

ENHANCING THE POTENTIAL OF PROBIOTIC BACTERIA TO IMPROVE LABNEH'S NUTRITIONAL VALUE

HANEEN NAYEF AL-TARAWNEH

Department of Food Science and Nutrition, Faculty of Agriculture, Jerash University, 26250 Jerash, Jordan.
Email: H.tarawneh@jpu.edu.jo

KHALED AHMAD AL-NAJJAR*

General Commission for Scientific Agricultural Research, GCSAR, 12573 Damascus, Syria.
*Corresponding Author Email: khnadj2011@yahoo.com

Abstract

Labneh is a popular probiotic-rich food, but adding probiotics while maintaining its texture is challenging. This study tested Labneh as a carrier for "*Lactobacillus acidophilus*" and "*Bifidobacterium spp.*". It examined their effects on chemical composition and sensory properties during storage at 5°C. Seven probiotic strains were tested, including "*Lactobacillus*", "*Bifidobacterium*", and "*Streptococcus thermophiles*". Pure cultures were isolated using serial dilution on Bromocresol Green Whey Agar (BGWA) under anaerobic conditions. Labneh was made using ultra-high temperature (UHT) skim milk with 2% active culture. Two groups were tested: probiotics alone and probiotics with "*S. thermophiles*" to speed up curd formation. The Labneh was strained and stored at 5°C. It was analyzed on days 0, 7, and 14 for bacterial count, pH, acidity, and salt content. Sensory evaluation used a 5-point scale with commercial Labneh as the control. Data were analyzed using one-way ANOVA. The results showed that all probiotic strains grew in cow's milk at 37°C. "*L. rhamnosus*" had the highest counts, while "*B. lactis*" and "*Bifidobacterium spp. 2*" failed to form curds. Straining increased probiotic counts to over 8 Log₁₀, with no significant changes during storage. Labneh met the Jordanian salt standard, with a pH of around 4.5. Adding "*S. thermophiles*" lowered pH and increased acidity. Sensory testing found "*L. acidophilus 1*" most acceptable, with other strains improving when "*S. thermophiles*" was included. Probiotic Labneh made with "*Lactobacillus*" and "*Bifidobacterium spp.*" maintains probiotic viability and meets standards for 14 days. "*L. acidophilus*" Labneh had the best sensory quality. Adding "*S. thermophiles*" improved less acceptable strains. BGWA was more effective than MRSA (de Man, Rogosa, Sharpe media) for bacterial enumeration.

Keywords: Fermented Milk, Curd Formation, Sensory Analysis, Refrigerated Storage, Bromocresol Whey Agar, De Man Rogosa Agar.

1. INTRODUCTION

Labneh, a Middle Eastern dairy product, is made by straining yogurt to remove whey. Traditional Labneh lasts up to two weeks, while firmer varieties can last longer [1]. Although cloth bags are still used for straining, modern mechanical methods are increasingly adopted for improved efficiency and quality.

With rising interest in functional foods, Labneh is gaining attention as a probiotic delivery vehicle. Naturally rich in lactic acid bacteria, it supports gut health, immunity, and cholesterol regulation [2]. Adding probiotic strains like "*Lactobacillus acidophilus*" and "*Bifidobacterium spp.*" could boost its nutritional value and shelf life [3].

Probiotic Labneh enhances traditional Labneh by adding probiotics for improved nutrition and taste. Modern techniques and strict hygiene practices address contamination risks and extend shelf life [4].

Labneh's high solids and fat content make it ideal for probiotic delivery, supporting digestion and immunity [5]. Modern methods like centrifugation and ultrafiltration enable large-scale production but challenge traditional texture and firmness [6].

Non-dairy probiotic products like fermented fruit, vegetable, and legume beverages are popular among those with lactose intolerance or seeking cholesterol-free options. Advances in food technology enable probiotics in juices, dried fruits, and fermented vegetables, providing added nutrients like vitamins, minerals, and fiber [7].

Probiotic chickpeas have been studied as a non-dairy probiotic carrier, maintaining viable counts $>10^7$ CFU/g for days under refrigeration, demonstrating potential as a functional food [8].

The most studies show growing interest in probiotic-rich dairy and non-dairy products for their health benefits. However, Labneh, a potential probiotic carrier, remains underutilized. While Labneh can deliver probiotics like "*Lactobacillus acidophilus*" and "*Bifidobacterium spp.*", research is limited. Despite advancements, maintaining Labneh's traditional texture and firmness remains challenging.

In Jordan, Mahasneh and Abbas [9] similarly highlight the role of *Lactobacillus* in fermented milk to improve gut health and reduce microbial toxins. Since then, the concept of probiotics has expanded to include beneficial bacteria such as *Lactobacillus*, *Bifidobacterium*, and *Saccharomyces boulardii* [10, 11, 12]. Moreover, households generally exhibit average food security, although food insecurity can influence household food spending and is impacted by factors such as the age and income of the head of the household [13].

Furthermore, probiotics offer various health benefits, including improved digestion, aid with lactose intolerance, enhanced immune function, and management of conditions like inflammatory bowel disease and high cholesterol [14]. In addition, dairy products, particularly yogurt, probiotic cheese, and supplements, remain primary sources of these beneficial microorganisms [15].

This study aims to develop a probiotic Labneh by exploring the feasibility of using traditional Labneh as a carrier for "*Lactobacillus acidophilus*" and selected "*Bifidobacterium spp.*". It also examines the impact of these probiotic additions on the chemical composition and sensory properties of Labneh during storage at 5°C.

2. MATERIALS AND METHODS

This study was conducted in the laboratories of the Faculty of Agriculture at Jerash University, Jordan, in 2023.

2.1 Probiotic Bacteria Used in Labneh Production

To develop probiotic Labneh, we selected seven probiotic bacterial strains from the "*Lactobacillus*" and "*Bifidobacterium*" genera. These strains were sourced from both established culture collections and various probiotic products, including dairy, infant

formula, and supplements. In addition to these probiotic strains, we included "Streptococcus thermophiles" (DELVO DSL, The Netherlands) in our formulation.

2.2 Isolation of Pure Cultures of Probiotic and Starter Culture Bacteria

We serially diluted each culture from 10^{-2} to 10^{-7} in peptone water and used the pour plate technique with Bromocresol Green Whey Agar (BGWA) to isolate bacterial colonies. The BGWA plates were prepared and incubated anaerobically at 37°C for 72 hours (Figure 1, A). Isolated colonies were characterized by catalase activity and cell morphology.

2.3 Activation and Maintenance of Probiotic and Starter Cultures Bacteria

Selected colonies with different morphological appearances were inoculated using a cooled pre-sterilized loop in pre-sterilized 12% non-fat dry milk (NFDM) tubes (115°C/ 15 min) with 0.05% L. cysteine. HCL. Anaerobic incubation was carried out using AnaeroGen (Oxoid Ltd., AN35 or AN25, UK) and anaerobic jar. Well-sealed tubes were incubated at 37°C for 72 h. Then, a cell morphology test was made under direct microscopic examination (DME) using methylene blue staining (Löffler's methylene blue, Fisher Scientific Co. USA, 0.002%) [16]. 2% of the inoculated isolate colonies were transferred into another autoclaved 12% NFDM (115°C/ 15 min) incubated at 37°C for 72 hours. This process was repeated weekly to have pure active cultures (2% working culture) for Labneh production (Figure 1, B). Cultures were kept at -20°C in 2% NFDM plus 20% glycerol [17], (Figure 1, C).

2.4 Production of Probiotic Labneh

Ultra-high temperature (UHT) fresh skim cow's milk (Nadec, Saudi Arabia) was sourced from a local market in Amman, Jordan. All milk originated from the same batch and was analyzed for pH, fat percentage, and total solids percentage before use [18]. Labneh was produced following the traditional method. UHT milk was heated using a well-cleaned stainless steel pot to 95°C for 5 min and poured into pre-sterilized 500 ml flasks, cooled to 40°C, inoculated with 2% active working culture of probiotic bacteria, and incubated at 37°C. Monitoring of curd formation, pH, and direct microscopic examination (DME) were carried out every 8 h. Flasks were then divided into two groups: The first: flasks with the selected active inoculated culture only were re-incubated at 37°C until curd formation occurred. The second: was flasks with active inoculated culture to which 1% "S. thermophiles" culture was added to accelerate curd formation. Cultures were monitored for curd formation, pH, and DME (Figure 2). Each experiment was made in duplicate. Flasks, in which curd formation was noticed, were transferred into the refrigerator and kept overnight. Before straining 1% table salt (NaCl) was added into each flask and stirred using a clean glass rod. The flask contents were strained using a commercially used cloth bag and left for 12 hours at 5°C. They were then emptied into three small press-to-close plastic containers and stored at 5°C to be analyzed microbiologically, chemically, and sensorial at (0, 7, and 14th days).

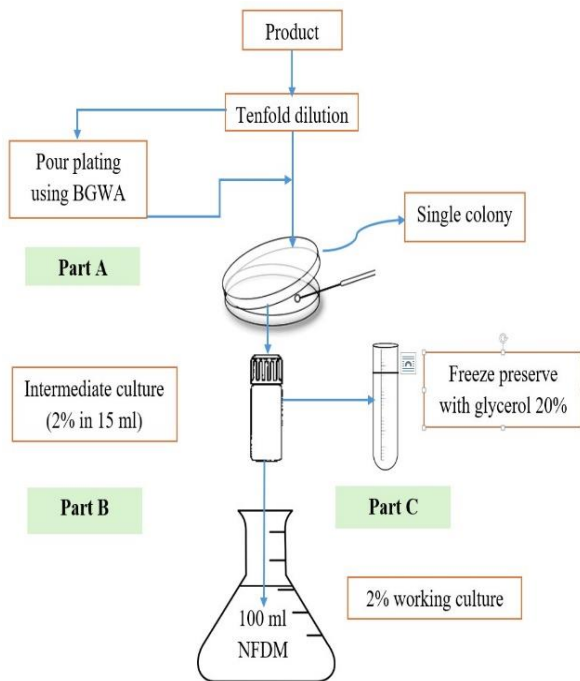


Figure 1: Preparation and Preservation of Probiotic Cultures for Labneh

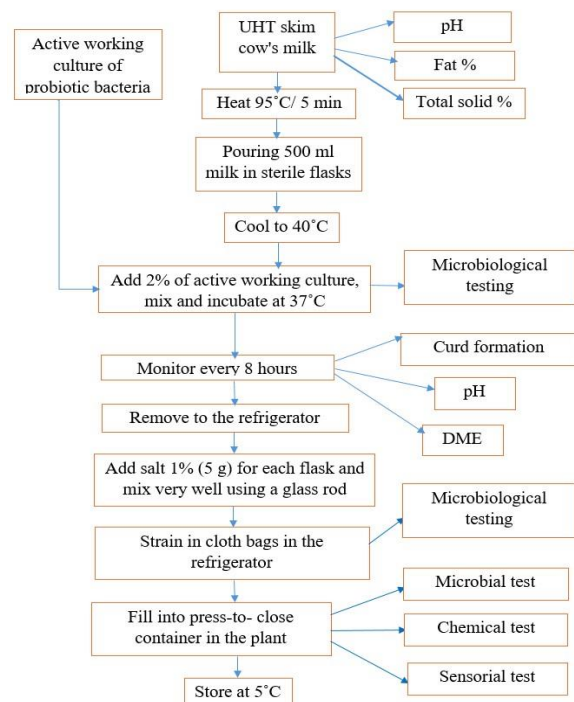


Figure 2: Steps of probiotic Labneh production

2.5 Microbiological Testing

2.5.1 Processing of Probiotic Labneh

At the start of incubation, composite samples were serially diluted (10^{-2} to 10^{-7}) in 0.15% peptone water and plated on BGWA and MRS agar (The media serves as a nutrient-rich agar). Plates were incubated anaerobically. Bacterial concentration was determined by counting colonies (CFU/g). Pre-straining samples were tested similarly.

2.5.2 Testing of Probiotic Labneh

At each time point, 11 grams of sample was mixed with 99 ml of 0.15% peptone water in a stomacher for 1 minute. Serial dilutions (10^{-2} to 10^{-7}) were prepared and plated on BGWA and MRS agar. Plates were incubated anaerobically at 37°C for 72 hours. Colony counts (CFU/g) were determined in duplicate.

2.6 Chemical Tests

2.6.1 Measuring of pH

pH-meter (HANNA instruments-USA) was used to measure the pH of probiotic Labneh samples at the assigned time intervals. It was used after calibration using buffers 4.0 and 7.0. The electrode was immersed directly into a beaker containing a homogenized Labneh sample [19].

2.6.2 Titratable Acidity (Lactic Acid %)

Between 2 to 5 grams of Labneh were weighed into a flask and gently mixed with 100 mL of distilled water using a glass rod [19]. Next, 0.5 mL of phenolphthalein indicator was added, and the sample was titrated with 0.1 N NaOH (Fisher Scientific Co., USA), stirring until the solution turned a permanent pink. Then the acidity was calculated as lactic acid percentage using the following formula:

$$\text{Acidity (lactic acid \%)} = \frac{\text{ml (NaOH)} \times (\text{Normality of NaOH}) \times 9}{\text{Sample weight in grams titrated}}$$

Where, 1 ml of 0.1 N NaOH = 0.009 gram of lactic acid.

2.6.3 Salt (NaCl %) in Probiotic Labneh

Dissolve 4 grams of Labneh in 100 mL of distilled water [20]. Titrate the solution with 0.1 N AgNO₃, using K₂CrO₄ as an indicator (Laboratory Rasayan, India). The endpoint is indicated by a persistent pale red-brown color [19]. The NaCl content is calculated using the following formula:

$$\text{NaCl (salt \%)} = \frac{\text{ml (AgNO}_3\text{)} \times (\text{Normality of AgNO}_3\text{)} \times 0.0585 \times 100}{\text{Sample weight}}$$

2.7 Sensory Evaluation of Probiotic Labneh

Sensory evaluation of Labneh was conducted immediately after processing and at regular intervals during refrigerated storage. Trained panelists assessed its qualities, using commercial Labneh from the Faculty of Agriculture, University of Jerash as the control. A 5-point hedonic scale was employed to evaluate five key attributes: appearance, smell, taste, texture, and overall acceptability [21].

2.8 Statistical Analysis

Log-transformed bacterial counts were subjected to one-way ANOVA, followed by a least significant differences test at a 95% confidence level [22]. All experiments were carried out in duplicate, and all the obtained data are expressed as average. Multiple range test was used to identify significant differences between the means [23].

3. RESULTS

3.1 Growth and Bacterial Counts

All probiotic bacteria tested were able to grow in cow's milk when incubated at 37 °C, with variations observed in their final counts (Table 1). The highest counts were recorded for "L. rhamnosus", while the lowest were for "L. acidophilus 1" and "Bifidobacterium spp 2". Nevertheless, all strains achieved counts exceeding 6 Log₁₀. Except for "B. lactis" (Sundown) and "Bifidobacterium spp 2", all strains could form curd in the milk after 48 hours of incubation (Table 2) and be strained directly in cloth bags to produce Labneh.

Direct microscopic examination of the curds proved that they contained pure cultures, as the typical shape and arrangement of the respective tested bacteria were observed.

Counts obtained using BGWA were generally higher than those of MRSA (Table 3) proving the suitability of this medium to enumerate probiotic bacteria.

Table 1: Initial probiotic counts in cultured milk (2 hours at 37°C)

Probiotic bacteria	Medium	Counts ($\text{Log}_{10} \pm \text{SE}$)
"Lactobacillus acidophilus 1" (Life plan)	BGWA	6.2 ^b ± 0.01
	MRSA	6.4 ^a ± 0.01
"L. acidophilus 2" (Lactibiane enfant)	BGWA	7.4 ^b ± 0.01
	MRSA	7.5 ^a ± 0.01
"L. rhamnosus" (cheese)	BGWA	7.7 ^a ± 0.01
	MRSA	7.4 ^b ± 0.01
"L. casei" (Shanena)	BGWA	6.8 ^a ± 0.01
	MRSA	6.3 ^b ± 0.01
"Bifidobacterium lactis" (Sundown)	BGWA	7.2 ^a ± 0.01
	MRSA	7.2 ^a ± 0.01
"Bifidobacterium spp 1" (Nido)	BGWA	7.1 ^b ± 0.01
	MRSA	7.9 ^a ± 0.01
"Bifidobacterium spp 2" (Cerelac)	BGWA	6.9 ^a ± 0.01
	MRSA	6.7 ^b ± 0.01

BGWA: Bromocresol green whey agar; **MRSA:** de Man, Rogosa, Sharpe media; **Initial probiotic counts** (BGWA, MRSA) in cultured milk before "S. thermophiles" addition; **Incubation:** probiotic alone (37 °C, 48-72 h) combined with "S. thermophiles" (37°C, 24 hours); Means with the same letter are not significantly different ($p>0.05$).

Table 2: Probiotic counts and curd formation in Labneh

Probiotic bacteria	Medium	Count (log_{10})±SE	Curd formation	
"Lactobacillus acidophilus 1" (Life plan)	Alone	BGWA	7.5 ^a ±0.10	+
		MRSA	7.6 ^a ± 0.10	
	With <i>S. thermophilus</i>	BGWA	8.4 ^b ± 0.01	+
		MRSA	8.8 ^a ± 0.01	
"L. acidophilus 2" (Lactibiane enfant)	Alone	BGWA	8.7 ^a ± 0.01	+
		MRSA	8.8 ^a ± 0.10	
	With <i>S. thermophilus</i>	BGWA	8.6 ^a ± 0.10	+
		MRSA	8.5 ^a ± 0.01	
"L. rhamnosus" (cheese)	Alone	BGWA	9.1 ^a ± 0.10	+
		MRSA	8.7 ^a ± 0.10	
	With <i>S. thermophilus</i>	BGWA	9.1 ^a ± 0.10	+
		MRSA	7.9 ^b ± 0.10	
"L. casei" (Shanena)	Alone	BGWA	8.1 ^a ± 0.10	+
		MRSA	8.0 ^a ± 0.20	
	With <i>S. thermophilus</i>	BGWA	9.0 ^a ± 0.10	+
		MRSA	8.6 ^b ± 0.01	
"Bifidobacterium lactis" (Sundown)	Alone	BGWA	8.3 ^a ± 0.10	-
		MRSA	8.3 ^a ± 0.10	
	With <i>S. thermophilus</i>	BGWA	8.0 ^a ± 0.10	+
		MRSA	7.6 ^b ± 0.01	
"Bifidobacterium spp1" (Nido)	Alone	BGWA	8.5 ^a ± 0.10	+
		MRSA	8.5 ^a ± 0.10	

Probiotic bacteria		Medium	Count (\log_{10}) \pm SE	Curd formation
"Bifidobacterium spp 2" (Cerelac)	With <i>S. thermophilus</i>	BGWA	8.3 ^a \pm 0.10	+
		MRSA	8.2 ^a \pm 0.01	
	Alone	BGWA	7.4 ^a \pm 0.01	-
		MRSA	7.3 ^b \pm 0.01	
	With <i>S. thermophilus</i>	BGWA	8.0 ^a \pm 0.01	+
		MRSA	6.8 ^b \pm 0.10	

BGWA: Bromocresol green whey agar media; **MRSA:** de Man, Rogosa, Sharpe media; Incubation of probiotic bacteria alone was at 37 °C/ 48-72 hours and 24 hours when combined with "*S. thermophilus*"; **Means** with the same letter in each column are not significantly different at 95% confidence.

Table 3: A Comparison of Probiotic Counts in Cultured Milk and Labneh at 5°C

Probiotic Bacteria Counts		BGWA	MRSA
Cultured milk	Range	7.4- 9.1	6.8- 8.7
	Average	8.4	8.1
Labneh at 0 day	Range	7.8- 9.4	6.8- 9.3
	Average	8.9	8.4
Labneh at 7 days	Range	7.9- 9.5	6.9- 9.5
	Average	9.0	8.6
Labneh at 14 days	Range	8.0- 9.6	7.1- 9.5
	Average	9.0	8.5

BGWA: Bromocresol green whey agar; **MRSA:** de Man, Rogosa, Sharpe media.

3.2 pH

The milk for Labneh had a starting pH of 6.5. After 48 hours at 37°C (Table 4), "*L. acidophilus*" and "*Bifidobacterium*" strains formed curds due to significant pH reduction. "*B. lactis*" and another "*Bifidobacterium*" strain did not, as their pH remained above 5.

Table 4: pH \pm SE of cultured milk (with or without *S. thermophilus*) and produced Labneh

Probiotic bacteria	pH \pm SE					
	Cultured milk			Probiotic Labneh		
	After			At		
	8 h	24 h	48 h	0 day	7 days	14 days
"L. acidophilus 1" (Life plan)						
Alone	6.3 ^a \pm 0.10	5.3 ^a \pm 0.01	4.6 ^d \pm 0.01	4.6 ^a \pm 0.10	4.5 ^a \pm 0.10	4.5 ^a \pm 0.10
With <i>S. thermophilus</i>	6.3 ^a \pm 0.10	3.9 ^b \pm 0.01	3.9 ^b \pm 0.01	3.7 ^b \pm 0.01	3.7 ^b \pm 0.01	3.6 ^b \pm 0.01
"L. acidophilus 2" (Lactibiane enfant)						
Alone	6.2 ^a \pm 0.10	5.3 ^a \pm 0.10	4.2 ^e \pm 0.01	4.4 ^a \pm 0.01	4.2 ^a \pm 0.01	4.2 ^a \pm 0.10
With <i>S. thermophilus</i>	6.2 ^a \pm 0.10	3.9 ^b \pm 0.010	3.9 ^b \pm 0.01	3.8 ^b \pm 0.01	3.7 ^b \pm 0.01	3.6 ^b \pm 0.01
"L. rhamnosus" (Cheese)						
Alone	5.9 ^a \pm 0.01	4.9 ^a \pm 0.01	4.7 ^c \pm 0.01	4.5 ^a \pm 0.10	4.3 ^a \pm 0.10	4.2 ^a \pm 0.01
With <i>S. thermophilus</i>	5.9 ^a \pm 0.01	4.2 ^b \pm 0.101	4.2 ^b \pm 0.10	3.9 ^b \pm 0.10	3.8 ^b \pm 0.10	3.8 ^b \pm 0.10
"L. casei" (Shanena)						
Alone	6.2 ^a \pm 0.01	5.2 ^a \pm 0.01	4.8 ^c \pm 0.10	4.6 ^a \pm 0.20	4.6 ^a \pm 0.10	4.6 ^a \pm 0.10
With <i>S. thermophilus</i>	6.2 ^a \pm 0.01	3.9 ^b \pm 0.01	3.9 ^b \pm 0.01	3.6 ^b \pm 0.01	3.6 ^b \pm 0.10	3.4 ^b \pm 0.01

Probiotic bacteria	pH ± SE					
	Cultured milk			Probiotic Labneh		
	After			At		
	8 h	24 h	48 h	0 day	7 days	14 days
"B. lactis" (Sundown)						
Alone	6.3 ^a ± 0.01	5.9 ^a ± 0.10	5.7 ^a ± 0.10	5.2 ^a ± 0.10	5.1 ^a ± 0.10	5.0 ^a ± 0.01
With <i>S. thermophilus</i>	6.2 ^b ± 0.01	4.0 ^b ± 0.01	4.0 ^b ± 0.01	3.9 ^b ± 0.01	3.8 ^b ± 0.01	3.8 ^b ± 0.10
"Bifidobacterium spp 1" (Nido)						
Alone	6.2 ^a ± 0.01	5.4 ^a ± 0.01	4.7 ^{cd} ± 0.01	4.6 ^a ± 0.01	4.6 ^a ± 0.10	4.6 ^a ± 0.10
With <i>S. thermophilus</i>	6.2 ^a ± 0.01	4.3 ^b ± 0.01	4.3 ^b ± 0.01	3.9 ^b ± 0.10	3.8 ^b ± 0.01	3.8 ^b ± 0.10
"Bifidobacterium spp 2" (Cerelac)						
Alone	6.3 ^a ± 0.10	5.9 ^a ± 0.10	5.5 ^a ± 0.10	5.2 ^a ± 0.01	5.2 ^a ± 0.01	5.1 ^a ± 0.01
With <i>S. thermophilus</i>	6.3 ^a ± 0.10	4.2 ^b ± 0.01	4.2 ^b ± 0.01	4.0 ^b ± 0.10	3.9 ^b ± 0.10	3.7 ^b ± 0.10

For each bacterial type, values in a column with the same letter are not significantly different at 95% confidence.

3.3 Probiotic Labneh Bacterial Counts

Straining probiotic milk led to a high concentration of probiotic bacteria in Labneh, with counts exceeding 8 log₁₀ in all tests. Storage at 5°C for 7 or 14 days showed no significant changes in bacterial counts. Direct microscopic examination (DME) confirmed the culture's purity, with BGWA counts higher than those of MRSA (Table 5).

Table 5: Probiotic bacteria counts in Labneh after straining (0, 7, and 14 days) at 5°C, measured by BGWA and MRSA

Probiotic bacteria	Media	Count (log ₁₀) ± SE		
		0 day	7 days	14 days
"Lactobacillus acidophilus 1" (Life plan)				
Alone	BGWA	8.3 ^a ± 0.01	8.3 ^a ± 0.10	8.3 ^a ± 0.01
	MRSA	8.3 ^a ± 0.01	8.4 ^a ± 0.10	8.3 ^a ± 0.01
With " <i>S. thermophiles</i> "	BGWA	9.0 ^b ± 0.10	9.0 ^b ± 0.01	9.0 ^a ± 0.01
	MRSA	9.3 ^a ± 0.01	9.2 ^a ± 0.01	9.1 ^a ± 0.10
"L. acidophilus 2" (Lactibiane enfent)				
Alone	BGWA	9.2 ^a ± 0.01	9.2 ^a ± 0.10	9.2 ^a ± 0.01
	MRSA	9.1 ^b ± 0.01	9.3 ^a ± 0.01	8.4 ^b ± 0.01
With " <i>S. thermophiles</i> "	BGWA	9.1 ^a ± 0.10	9.1 ^a ± 0.01	9.1 ^a ± 0.01
	MRSA	8.7 ^b ± 0.01	8.8 ^b ± 0.01	8.8 ^b ± 0.01
"L. rhamnosus" (cheese)				
Alone	BGWA	9.2 ^a ± 0.01	9.5 ^a ± 0.10	9.6 ^a ± 0.10
	MRSA	8.9 ^b ± 0.10	9.5 ^a ± 0.10	9.5 ^a ± 0.01
With " <i>S. thermophiles</i> "	BGWA	9.4 ^a ± 0.10	9.5 ^a ± 0.10	9.6 ^a ± 0.01
	MRSA	8.5 ^b ± 0.10	9.4 ^a ± 0.01	9.2 ^b ± 0.10
"L. casei" (Shanena)				
Alone	BGWA	8.7 ^a ± 0.11	8.6 ^a ± 0.01	8.7 ^a ± 0.10
	MRSA	8.6 ^a ± 0.01	8.3 ^b ± 0.01	8.7 ^a ± 0.10
With " <i>S. thermophiles</i> "	BGWA	9.4 ^a ± 0.10	9.3 ^a ± 0.01	9.3 ^a ± 0.01
	MRSA	8.9 ^b ± 0.01	9.0 ^b ± 0.01	9.3 ^a ± 0.10
"Bifidobacterium lactis" (Sundown)				
Alone	BGWA	8.3 ^a ± 0.10	8.5 ^a ± 0.01	8.6 ^a ± 0.10

Probiotic bacteria	Media	Count (log ₁₀) ± SE		
		0 day	7 days	14 days
With " <i>S. thermophiles</i> "	MRSA	8.1 ^a ± 0.10	8.2 ^b ± 0.01	8.1 ^b ± 0.10
	BGWA	8.4 ^a ± 0.01	8.9 ^a ± 0.01	9.1 ^a ± 0.01
	MRSA	7.1 ^b ± 0.10	7.1 ^b ± 0.01	7.1 ^b ± 0.01
"Bifidobacterium spp 1" (Nido)				
Alone	BGWA	9.4 ^a ± 0.01	9.4 ^a ± 0.01	9.2 ^a ± 0.01
	MRSA	9.3 ^a ± 0.10	9.4 ^a ± 0.10	9.2 ^a ± 0.01
With " <i>S. thermophiles</i> "	BGWA	9.2 ^a ± 0.10	9.3 ^a ± 0.01	9.3 ^a ± 0.01
	MRSA	8.9 ^a ± 0.10	9.1 ^b ± 0.10	8.9 ^b ± 0.01
"Bifidobacterium spp 2" (Cerelac)				
Alone	BGWA	7.8 ^a ± 0.01	7.9 ^a ± 0.10	8.0 ^a ± 0.10
	MRSA	6.8 ^b ± 0.10	6.9 ^b ± 0.10	7.1 ^b ± 0.01
With " <i>S. thermophiles</i> "	BGWA	9.1 ^a ± 0.11	9.1 ^a ± 0.01	9.3 ^a ± 0.10
	MRSA	7.6 ^b ± 0.01	7.6 ^b ± 0.01	7.6 ^b ± 0.01

BGWA: bromcresol green whey agar; **MRSA**: de Man Rogosa agar; **Means** for each bacterial type in each column with the same letter are not significant difference with up at 95% Confidence Level.

3.4 Salt (NaCl %)

Table (6) shows salt content (1.1–1.3% NaCl) in probiotic Labneh after straining, whether using probiotics alone or with "*S. thermophiles*". All batches met the Jordanian Labneh standard (≤1.5%, JISM108: 2003) with no significant differences.

Table 6: Salt content (NaCl %)-in probiotic Labneh after straining, with or without *Streptococcus thermophilus*

Probiotic bacteria		Value±SE
		NaCl% at 0 day
" <i>L. acidophilus</i> 1" (Life plan)	Alone	1.1 ^a ± 0.01
	with	1.2 ^a ± 0.01
" <i>L. acidophilus</i> 2" (Lactibiane enfant)	Alone	1.1 ^a ± 0.10
	with	1.3 ^a ± 0.01
" <i>L. rhamnosus</i> " (Cheese)	Alone	1.3 ^a ±0.10
	with	1.2 ^a ± 0.01
" <i>L. casei</i> (Shanena)"	Alone	1.1 ^a ± 0.20
	with	1.3 ^a ± 0.01
" <i>B. lactis</i> (Sundown)"	Alone	1.1 ^a ± 0.10
	with	1.2 ^a ± 0.20
" <i>Bifidobacterium spp 1</i> " (Nido)	Alone	1.0 ^a ± 0.01
	with	1.2 ^a ±0.11
" <i>Bifidobacterium spp 2</i> " (Cerelac)	Alone	1.1 ^a ± 0.01
	with	1.2 ^a ± 0.10

Means with the same letter in each column for each bacterial type are not significantly different at 95% confidence.

3.5 pH and Acidity

Except for "*B. lactis*" and "*Bifidobacterium spp 2*", Labneh's pH after straining was around 4.5 (Table 7). The lowest pH occurred with "*L. acidophilus 2*", and the highest with "*B. lactis*" and "*Bifidobacterium spp 2*".

The same trend applied to acidity (Table 7). Combining probiotics with "*S. thermophiles*" significantly lowered pH and increased acidity, with no significant changes after 7 or 14 days at 5°C.

Table 7: pH and acidity of probiotic Labneh after straining, and after 7 and 14 days at 5°C, with or without *Streptococcus thermophilus*

Probiotic bacteria	Days	pH ± SE		Acidity ± SE	
		Alone	With " <i>S. thermophilus</i> "	Alone	With " <i>S. thermophilus</i> "
<i>"L. acidophilus 1"</i> (Life plan)	0	4.6 ^a ± 0.10	3.7 ^a ± 0.01	1.2 ^b ± 0.10	2.4 ^c ± 0.01
	7	4.5 ^a ± 0.10	3.7 ^a ± 0.01	1.2 ^{ab} ± 0.01	2.6 ^b ± 0.10
	14	4.5 ^a ± 0.10	3.6 ^b ± 0.01	1.4 ^a ± 0.10	2.8 ^a ± 0.01
<i>L. acidophilus 2</i> (Lactibiane enfert)	0	4.4 ^a ± 0.01	3.8 ^a ± 0.01	1.2 ^c ± 0.10	2.3 ^b ± 0.01
	7	4.2 ^b ± 0.01	3.7 ^b ± 0.01	1.4 ^b ± 0.01	2.4 ^b ± 0.10
	14	4.2 ^b ± 0.01	3.6 ^c ± 0.01	1.7 ^a ± 0.10	2.8 ^a ± 0.10
<i>"L. rhamnosus"</i> (Cheese)	0	4.5 ^a ± 0.10	3.9 ^a ± 0.10	1.4 ^a ± 0.10	2.4 ^b ± 0.10
	7	4.3 ^b ± 0.10	3.8 ^a ± 0.10	1.5 ^a ± 0.10	2.7 ^{ab} ± 0.10
	14	4.2 ^b ± 0.01	3.8 ^a ± 0.10	1.8 ^a ± 0.10	2.9 ^a ± 0.10
<i>"L. casei</i> (Shanena)"	0	4.6 ^a ± 0.20	3.6 ^a ± 0.01	1.4 ^b ± 0.10	1.9 ^b ± 0.10
	7	4.6 ^a ± 0.10	3.6 ^a ± 0.10	1.6 ^{ab} ± 0.10	2.1 ^a ± 0.01
	14	4.6 ^a ± 0.10	3.4 ^b ± 0.01	1.7 ^a ± 0.10	2.1 ^a ± 0.01
<i>"B. lactis"</i> (Sundown)	0	5.2 ^a ± 0.10	3.9 ^a ± 0.01	0.8 ^a ± 0.10	1.6 ^b ± 0.01
	7	5.1 ^a ± 0.10	3.8 ^{ab} ± 0.01	0.9 ^a ± 0.10	1.8 ^{ab} ± 0.10
	14	5.0 ^a ± 0.01	3.8 ^b ± 0.10	0.9 ^a ± 0.01	1.9 ^a ± 0.10
<i>"Bifidobacterium spp 1"</i> (Nido)	0	4.6 ^a ± 0.01	3.9 ^a ± 0.10	1.3 ^a ± 0.10	1.6 ^c ± 0.01
	7	4.6 ^a ± 0.10	3.8 ^a ± 0.01	1.3 ^a ± 0.01	1.7 ^b ± 0.01
	14	4.6 ^a ± 0.10	3.8 ^a ± 0.10	1.3 ^a ± 0.10	1.7 ^b ± 0.01
<i>"Bifidobacterium spp 2"</i> (Cerelac)	0	5.2 ^a ± 0.01	4.0 ^a ± 0.10	0.8 ^b ± 0.10	1.5 ^b ± 0.10
	7	5.2 ^a ± 0.01	3.9 ^a ± 0.10	0.9 ^{ab} ± 0.10	1.6 ^{ab} ± 0.10
	14	5.1 ^b ± 0.10	3.7 ^a ± 0.10	1.0 ^a ± 0.01	1.8 ^a ± 0.10

For each bacterial type, means in the same column with the same letter show no significant difference at the 95% confidence level.

3.6 Sensory Testing of Labneh

When used alone, "*L. acidophilus 1*" had the highest sensory acceptability, comparable to commercial Labneh. Labneh with "*L. rhamnosus*", "*B. lactis*", and "*Bifidobacterium spp 2*" scored poorly, while "*Bifidobacterium spp 1*" was moderately acceptable. Adding "*S. thermophilus*" improved the sensory quality of poorly rated batches "*L. rhamnosus*", "*L. casei*", and "*bifidobacteria*" (Table 8).

Table 8: Sensory scores (Mean ± SE) of probiotic Labneh after straining, and after 7 and 14 days at 5°C, compared to commercial control Labneh

Probiotic bacteria	Days	Appearance	Smell	Consistency	Taste	Overall acceptability
"L. acidophilus 1" (Life plan)						
Alone	0	9.0 ^a ± 0.01	9.0 ^a ± 0.01	9.0 ^a ± 0.01	9.0 ^a ± 0.01	9.0 ^a ± 0.01
	7	9.0 ^a ± 0.01	9.0 ^a ± 0.01	9.0 ^a ± 0.01	9.0 ^a ± 0.01	9.0 ^a ± 0.01
	14	9.0 ^a ± 0.01	9.0 ^a ± 0.01	9.0 ^a ± 0.30	9.0 ^a ± 0.01	9.0 ^a ± 0.01
With "S. thermophiles"	0	9.0 ^a ± 0.01	9.0 ^a ± 0.10	8.9 ^a ± 0.01	8.1 ^a ± 0.50	8.8 ^a ± 0.10
	7	9.0 ^a ± 0.10	9.0 ^a ± 0.20	8.7 ^b ± 0.01	7.7 ^b ± 0.01	8.6 ^b ± 0.01
	14	9.0 ^a ± 0.01	9.0 ^a ± 0.10	8.7 ^b ± 0.10	7.3 ^c ± 0.11	8.5 ^c ± 0.30
"L. acidophilus 2" (Lactibiane enfert)						
Alone	0	9.0 ^a ± 0.01	9.0 ^a ± 0.01	8.2 ^c ± 0.01	8.0 ^b ± 0.01	8.6 ^c ± 0.01
	7	9.0 ^a ± 0.01	9.0 ^a ± 0.01	8.5 ^b ± 0.01	8.3 ^a ± 0.01	8.7 ^a ± 0.01
	14	8.7 ^b ± 0.30	8.7 ^b ± 0.10	8.7 ^a ± 0.01	8.3 ^a ± 0.10	8.6 ^b ± 0.30
With "S. thermophiles"	0	9.0 ^a ± 0.01	9.0 ^a ± 0.01	8.4 ^c ± 0.01	8.4 ^a ± 0.01	8.7 ^b ± 0.01
	7	9.0 ^a ± 0.10	9.0 ^a ± 0.30	8.6 ^b ± 0.01	8.3 ^b ± 0.10	8.7 ^a ± 0.20
	14	9.0 ^a ± 0.01	9.0 ^a ± 0.01	8.7 ^a ± 0.01	8.0 ^c ± 0.01	8.8 ^c ± 0.01
"L. rhamnosus" (Cheese)						
Alone	0	8.1 ^c ± 0.10	6.2 ^a ± 0.20	3.8 ^c ± 0.01	2.2 ^c ± 0.20	5.1 ^b ± 0.20
	7	8.3 ^b ± 0.01	5.3 ^b ± 0.01	4.0 ^b ± 0.01	2.6 ^b ± 0.01	5.2 ^c ± 0.01
	14	8.7 ^a ± 0.20	5.0 ^c ± 0.01	4.3 ^a ± 0.20	2.7 ^a ± 0.20	5.3 ^a ± 0.40
With "S. thermophiles"	0	8.7 ^c ± 0.01	5.3 ^c ± 0.01	8.0 ^b ± 0.01	5.6 ^c ± 0.01	6.9 ^c ± 0.01
	7	8.9 ^b ± 0.30	6.0 ^b ± 0.01	8.3 ^a ± 0.30	5.9 ^b ± 0.30	7.3 ^b ± 0.30
	14	9.0 ^a ± 0.01	7.3 ^a ± 0.01	8.3 ^a ± 0.01	6.7 ^a ± 0.01	7.8 ^a ± 0.01
L. casei (Shanena)						
Alone	0	9.0 ^a ± 0.20	3.7 ^c ± 0.01	9.0 ^a ± 0.30	5.6 ^a ± 0.10	6.8 ^a ± 0.30
	7	8.6 ^b ± 0.10	4.5 ^a ± 0.01	9.0 ^a ± 0.10	5.1 ^b ± 0.20	6.8 ^b ± 0.50
	14	8.0 ^c ± 0.01	4.0 ^b ± 0.01	9.0 ^a ± 0.10	4.7 ^c ± 0.01	6.4 ^c ± 0.10
With "S. thermophiles"	0	9.0 ^a ± 0.10	5.0 ^c ± 0.60	8.6 ^b ± 0.01	8.5 ^a ± 0.60	7.8 ^c ± 0.01
	7	9.0 ^a ± 0.20	6.3 ^b ± 0.01	9.0 ^a ± 0.20	7.4 ^b ± 0.01	7.9 ^b ± 0.20
	14	9.0 ^a ± 0.01	7.3 ^a ± 0.01	9.0 ^a ± 0.01	6.3 ^c ± 0.01	7.8 ^a ± 0.01
"B. lactis" (Sundown)						
Alone	0	1.0 ^a ± 0.01	4.0 ^a ± 0.10	1.3 ^a ± 0.01	2.5 ^c ± 0.20	2.2 ^b ± 0.10
	7	1.0 ^a ± 0.01	3.7 ^b ± 0.01	1.3 ^a ± 0.01	2.8 ^b ± 0.01	2.2 ^b ± 0.01
	14	1.0 ^a ± 0.01	3.7 ^b ± 0.30	1.3 ^a ± 0.01	3.0 ^a ± 0.30	2.3 ^a ± 0.10
With "S. thermophiles"	0	9.0 ^a ± 0.01	8.4 ^b ± 0.01	8.9 ^a ± 0.01	9.0 ^a ± 0.01	8.8 ^a ± 0.01
	7	8.8 ^b ± 0.10	8.7 ^a ± 0.01	8.4 ^c ± 0.20	8.7 ^b ± 0.01	8.7 ^b ± 0.20
	14	8.0 ^c ± 0.01	8.0 ^c ± 0.01	8.7 ^b ± 0.01	8.0 ^c ± 0.01	8.2 ^c ± 0.01
"Bifidobacterium spp 1" (Nido)						
Alone	0	8.7 ^a ± 0.01	5.8 ^a ± 0.40	9.0 ^a ± 0.01	5.4 ^c ± 0.40	7.2 ^a ± 0.40
	7	7.9 ^b ± 0.30	5.5 ^b ± 0.01	8.2 ^b ± 0.30	5.7 ^b ± 0.30	6.8 ^b ± 0.30
	14	7.7 ^c ± 0.01	5.3 ^c ± 0.01	8.0 ^c ± 0.01	6.3 ^a ± 0.01	6.8 ^b ± 0.01
With "S. thermophiles"	0	9.0 ^a ± 0.10	7.0 ^b ± 0.01	8.5 ^a ± 0.10	8.7 ^a ± 0.01	8.3 ^a ± 0.10
	7	8.5 ^c ± 0.10	7.5 ^a ± 0.01	8.0 ^c ± 0.01	8.2 ^c ± 0.01	8.1 ^b ± 0.10
	14	8.7 ^b ± 0.01	6.3 ^c ± 0.01	8.3 ^b ± 0.01	8.3 ^b ± 0.01	7.9 ^c ± 0.01
"Bifidobacterium spp 2" (Cerelac)						
Alone	0	1.7 ^a ± 0.10	5.3 ^c ± 0.01	1.0 ^b ± 0.10	4.3 ^b ± 0.01	3.1 ^c ± 0.10
	7	1.7 ^a ± 0.01	5.8 ^b ± 0.30	1.3 ^a ± 0.01	4.0 ^c ± 0.30	3.2 ^b ± 0.01

Probiotic bacteria	Days	Appearance	Smell	Consistency	Taste	Overall acceptability
With "S. thermophiles"	14	1.7 ^a ± 0.01	6.3 ^a ± 0.01	1.3 ^a ± 0.01	5.7 ^a ± 0.01	3.8 ^a ± 0.10
	0	8.7 ^a ± 0.10	5.8 ^c ± 0.01	9.0 ^a ± 0.10	7.8 ^a ± 0.01	7.8 ^a ± 0.01
	7	8.4 ^b ± 0.01	6.4 ^a ± 0.01	8.6 ^c ± 0.01	7.0 ^b ± 0.50	7.6 ^b ± 0.50
	14	8.0 ^c ± 0.30	6.0 ^b ± 0.01	8.7 ^b ± 0.30	6.3 ^c ± 0.01	7.3 ^c ± 0.30
Control	0	9.0 ^a ± 0.01	9.0 ^a ± 0.01	9.0 ^a ± 0.01	9.0 ^a ± 0.01	9.0 ^a ± 0.01
	7	9.0 ^a ± 0.01	9.0 ^a ± 0.01	9.0 ^a ± 0.01	9.0 ^a ± 0.01	9.0 ^a ± 0.01
	14	9.0 ^a ± 0.01	9.0 ^a ± 0.01	8.7 ^b ± 0.30	8.6 ^b ± 0.60	8.3 ^b ± 0.30

Means with the same letter in a column show no significant difference at 95% confidence.

4. DISCUSSION

Labneh can be made with all tested probiotic bacteria using the traditional in-bag straining method, but sensory differences in smell, taste, and acceptability were noted. Labneh with "L. acidophilus" was the most preferred with or without "S. thermophiles". In contrast, Labneh made with "L. rhamnosus", "L. casei", or "bifidobacteria" strains was acceptable only when "S. thermophiles" was included. These differences likely stem from variations in bacterial growth and flavor compound production. Indigenous lactic acid bacteria (LAB) strains, commonly used in dairy fermentation, thrive in milk and produce abundant flavor compounds, enhancing acceptability.

"L. acidophilus", known for its health benefits, including treating infectious enteritis, lactose intolerance, cholesterol control, and anti-cancer effects [24], was the first probiotic bacteria to be used commercially. It was utilized in 1922 to produce "Acidophilus-Milch," commercially produced in Germany and other European countries since the 1980s [25]. Today, "L. acidophilus" is found in products such as acidophilus milk, infant formula, and dietary supplements. Due to its slow growth, milk used for acidophilus milk production is typically heat-treated, which is why UHT was used in this study.

A pH below 4.8 is crucial for stable curd formation from coagulated milk proteins [25], which facilitates Labneh production. All tested probiotic bacteria formed curd within 48 hours, except "B. lactis" (Sundown) and "Bifidobacterium spp 2". This aligns with findings by Wróblewska et al., [26], who reported that "Bifidobacterium spp". Grow slowly in milk and often lose viability before the end of the product's shelf life, likely due to their limited proteolytic activity, which impairs growth and survival [27].

Labneh had higher "L. acidophilus" counts than the cultured milk used for production, as straining concentrated probiotics above the >10⁶ CFU/g health-benefit threshold [28]. A similar increase in yogurt starter bacteria was observed during Labneh production from set yogurt [29]. Labneh is an ideal vehicle for probiotic bacteria, as their storage stability remains satisfactory throughout the product's shelf life. Its popularity, especially at breakfast, further enhances its effectiveness.

Probiotic bacteria counts increased after straining in cloth bags to produce Labneh, as the bacteria were retained in the curd while whey was removed [30]. This supports findings by Khider et al., [31], who noted that Labneh's high total solids make it an

excellent matrix for probiotics, providing protection and maintaining their viability during storage. Similarly, Pereira et al., [32] reported variations in the viability of five "L. acidophilus" strains in yogurt and fermented buttermilk stored at 5–7°C, while "L. casei" showed no loss of viability under the same conditions.

The Acidophilus Labneh produced in this study shows good potential, as it is sensorial acceptable, contains sufficient concentrations of viable probiotic bacteria throughout its shelf life, and meets the requirements of the Jordanian Standard for Labneh (JS 108/2003). Bifidobacterium Labneh was less satisfactory than acidophilus Labneh. Two of three strains failed to lower the pH enough for curd formation, likely due to weaker growth in milk. While "Bifidobacterium spp 1" was sensory acceptable, acetic acid production may reduce acceptability. Marcos-Fernández et al., [33] found that bifidobacteria in 12% skim milk at 37°C for 8 hours produced a final pH of 5.1–6.2 (average 5.8) and acetic-to-lactic acid ratios of 0.4:1 to 3.0:1.

Using "S. thermophiles" with "bifidobacteria" enabled curd formation within 8 hours, producing acceptable Labneh, likely due to limited "bifidobacteria" growth in milk. Baliyan et al., [34] found bifidobacteria strains insufficient to lower pH for curd formation in 12% skim milk after 8 hours at 37°C. Similarly, Pandey [35] reported a pH of 5.6 after 24 hours, above the curd-forming threshold.

Incorporating "S. thermophiles", a widely used dairy starter, addresses the limitations of bifidobacteria and "L. acidophilus". This indigenous milk bacterium grows rapidly, producing lactic acid for quick curd formation and flavor compounds like acetaldehyde, a key component in yogurt and cheese [36]. It enhances probiotic Labneh production, particularly when combined with bifidobacteria, which alone do not support milk processing. Adding "S. thermophiles" after 8 hours of probiotic incubation promotes optimal growth levels. Its interactions with bifidobacteria and "L. acidophilus" may resemble its synergistic role with "L. bulgaricus" in yogurt, warranting further investigation.

Bromocresol green whey agar (BGWA) is a selective medium for counting yogurt starter bacteria and probiotic strains in products like yogurt, cheese, and supplements [37]. Its whey-based composition, rich in lactose, glucose, galactose, calcium, and non-protein nitrogen, supports the growth of dairy lactic acid bacteria (LAB). The addition of L-cysteine, HCl, tryptone, and yeast extract further enhances LAB growth [38]. BGWA offers high bacterial counts, improved colony differentiation, and is easy to prepare, making it ideal for routine LAB and probiotic enumeration.

The future of probiotic Labneh lies in optimizing probiotic combinations like "S. thermophiles" with "L. acidophilus" and bifidobacteria to improve taste and probiotic viability. Research should focus on understanding how these strains interact with indigenous LAB to enhance fermentation and flavor production. Advanced fermentation methods and media like BGWA can boost probiotic stability. As demand for functional foods grows, studies on consumer preferences will be crucial to ensure Labneh remains a popular and effective probiotic product.

5. CONCLUSION

Probiotic Labneh can be successfully produced using the conventional method of in-bag straining of cultured milk with strains of *Lactobacillus* and "*Bifidobacterium* spp". Labneh serves as an effective vehicle for probiotic bacteria, maintaining their viability throughout its shelf life, thereby qualifying it as a functional probiotic food. Among the tested variants, "*L. acidophilus*" Labneh was the most acceptable in sensory quality, followed by "*L. casei*" and "*Bifidobacterium* spp 1", while "*L. rhamnosus*" and "*Bifidobacterium* spp 2" scored lower. The inclusion of "*S. thermophiles*" enhanced the sensory characteristics of probiotic Labneh. The product met the Jordanian standards for Labneh and remained acceptable for up to 14 days under refrigerated storage. Additionally, BGWA proved more effective than MRSA in enumerating probiotic bacteria in Labneh.

Commercial probiotic Labneh can be developed based on the process established in this study, using BGWA for the enumeration of probiotic bacteria. Additionally, further studies should explore potential interactions between "*S. thermophiles*" and probiotic bacteria during milk fermentation to enhance understanding and optimize production.

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Conflict of interest

The authors report no conflicts of interest.

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