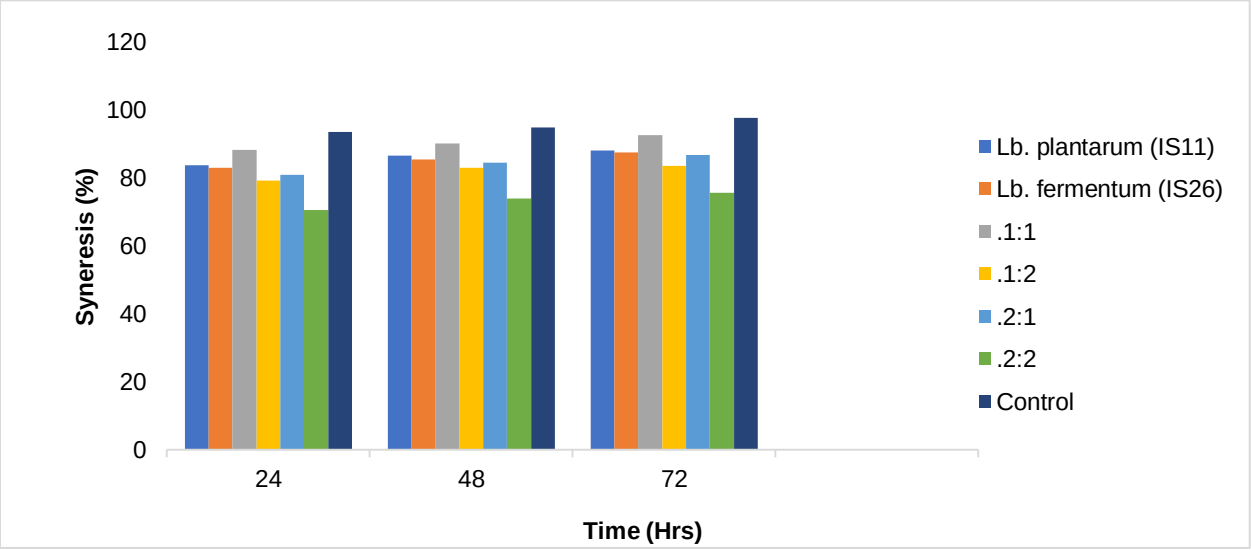

Fig. 9. Syneresis of the Soymilk Yoghurt using the selected starters (*Lb. plantarum* and *Lb. fermentum*) isolated from *Ogi*, *Wara*, and Yoghurt.

The viscosity data obtained from the analysis of variance showed significant differences ($p<0.05$) between the starter-produced Soymilk Yoghurt and the spontaneously fermented soymilk, as shown in Table 7.

The results obtained from the proximate composition of laboratory-produced Soymilk Yoghurt using the starters (*Lb*. *plantarum* and *Lb*. *fermentum*), spontaneously fermented soymilk, and cow milk yoghurt showed substantial variations as regards the protein content, ash content, fat content, moisture content, fibre content and total carbohydrate content. The highest value of protein content, fat content, and ash content was observed in the cow milk yoghurt compared to the spontaneously fermented soymilk and starter-produced soymilk yoghurt. In contrast, the highest fibre content and carbohydrate content were observed in the Starter-produced soymilk yoghurt using the selected starters compared to the cow milk yoghurt and spontaneously fermented soymilk. In contrast, the highest moisture content was recorded in the spontaneously fermented soymilk compared to the cow milk yoghurt and starter-produced soymilk yoghurt. The results are represented in Table 8.

Table 7. The viscosity of starter-produced Soymilk Yoghurt using the selected starters (*Lb. plantarum* and *Lb. fermentum*) isolated from Ogi, Wara, and Yoghurt.

Sample	Viscosity (MPA's)
Lb. *plantarum*	101.00 ± 1.41^{c}
Lb. *fermentum*	141.00 ± 1.41^{b}
1:1	202.50 ± 3.54^{a}
1:2	80.00 ± 0.00^{d}
2:1	59.00 ± 1.41^{e}
2:2	50.00 ± 1.41^{f}

Means along the rows with distinct superscripts are substantially distinct from each other at $\alpha = 0.05$

Key: *Lb*. *plantarum*: *Lactiplantibacillus plantarum*, *Lb*. *fermentum*: *Limosilactobacillus fermentum*.

Table 8. Proximate Composition of Starter-produced Soymilk Yoghurt.

Sample	Moisture	Protein	Fat	Fiber	Ash	CHO
Produced Soymilk Yoghurt	85.25 ± 0.35^{c}	5.34 ± 0.09^{ab}	0.04 ± 0.00^{c}	0.37 ± 0.03	0.34 ± 0.02^{b}	9.13 ± 0.36^{a}
Cow milk Yoghurt	87.9 ± 0.14^{b}	5.82 ± 0.25^{a}	0.92 ± 0.02^{a}	0.00 ± 0.00	1.22 ± 0.04^{a}	4.15 ± 0.42^{b}
Spontaneous fermented soymilk (control)	90.40 ± 0.14^{a}	$4.98\pm o.64^{b}$	0.36 ± 0.06^{b}	0.33 ± 0.02	0.11 ± 0.02^{c}	4.16 ± 0.00^{b}

Means along the rows with distinct superscripts are substantially distinct from each other at $\alpha = 0.05$

Key: Control: (spontaneously fermented soymilk), CHO: carbohydrate

Table 9 represents the anti-nutrient composition of the starter-produced Soymilk Yoghurt and spontaneously fermented soymilk. The utilisation of the joined starters *Lb*. *fermentum*, and *Lb*. *plantarum* in Soymilk Yoghurt production recorded the lowest anti-nutrient components (tannin, phytate, and alkaloids) in contrast to the spontaneously fermented soymilk (control). In correspondence to the outcome of the analysis of variance, the anti-nutrient composition of the spontaneously fermented soymilk (control) recorded the highest anti-nutrient components, which showed significant differences in the anti-nutrient composition of the starter-produced Soymilk Yoghurt. The lowest tannin, phytate, and alkaloids were observed in the starter-produced Soymilk Yoghurt compared to the spontaneously fermented soymilk (control).

Figure 10 shows the organoleptic properties of the soymilk yoghurt produced using the LAB starters. The outcome of the variance analysis disclosed remarkable changes in mean scores of preferences (flavour, appearance, aroma, pungency, and general overall acceptability) in the starter-produced soymilk Yoghurt. The highest average score of first choice (appearance, flavour, aroma, pungency, and general acceptability) was observed in the starter-produced Soymilk Yoghurt. In contrast, the lowest mean score of preferences (appearance, flavour, aroma, pungency, and general acceptability) was recorded from the spontaneously fermented soymilk.

Table 9. Anti-nutrient composition of starter-produced Soymilk Yoghurt.

Sample	Alkaloid (%)	Phytate (%)	Tannin (%)
Produced Soymilk Yoghurt	1.39±0.01[a]	98.25±0.08[a]	208.15±2.62[a]
Spontaneously fermented soymilk	1.82±0.05[b]	108.98±0.03[a]	384.00±1.41[b]

Means along the rows with distinct superscripts are substantially different from each other at α = 0.05

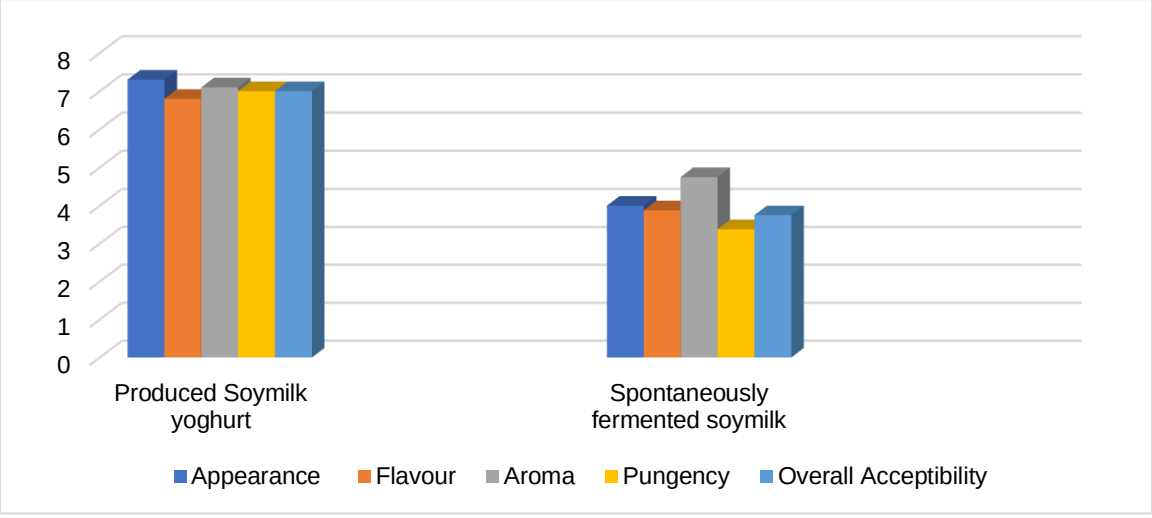

Fig. 10. Organoleptic properties of starter-produced Soymilk Yoghurt.

DISCUSSION

In this study, different species of LAB were isolated from yoghurt, maize gruel (*Ogi*), and *wara*. Lactic acid bacteria are often isolated from different fermented food sources (Bansal *et al*., 2013). Adesulu-Dahunsi et al. (2022) recorded the occurrence of LAB species in various fermented foods, and their report presented *Lactobacillus* as the most prevalent.

Lactic acid bacteria isolated from *Ogi*, *Wara*, and Yoghurt were identified as *Lactobacillus acidophilus*, *Limosilactobacillus fermentum*, *Lactiplantibacillus plantarum*, as well as *Lactococcus lactis* using morphological, biochemical, and molecular techniques. The predominant LAB species isolated was *Lactobacillus acidophilus*.

Olasupo et al. (1997) reported isolating *Lb. plantarum*, *Lb. fermentum*, *L. lactis*, and *Lb. acidophilus* from *Wara* and *Ogi*, noting that *Lb. acidophilus* was the most dominant LAB from indigenous fermented foods. This agrees with the results of this research, which also found *Lb. acidophilus* to be the most predominant LAB.

The physiological response of the tested LAB to acid stress at pH 2, 3, 4, and 5 showed that the growth of the tested LAB gradually reduced as the pH decreased. LAB are neutrophils, which are capable of growing optimally in a pH range from 5 to 9. In this study, *Lb*. *fermentum* was more tolerant to low pH (pH 2 and 3) than other LAB isolates. *Lb*. *fermentum* is recognised to survive acidic environments. For example, two different strains of *Lb*. *fermentum* could survive an acidic pH of 4-5 (Chaka, 2020). These traits enable strains of *Lb*. *fermentum* to take part in the latter phase of spontaneous fermentation of food produce (Olasupo *et al*., 1997).

In this research, *Lb*. *plantarum* and *Lb*. *fermentun* isolated from *Ogi*, *Wara*, and Yoghurt produced a relatively high amount of lactic acid and diacetyl in the growth medium. Lactic acid, classified as an organic acid, is recognised as safe by the U.S. Food and Drug Administration under the GRAS (generally regarded as safe) designation (Abedi & Hashemi, 2020). Lactic acid bacteria are capable of fermenting carbohydrates to generate lactic acid, which makes them an important part of the food sector (Wang *et al*., 2021). The highest lactic acid production was from *Lb*. *fermentum* (1.00g/mL) after 72 hours of fermentation. Similar results have been reported from studies conducted by Fu and Mathews (1999), where a synthetic lactose medium was used in culturing *Lb*. *plantarum* for the production of lactic acid.

Some *lactobacilli* have shown a correlation between their adhesion capability and hydrophobicity (Kos *et al*., 2003). From the results of this study, *Lb*. *plantarum* exhibited a high auto-aggregative percentage compared to *Lb*. *fermentum*. Adhesion capacity is an important criterion for the selection of probiotic LAB strains. These findings are in agreement with the study conducted by Tuo et al. (2013) on the aggregation and adhesion properties of *Lb*. *plantarum*.

The participation of LAB as a starter in soybean milk fermentation agreed with Li et al. (2021), who found *Lb. plantarum* is involved in acidification in soymilk fermentation. This process increases acidification, promoting soymilk yoghurt production (Chaka, 2020). Sharma et al. (2020) noted that LAB produces lactic acid, lowering the pH and inhibiting harmful bacteria during fermentation.

A meaningful reduction in pH and a concurrent increase in acidity in the substrate during the fermentation using *Lb*. *plantarum* and *Lb*. *fermentum* in the production of soymilk yoghurt was observed. Previously, Ogunbanwo et al. (2013) reported a considerable decline in the pH and a concomitant rise in the medium's acidity when fermenting sorghum grains with *Lb*. *fermentum* for the production of *Burukutu*.

Variations in yoghurt's rheological characteristics are linked to milk's chemical composition, primarily total solids and protein content (Kaur & Riar, 2020). Previous studies have examined the viscosity of dairy and non-dairy yoghurts using viscometers or rheometers (Lee & Lucey, 2010).

The viscosity of starter-produced soy milk yoghurt significantly decreased when *Lb. plantarum* and *Lb. fermentum* was combined in a 2:1 ratio compared to other combinations.

Results from the syneresis of the starter-produced soy milk yoghurt revealed an increase in syneresis at different hours of storage when the fermentation was done using the single starters and the combination of both. The ratio at which the starters were used significantly impacted the outcome of the yoghurt. This may be due to the differences in the production of exopolysaccharides from both starters. The results from this study align significantly with the study of Penna et al. (2006), where the total solids in milk significantly impacted the physical characteristics of yoghurt. Syneresis, or whey separation, occurs due to factors like low protein content (<3.4%), low fat, high mineral content in milk, and heating of the coagulum during or after incubation (Kılıç *et al*., 2022).

The water-holding capacity of soybean milk is influenced by the type of LAB used for its fermentation. *Limosilactobacillus fermentum* and *Lactiplantinacillus plantarum* enhance water retention in soymilk yoghurt by producing exopolysaccharides (EPS) and organic acids, respectively. Combining these LABs can synergistically improve water-holding capacity. Proteolytic activity and inoculation rate also affect water-holding capacity and syneresis (Arab *et al*., 2022).

The proximate analysis showed that using combined starters for Soymilk Yoghurt production decreased fat content compared to cow milk yoghurt and spontaneously fermented soymilk. This reduction is likely due to lipolytic enzyme activity during fermentation, consistent with Obi et al. (2023).

The high moisture content in the yoghurt samples indicates high water activity, promoting microbial growth but reducing shelf life. Therefore, starter-produced Soymilk Yoghurt should be consumed quickly and kept refrigerated to prevent spoilage. The highest moisture was found in the spontaneously fermented soymilk (control) compared to starter-produced Soymilk Yoghurt and cow milk yoghurt.

From this work, the result of the protein content using the combined starters significantly increased ($P \leq 0.05$) compared to the spontaneously fermented soymilk (control). These results suggest that such yoghurt could serve as a valuable protein source, potentially substituting animal protein, especially in rural regions where animal protein costs are elevated. This observation aligns with the conclusions drawn by Akoma et al. (2000), Bamishaiye and Bamishaiye (2011), and Gambo and Da'u (2014).

Notably, there was no significant difference in the fibre content of the starter-produced soymilk yoghurt and the spontaneously fermented soymilk (control, no introduction of starter culture) ($P \leq 0.05$). In contrast, no fibre content in the cow milk was found since it is of animal origin and not plant. Significant benefits of consuming dietary fibre include: control of body weight, thereby improving satisfaction (Kristensen & Jensen, 2011). This is also a notable advantage that yoghurts produced from tiger nut and soy milk possess in contrast to dairy yoghurt (Obi *et al*., 2023).

The cow milk yoghurt had the highest ash content (1.22) compared to the soymilk yoghurt with *Lb. fermentum* and *Lb. plantarum* (0.34) and spontaneously fermented soymilk (0.11), indicating higher mineral levels essential for bodily functions. This increase in ash content may result from microbial breakdown and mineralisation during fermentation (De *et al*., 2022).

The starter-produced soymilk yoghurt had significantly higher carbohydrate content (9.13) than spontaneously fermented soymilk (4.15) and cow milk yoghurt (4.16). This makes it a suitable energy source for lactose-intolerant individuals due to its lactose-free profile (Nelson *et al*., 1976).

Fermenting soymilk with Lb. fermentum and *Lb. plantarum* reduced anti-nutrient components (tannins, phytate, and total alkaloids) compared to the control. Anti-nutrients hinder nutrient absorption and protein breakdown, affecting the body's nutrient utilisation (Mueller-Harvey, 2006). Ogunbanwo et al. (2013) reported a decrease in anti-nutritional compounds such as polyphenols, phytate, and tannins in *Burukutu* when a combination of *Lactobacillus fermentum* and Saccharomyces cerevisiae was used in its production.

CONCLUSION

Lactic acid bacteria fermentation presents a promising approach to enhancing the nutritional profiles of plant-based yoghurts. By leveraging the metabolic activities of LAB, it is possible to produce plant-based yoghurts that are nutritious, palatable, and appealing to consumers. Production of soy-based yoghurt utilising both organisms as starters reduced anti-nutrients while improving the proximate composition and enhancing the organoleptic properties of the soy yoghurt. Future research should optimise fermentation conditions and explore a broader range of LAB strains to maximise nutritional benefits. Future studies should focus on optimising fermentation parameters, evaluating diverse LAB strains, and assessing their functional and probiotic properties to improve further the nutritional and health-promoting potential of soy-based and other plant-based yoghurts.

RECOMMENDATIONS

The use of selected LAB strains, particularly *Limosilactobacillus fermentum* and *Lactiplantibacillus plantarum*, is recommended for the production of soy-based yoghurt, as they significantly improve nutritional composition, reduce anti-nutritional factors, and enhance sensory qualities compared to spontaneous fermentation. Further application of these strains in large-scale production could provide a viable non-dairy alternative with improved consumer acceptability and health benefits.

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CONFLICT OF INTEREST

There is no conflict of interest among the authors regarding this publication.

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