https://doi.org/10.52973/rcfcv-e362895 Revista Científica, FCV-LUZ / Vol. XXXVI

Pathogen survival in dairy and plant-based probiotic fermented beverages containing *Lacticaseibacillus rhamnosus* GG

Supervivencia de patógenos en bebidas fermentadas probióticas lácteas y de origen vegetal que contienen *Lacticaseibacillus rhamnosus* GG

Esra Yılmaz Başar*, *Arife Ezgi Telli*

Selcuk University, Faculty of Veterinary Medicine, Department of Food Hygiene and Technology. 42130, Konya, Türkiye.
**Corresponding author: ezgiyilmaz@selcuk.edu.tr*

ABSTRACT

The survival of *Lacticaseibacillus rhamnosus* GG and two foodborne pathogens (*Staphylococcus aureus* and *Escherichia coli*) was monitored in *L. rhamnosus* GG – supplemented dairy milk and three plant-based alternatives (soy, almond, and coconut). Microbiological measurements were taken during fermentation (24 hours) and refrigerated storage (21 days, 4°C), using the *L. rhamnosus* GG – supplemented dairy group as the reference matrix for comparisons. Across all matrices, *L. rhamnosus* GG maintained counts above 7 log CFU·mL^{-1} throughout the experimental period. *L. rhamnosus* GG numbers did not differ significantly between dairy and plant-based milks (P>0.05). Although time, milk type, and their interaction were statistically significant (P<0.05), the observed shifts did not translate into a loss of *L. rhamnosus* GG culturability, and counts remained stable across milk types during storage. By day 21, *S. aureus* and *E. coli* counts were lower in almond and coconut milk than in soy and dairy milk (P<0.05). Overall, plant-based milk alternatives particularly almond and coconut appeared to be effective carriers for *L. rhamnosus* GG and performed comparably to dairy milk in sustaining probiotic viability. Because pathogen reduction was matrix-dependent, future work should examine underlying matrix-microbe interactions and validate these findings at industrial scale using integrated microbiological, physicochemical, and compositional analyses.

Key words: Plant-based milk alternatives; *Lacticaseibacillus rhamnosus* GG; probiotic viability; pathogen inhibition; fermented functional beverages

RESUMEN

La supervivencia de *Lacticaseibacillus rhamnosus* GG y de dos patógenos transmitidos por alimentos (*Staphylococcus aureus* y *Escherichia coli*) se evaluó en leche láctea suplementada con *L. rhamnosus* GG y en tres alternativas vegetales a la leche (soja, almendra y coco). Las determinaciones microbiológicas se realizaron durante la fermentación (24 horas) y el almacenamiento refrigerado (21 días, 4°C), utilizando el grupo de leche de vaca suplementada con *L. rhamnosus* GG como matriz de referencia para la comparación. En todas las matrices, *L. rhamnosus* GG mantuvo recuentos superiores a 7 log UFC·mL^{-1} durante todo el período experimental. No se observaron diferencias significativas en los recuentos de *L. rhamnosus* GG entre la leche de vaca y las leches de origen vegetal (P>0,05). Aunque el tiempo, el tipo de leche y su interacción mostraron efectos estadísticamente significativos (P<0,05), estos cambios no se tradujeron en una pérdida de la viabilidad de *L. rhamnosus* GG, manteniéndose los recuentos estables en todos los tipos de leche durante el almacenamiento. Al día 21, los recuentos de *S. aureus* y *E. coli* fueron menores en las leches de almendra y coco en comparación con la leche de soja y la leche láctea (P<0,05). En conjunto, las alternativas vegetales a la leche en particular las de almendra y coco mostraron ser vehiculos eficaces de *L. rhamnosus* GG y presentaron un desempeño comparable al de la leche de vaca en el mantenimiento de la viabilidad probiótica. Dado que la reducción de patógenos fue dependiente de la matriz, futuros estudios deberían profundizar en las interacciones matriz-microorganismo y validar estos hallazgos a escala industrial mediante análisis microbiológicos, fisicoquímicos y composicionales integrados.

Palabras clave: Alternativas a la leche de origen vegetal; *Lacticaseibacillus rhamnosus* GG; viabilidad probiótica; inhibición de patógenos; bebidas funcionales fermentadas

Received: 08/01/2026 Accepted: 21/03/2026 Published: 03/04/2026

INTRODUCTION

Plant-based milk alternatives such as soy *(Glycine max)*, almond (*Prunus dulcis*), and coconut (*Cocos nucifera*) beverages have increasingly been explored as probiotic carriers, particularly in lactose-free food systems. These beverages are considered among the most popular alternatives due to their distinct nutrient profiles and potential prebiotic properties that support the survival and development of probiotics [1, 2].

Although research on probiotic viability in plant-based milk alternatives continues at an increasing pace, there is still a gap in understanding how these substrates directly affect the antimicrobial activity of probiotic bacteria against foodborne pathogens. While some studies highlight the supportive role of bioactive compounds in plant-based milk alternatives on probiotic survival, others report that the lack of milk-derived growth factors may compromise the culturable viability and functionality of probiotic strains through fermentation and storage [3, 4].

Pathogen contamination in plant based alternatives production can be caused by factors such as the microbial load of the raw material, inadequate pasteurization, unhygienic conditions during production, contamination from water and equipment, lack of employee hygiene, and improper storage processes [5, 6]. In these processes, *Eschericha coli* and *Staphylococcus aureus* are among the important pathogens that can cause contamination. *S. aureus* and *E. coli* are two of the most common bacterial pathogens responsible for foodborne illness worldwide [7].

Staphylococcus aureus is a Gram-positive bacterium capable of producing heat-stable enterotoxins that are one of the leading causes of food poisoning [8]. Similarly, *E. coli*, particularly pathogenic strains such as *E. coli* O157:H7, pose a significant public health threat due to their ability to cause severe gastrointestinal infections that can result in hemorrhagic colitis and hemolytic uremic syndrome [9].

Several *Lactobacillus* species have been reported to inhibit foodborne pathogens through acidification, metabolite production, and competitive interactions [10]. Previous studies have suggested that the type of milk substrate may influence the antimicrobial activity of probiotics, depending on its composition and physicochemical properties.

The main objective of this experimental study was to evaluate the viability of *Lacticaseibacillus rhamnosus* GG (*Lb*GG) in soy, coconut, almond, and dairy milk during a 24–hour fermentation period, followed by 21 days of storage at 4°C. Additionally, to assess the inhibitory effects of these probiotic-enriched beverages against *S. aureus* and *E. coli* during fermentation and storage, as well as to examine the impact of pH and acidity changes on probiotic viability and pathogen growth inhibition throughout this period.

MATERIAL AND METHODS

Experimental design and sample grouping

The experimental setup was designed to investigate pathogen behavior in *Lb*GG-supplemented milk alternatives. Commercially available soy milk, almond milk, coconut milk, and low fat dairy milk was obtained to be used in the production of probiotic-added plant-based beverages. The nutritional values of the milks used in the study are presented in TABLE I.

TABLE I
Basic nutritional composition of milk samples used in the study (100 mL)

Nutrition Facts	Dairy milk	Soy milk	Almond milk	Coconut milk
Energy	44 kcal	30 kcal	22 kcal	25 kcal
Carbohydrate	4.7 g	2.4 g	2.4 g	2.7 g
Protein	3.0 g	2.0 g	0.4 g	0.1 g
Fat	1.5 g	1.3 g	1.1 g	1.5 g
Salt	0.1 g	0.1 g	0.1 g	0.0 g

Dairy milk served as the main control for comparing probiotic survival across milk types. Each milk type included three experimental groups: (i) *Lb*GG + *E. coli*, (ii) *Lb*GG + *S. aureus*, and (iii) *Lb*GG-only control. Since all groups contained *Lb*GG, the study does not isolate the probiotic's direct inhibitory effect on pathogens. The study rather examines how pathogen viability varies among different milk types in the presence of *Lb*GG. Additionally, *Lb*GG survival was compared across plant based milk alternatives, with dairy milk acting as a reference. The experiment was conducted in triplicate at different time intervals.

Inoculation of the microorganisms

Prior to inoculation into plant-based milk alternatives, the bacterial cultures *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213, and *Lb*GG ATCC 53103 were revived under appropriate conditions to ensure viability and optimal growth. *E. coli* and *S. aureus* were first transferred into Tryptic Soy Broth (TSB) and incubated in a shaking incubator (Biosan ES–20, Türkiye) at 37°C for 24 hours (h), while *Lb*GG was cultured in De Man, Rogosa, and Sharpe (MRS) broth at 37°C.

The cultures were passaged back into fresh TSB to promote the exponential growth phase. The bacterial density of the final cultures was determined using an optical density (OD) measurement (Allsheng-Nano–200, Deepak Biological House, India) at 600 nm (OD600) and adjusted to an OD600 value corresponding to ~10^9 CFU·mL^{-1} [11]. The suspensions were then diluted to 10^8 CFU·mL^{-1} prior to inoculation. Subsequently, 10 mL of the standardized culture (10^8 CFU·mL^{-1}) was added to 90 mL of each milk matrix, resulting in a final inoculation level of approximately 10^7 CFU·mL^{-1}. This ratio also aligns with regulatory standards requiring probiotics to remain above 10^7 CFU·mL^{-1}. *E. coli* and *S. aureus* were inoculated at 10^5 CFU·mL^{-1} to represent realistic contamination levels.

Microbiological analysis

Microbiological analyses were performed on designated for the fermentation and storage periods. Prior to sampling, each tube was gently inverted and homogenized by vortexing (Labart MVS–1), for 15 seconds (s). A 10 mL aliquot was taken from sterile glass bottles and mixed with 90 mL of Ringer's solution. The mixture was homogenized using a stomacher for 90 s. Decimal dilutions (10^{-1}

to 10^{-10}) were prepared by transferring 1 mL of the homogenate into 9 mL of Ringer's solution in sterile tubes. Based on preliminary trials indicating high post-fermentation bacterial densities, extended dilution levels were included when necessary to ensure countable plates within the acceptable enumeration range. From each dilution, 100 µL was spreaded onto selective media. For the enumeration of *Lb*GG de Man, MRS agar was used. The plates were incubated at 30°C for 24–48 h, and typical colonies were counted and expressed as log CFU·mL^{-1}.

For *E. coli* enumeration, Tryptone Bile X-glucuronide agar was used. Plates were incubated at 37°C for 24 h, and green-blue, smooth, round colonies formed on the agar surface were counted and reported as log CFU·mL^{-1}. *S. aureus* was enumerated using Baird-Parker Agar. Plates were incubated aerobically at 37°C for 24–48 h, and black or grey-black colonies with a clear halo were counted and expressed as log CFU·mL^{-1}.

Measurement of titratable acidity and pH

In order to determine the acidity, 10 mL of milk samples were taken and transferred into flasks. 0,5 mL of 1 % phenolphthalein indicator were added to the milk. For titration, 0.1 N sodium hydroxide (NaOH) was added to the samples with a pipette until a light pink color was observed. When pink color was observed, the titration process was terminated and the amount of NaOH consumed was recorded. The titratable acidity was calculated as percent lactic acid using the following equation [12].

$$\text{Acidity } (\%) = \left(0.009 \times \frac{V}{S}\right) \times 100$$

In this equation, 0.009 represents the amount (in grams) of lactic acid neutralized by 1 mL of 0.1 N NaOH; V is the volume (mL) of NaOH used during titration; and S is the volume (mL) of milk sample analyzed. A benchtop pH meter (WTW, inolab 720, Germany) was used for pH analysis.

Statistical analysis

Descriptive statistics were made for the measured milk samples and shown as Arithmetic Mean ± Standard Error. The conformity of the data to normal distribution was evaluated according to Shapiro Wilk results. For comparisons between milk types and time points, the General Linear Model was used, as the same samples were measured at multiple time intervals (0, 7, 14, and 21 d for microbiological data; 0, 8, 24 h and d 0, 7, 14, 21 for pH and acidity).

The Bonferroni post-hoc tes was applied to determine pairwise differences between groups. All analyses were performed using the SPSS Statistics version 22.0 software (IBM Corp., Armonk, NY, USA). A *P*-value less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Viability of *Lacticaseibacillus rhamnosus* GG in different milk types during storage

In all milk types, *Lb*GG exhibited a marked increase after 24 h of fermentation compared to the initial inoculation level (d 0), reaching high-density populations exceeding 9.5 log$_{10}$ CFU·mL^{-1}. Throughout the 21 d refrigerated storage period at 4°C, *Lb*GG maintained viable counts well above the functional probiotic threshold of 7 log$_{10}$ CFU·mL^{-1} (TABLE II).

TABLE II
Viability of *Lacticaseibacillus rhamnosus* GG in different milk types over time (log$_{10}$ CFU·mL^{-1})

Milk Type	Day 0	Day 1*	Day 7	Day 14	Day 21
Dairy milk	6.76 ± 0.00	> 9.5	> 9.5	> 9.5	> 9.5
Soy milk	7.08 ± 0.10	> 9.5	> 9.5	> 9.5	> 9.5
Coconut milk	7.11 ± 0.28	> 9.5	> 9.5	> 9.5	> 9.5
Almond milk	6.84 ± 0.31	> 9.5	> 9.5	> 9.5	> 9.5

*: 24 fermentation

Given that dairy milk served as the reference matrix and experimental conditions were standardized across treatments, the sustained high-density viability of *Lb*GG in almond, coconut, and soy milks indicates that plant-based alternatives can effectively support probiotic maintenance under the tested conditions. *Lb*GG viability significantly increased during the first 24 h of fermentation. Following the initial increase during fermentation, *Lb*GG reached high-density populations (≥ 9 log$_{10}$ CFU·mL^{-1}) and remained at elevated viability levels throughout storage (d 7, 14, and 21). Although exact quantification at very high cell densities was limited by plate count constraints, viable counts consistently remained well above the functional probiotic threshold (> 7 log$_{10}$ CFU·mL^{-1}) during refrigerated storage.

Similar trends have been reported in fermented plant-based beverages. Kılınç *et al.* [13] observed that several probiotic strains, including *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus*, and *L. acidophilus*, maintained viability above 6 log$_{10}$ CFU·mL^{-1} during 21 d of storage at 4°C. Comparable stability has also been documented for *Lb*GG grown in various non-dairy substrates [6, 11, 14], supporting the sustained viability observed in the present study.

Survival of *Staphylococcus aureus* and *Escherichia coli* in *Lacticaseibacillus rhamnosus* GG-containing milk samples

An interaction was observed between time and milk type (P<0.001), indicating that the survival of *S. aureus* varied depending on the milk matrix. While *S.aureus* counts remained relatively high in dairy and soy milk throughout storage, lower counts were detected in almond and coconut milk. *S. aureus* was not detectable in coconut milk by d 21, which may suggest a possible inhibitory effect associated with this matrix (TABLE III).

The survival of *E. coli* was influenced by the type of milk used (P<0.001), suggesting that differences in milk composition may have affected the viability of the pathogen during storage. Although *E. coli* observed to have high viability in dairy and soy milk, a more noticeable decrease in viability was observed in coconut and almond milk samples. By d 21, *E. coli* counts in these plant-based milks had declined to approximately 4 log$_{10}$ CFU·mL^{-1}, indicating a relatively lower persistence compared to other milk types (TABLE IV, FIG. 1.).

TABLE III
Survival of *Staphylococcus aureus* in *Lacticaseibacillus rhamnosus* GG-containing milk samples

S. aureus (log CFU·mL^{-1})	Sampling Time (days)					*P*-value		
	Day 0	Day 1	Day 7	Day 14	Day 21	Time	Milk	Time*milk
Dairy milk	$4.67 \pm 0.40^{a,A}$	$6.58 \pm 0.23^{b,C}$	$7.19 \pm 0.25^{c,D}$	$6.04 \pm 0.18^{c,B}$	$5.36 \pm 0.17^{c,A}$	< 0.001	< 0.001	< 0.001
Soy milk	$4.55 \pm 0.06^{a,A}$	$7.53 \pm 0.04^{c,C}$	$7.66 \pm 0.78^{d,C}$	$6.28 \pm 0.08^{c,B}$	$5.14 \pm 0,27^{c,A}$			
Coconut milk	$4,70 \pm 0,21^{a,D}$	$6.07 \pm 0.11^{a,E}$	$3.15 \pm 0.08^{a,C}$	$1.64 \pm 0.06^{a,B}$	$0.00 \pm 0.00^{a,A}$			
Almond milk	$4,34 \pm 0,36^{a,B}$	$6.46 \pm 0.19^{ab,D}$	$5.12 \pm 0.04^{b,C}$	$3.89 \pm 0.26^{b,B}$	$3.34 \pm 0.35^{b,A}$			

The letters a, b, c, d in the table are used to express the differences between rows; the letters A, B, C, D, E are used to express the differences between columns. Different letters in the same column/row are significant (P<0.05)

TABLE IV
Survival of *Escherichia coli* in *Lacticaseibacillus rhamnosus* GG-containing milk samples

E. coli (log CFU·mL^{-1})	Sampling Time (days)					*P*-value		
	Day 0	Day 1	Day 7	Day 14	Day 21	Time	Milk	Time*milk
Dairy milk	$4.57 \pm 0.06^{a,A}$	$9.64 \pm 0.10^{c,C}$	$9.48 \pm 0.45^{b,C}$	$9.61 \pm 0.29^{b,C}$	$8.13 \pm 0.23^{b,B}$	< 0.001	< 0.001	< 0.001
Soy milk	$4.73 \pm 0.14^{a,A}$	$8.81 \pm 0.43^{b,BC}$	$8.95 \pm 0.23^{b,C}$	$9.69 \pm 0.37^{b,C}$	$8.21 \pm 0.23^{b,B}$			
Coconut milk	$4.64 \pm 0.18^{a,B}$	$7.64 \pm 0.05^{a,C}$	$7.41 \pm 0.28^{a,C}$	$7.31 \pm 0.35^{a,C}$	$3.87 \pm 0.29^{a,A}$			
Almond milk	$4.65 \pm 0.12^{a,B}$	$7.77 \pm 0.22^{a,C}$	$7.59 \pm 0.14^{a,C}$	$7.46 \pm 0.31^{a,C}$	$3.91 \pm 0.50^{a,A}$			

The letters a, b, c, d in the table are used to express the differences between rows; the letters A, B, C, D, E are used to express the differences between columns. Different letters in the same column/row are significant (P<0.05)

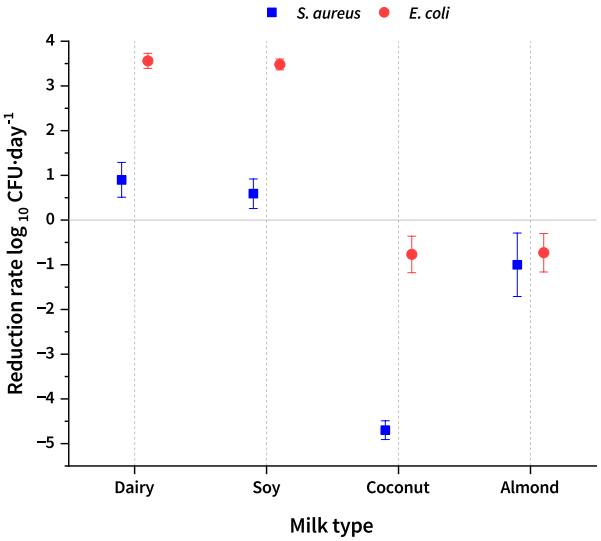

FIGURE 1. Pathogen reduction rates in different milk types

Greater reductions in pathogen culturability were observed in coconut and almond milks compared to dairy and soy, which may be influenced by differences in matrix composition. Although the current study did not analyze the specific compositional features of the milk matrices, previous research has reported that almond and coconut milks may contain medium-chain fatty acids and polyphenolic compounds with antimicrobial activity [15, 16]. It has been shown that polyphenols extracted from almond skins exhibit antimicrobial activity against foodborne pathogens [17].

Paul *et al.* [1] emphasized that soy, coconut, and almond milks contain bioactive compounds like phenolic acids and natural fibers that contribute improved microbial viability during fermentation and storage. The high linoleic and α–linolenic acid content of almond milk may also decrease the viable count of *S. aureus* that decreased significantly after d 1 (P<0.05), while a significant reduction in *E. coli* was observed only on d 21 (P<0.05). Taken together linoleic acid present in almond milk, may be considered one of the contributors that might exert an inhibitory effect on both *S. aureus* and *E. coli*. Coconut milk is a rich source of medium-chain fatty acids, particularly lauric acid, and also contains amino acids such as L-arginine [18]. The lauric acid and its monoglyceride form monolaurin, are known for their antibacterial properties, especially against Gram-positive pathogens [19]. The high content of these fatty acids may have contributed to the inhibition of *S. aureus* observed exclusively in coconut milk in the present study.

In this context, Shori and Zahrani [20] have also reported that the fiber and sugar content of plant-based milks plays a key role in promoting probiotic growth and supporting their survival during refrigerated storage. These compounds may have synergistic effect to enhance the nutritional and functional properties of plant-based probiotic beverages. Jogi *et al.* [21] similarly evaluated the antimicrobial activity of probiotic strains isolated from plant-based milks and reported that *L. plantarum*, *L. acidophilus*, *L. rhamnosus*, *L. casei*, *L. lactis*, and *B. longum* exhibited antagonistic activity against *E. coli*, *Shigella*, *Salmonella*, and *S. aureus* in almond, coconut, and soy milks. Their findings also showed that milk composition can influence both probiotic viability and pathogen inhibition. In this regard, fermentation conditions and the specific microbial interactions during the fermentation process found to be attributable in determining the inhibitory effect in this study.

Similar results by the same researchers, Zahrani and Shori [2] compared probiotic viability and antioxidant activity in soy and almond milks fermented with *Lb*GG. The researchers observed that the strains maintained their viability during 21 d of storage consistent with the findings of the present study. They emphasized the role of synergistic matrix effects in supporting probiotic functionality.

The initial pH levels differed among milk types prior to *Lb*GG inoculation ($P<0.05$). After 24 h of fermentation, the lowest pH value was measured in dairy milk (4.62 ± 0.02), indicating greater acidification compared to the others.

During the cold storage period (0–21 d), pH values either continued to decrease or remained relatively stable, depending on the milk type. Almond milk showed the highest decrease in pH, reaching 4.31 ± 0.02 by d 21. In contrast, soy milk retained higher pH values throughout storage, ending at 4.97 ± 0.02. These results suggest that the extent of acidification during storage is influenced by both the milk matrix and the post-fermentation pH level (TABLE V).

The acidity values were analyzed before fermentation; the dairy milk was measured at the highest value ($P<0.05$). The acidity increased significantly on the last d of storage (d 21) compared to the first measurement (d 0) in all milk samples except soy milk. In soy milk, an increase was first observed for acidity after the d 0 measurement, and no change was observed on the 7th and 14th d of storage. On d 21, it decreased and reached a level lower than the d 0 measurement (TABLE VI).

Beyond the influence of milk composition, the extended fermentation period in this study (24 h) may have also contributed to the observed microbial behavior and metabolite production. During extended fermentation periods, LAB typically enter a stationary growth phase, which enables the activation of secondary metabolic pathways and the onset of mixed acid fermentation [22].

In a study by Montemurro *et al.* [23], the production of soy-based yogurt through a 6 hour fermentation with LAB resulted in lower levels of digestible protein compared to unfermented soy milk. This was attributed to the weak proteolytic activity during the early stages of fermentation, when LAB preferentially utilize free amino acids available in the substrate.

Yuyama *et al.* [24] tested the antibiofilm effects of various fatty acids, including linoleic acid. They reported that Gram-positive bacteria were more susceptible to the inhibitory effects of long-chain fatty acids compared to Gram-negative bacteria. Similarly, Kusumah *et al.* [25] demonstrated that both linoleic and α–linolenic acids exhibited antibacterial activity against *S. aureus* and *Bacillus subtilis*, significantly impairing bacterial growth. In this study, a similar mechanism might also explain the antimicrobial effect observed in coconut milk, which compared to dairy milk showed greater suppression of *S. aureus* and is characterized by its distinct medium-chain fatty acid profile.

Considering that the fermentation period in the present study was extended to 24 h, it is possible that proteolytic activity may have increased following the initial depletion of free amino acids. The prolonged fermentation may allow for more extensive protein degradation and secondary metabolite production. Compared to dairy milk, the observed trends in plant-based alternatives suggest that under equivalent fermentation conditions, they can support similar microbial activity and metabolite development.

When compared with dairy milk, plant-based milks particularly almond and coconut showed more pronounced pH declines by the end of storage, implying comparable fermentation dynamics. But in contrast, Demircan [26] reported a lower post-fermentation pH

TABLE V
pH Values of milk samples during fermentation and cold storage

Milk Type	0 h (F)	8 h (F)	24 h (F)	Day 0 (S)	Day 7 (S)	Day 14 (S)	Day 21 (S)
Dairy milk	$6.84 \pm 0.02^{b,A}$	$6.78 \pm 0.02^{c,B}$	$4.62 \pm 0.02^{a,C}$	$4.62 \pm 0.02^{a,C}$	$4.51 \pm 0.02^{a,B}$	$4.46 \pm 0.03^{b,A}$	$4.61 \pm 0.02^{b,C}$
Soy milk	$7.26 \pm 0.01^{c,A}$	$6.62 \pm 0.02^{b,B}$	$5.65 \pm 0.02^{c,C}$	$5.65 \pm 0.02^{c,C}$	$5.20 \pm 0.03^{c,B}$	$4.99 \pm 0.02^{c,A}$	$4.97 \pm 0.02^{c,A}$
Coconut milk	$6.73 \pm 0.01^{a,A}$	$6.27 \pm 0.03^{a,B}$	$4.77 \pm 0.07^{b,C}$	$4.77 \pm 0.07^{b,C}$	$4.56 \pm 0.01^{b,B}$	$4.50 \pm 0.01^{b,A}$	$4.61 \pm 0.01^{b,B}$
Almond milk	$7.65 \pm 0.02^{d,A}$	$6.91 \pm 0.02^{d,B}$	$4.66 \pm 0.02^{ab,C}$	$4.66 \pm 0.02^{a,C}$	$4.48 \pm 0.02^{a,B}$	$4.35 \pm 0.02^{a,A}$	$4.31 \pm 0.02^{a,A}$

F: fermentation, S: storage. Different lowercase letters (a, b, c, d) within rows indicate significant differences over time ($P<0.05$). Uppercase letters (A, B, C, D) within columns indicate differences between milk types at each time point

TABLE VI
Titratable acidity values of milk samples during fermentation and cold storage

Milk Type	0 h (F)	8 h (F)	24 h (F)	Day 0 (S)	Day 7 (S)	Day 14 (S)	Day 21 (S)
Dairy milk	$0.209 \pm 0.002^{d,A}$	$0.230 \pm 0.004^{d,B}$	$0.261 \pm 0.004^{c,C}$	$0.261 \pm 0.004^{c,A}$	$0.430 \pm 0.002^{c,B}$	$0.566 \pm 0.000^{d,C}$	$0.713 \pm 0.007^{b,BC}$
Soy milk	$0.053 \pm 0.003^{c,A}$	$0.120 \pm 0.002^{c,B}$	$0.140 \pm 0.004^{b,C}$	$0.140 \pm 0.004^{b,A}$	$0.162 \pm 0.005^{b,B}$	$0.163 \pm 0.004^{c,B}$	$0.120 \pm 0.002^{a,AB}$
Coconut milk	$0.022 \pm 0.000^{b,A}$	$0.067 \pm 0.004^{b,B}$	$0.066 \pm 0.003^{a,B}$	$0.066 \pm 0.003^{a,A}$	$0.079 \pm 0.002^{a,B}$	$0.070 \pm 0.002^{a,AB}$	$0.274 \pm 0.352^{ab,AB}$
Almond milk	$0.009 \pm 0.00^{a,A}$	$0.049 \pm 0.001^{a,B}$	$0.058 \pm 0.003^{a,B}$	$0.058 \pm 0.003^{a,A}$	$0.073 \pm 0.004^{a,B}$	$0.101 \pm 0.002^{b,C}$	$0.129 \pm 0.003^{a,ABC}$

F: fermentation, S: storage. Different lowercase letters (a, b, c, d) within rows indicate significant differences over time ($P<0.05$). Uppercase letters (A, B, C, D, E) within columns indicate differences between milk types at each time point

of 4.2 in soy milk fermented with LAB at 37°C for 6 h. Variations in pH and acidity observed appear to be influenced by factors such as the type of probiotic strains used, fermentation duration, and the composition of plant-based milks.

A similar study by Deziderio *et al.* [27] reported minimal changes in pH and acidity in almond and soy milk after 12 h of fermentation. In contrast, the present study resulted in more pronounced decreases in pH and increases in titratable acidity. The prolonged fermentation period may have been the difference between studies that highligting the importance of fermentation time in physicochemical properties. Erik and Ergenekon [28] observed a gradual increase in pH and a decrease in acidity in whey cheese during storage, whereas the opposite trend was noted in probiotic milk samples in this study. These discrepancies could be influenced by differences in milk types and product type.

The pH of soy milk after 24 h of fermentation was higher than that of cow milk ($P<0.05$), highlighting a distinct buffering response. Similarly, Okur [29] reported higher pH values in soy milk-based beverages, which were attributed to the high buffering capacity of soy milk. The buffering capacity may influence fermentation dynamics and acidification behavior. It is known as a key factor affecting changes in acidity and pH. The protein content and intrinsic pH level of the food are primary determinants of its buffering potential.

In a related study, Wang *et al.* [30] fermented dairy milk, soy milk, and oat milk until their pH values reached 4.5. Although the initial pH of dairy milk was higher than that of soy milk, it reached the target pH more rapidly than soy milk, suggesting that dairy milk had a lower buffering capacity under the same conditions. The more pronounced acidification observed in dairy milk may be partially explained by the presence of lactose, a readily fermentable carbohydrate efficiently utilized by lactic acid bacteria such as *Lb*GG, whereas plant-based alternatives lack lactose and differ in carbohydrate composition.

This relationship between protein content and buffering capacity has also been linked to fermentation duration in various plant-based milks. Masia *et al.* [31] stated that plant-based milks containing high-molecular-weight proteins exhibit stronger buffering capacities, resulting in prolonged fermentation times. Consistent with this higher protein content and buffering potential, soy milk maintained a higher pH value after 24 h of fermentation compared to dairy milk [32], suggesting that it resisted acidification more effectively under similar conditions.

A limitation of this study is the absence of pathogen-only controls within each milk matrix. Therefore, the observed reductions should be interpreted as matrix-associated effects in *Lb*GG-containing systems rather than being attributed exclusively to probiotic activity. Future studies may further clarify the relative contribution of matrix composition and microbial interactions.

CONCLUSION

The reductions observed in *S. aureus* and *E. coli* counts in almond and coconut milks may result from a combined effect of *Lb*GG activity and matrix-related characteristics. Although this study did not aim to isolate the specific contribution of each factor, the results provide a meaningful basis for future research focusing on targeted probiotic-pathogen-matrix interactions.

Overall, the findings highlight that certain plant-based milk alternatives may serve as effective probiotic carriers, offering functional potential similar to dairy milk. Further studies are needed incorporating detailed compositional, nutritional, and sensory evaluations to clarify the mechanisms underlying these effects and support the development of optimized non-dairy probiotic beverages.

ACKNOWLEDGEMENTS

This study was financially supported by Selcuk University Scientific Research Projects Coordination Unit under project number 24202005.

Conflicts of Interest

The authors declare no conflicts of interest.

Author contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by both authors. The first draft of the manuscript was written by EYB and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

This article does not contain any studies with human participants or animals performed by any of the authors.

Data availability

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

BIBLIOGRAPHICS REFERENCES

[1] Paul AA, Kumar S, Kumar V, Sharma R. Milk Analog: Plant based alternatives to conventional milk, production, potential and health concerns. Crit. Rev. Food Sci. Nutr. [Internet]. 2020; 60(18):3005–3023. doi: https://doi.org/gj33hn

[2] Zahrani AJA, Shori AB. Viability of probiotics and antioxidant activity of soy and almond milk fermented with selected strains of probiotic *Lactobacillus* spp. LWT 2023; 176:114531. doi: https://doi.org/g6jmr8

[3] Ranadheera RDCS, Baines SK, Adams MC. Importance of food in probiotic efficacy. Food Res. Int. [Internet]. 2010; 43(1):1–7. doi: https://doi.org/cwcsx8

[4] Craig WJ, Fresán U. International analysis of the nutritional content and a review of health benefits of non-dairy plant-based beverages. Nutrients [Internet]. 2021; 13(3): 842. doi: https://doi.org/gqzs25

[5] Kyrylenko A, Eijlander RT, Alliney G, Lucas-van de Bos E, Wells-Bennik MHJ. Levels and types of microbial contaminants in different plant-based ingredients used in dairy alternatives. Int. J. Food Microbiol. [Internet]. 2023; 407:110392. doi: https://doi.org/gszqzb

[6] Rasika DMD, Vidanarachchi JK, Luiz SF, Azeredo DRP, Cruz AG, Ranadheera CS. Probiotic delivery through non-dairy plant-based food matrices. Agriculture [Internet]. 2021; 11(7):599. doi: https://doi.org/qx29

[7] Abebe E, Gugsa G, Ahmed M. Review on major food–borne zoonotic bacterial pathogens. J. Trop. Med. [Internet]. 2020; 2020:4674235. doi: https://doi.org/gmq43k

[8] Fetsch A, Johler S. *Staphylococcus aureus* as a foodborne pathogen. Curr. Clin. Microbiol. Rep. [Internet]. 2018; 5:88–96. doi: https://doi.org/qx3b

[9] Lorenzo JM, Munekata PE, Dominguez-Valencia R, Pateiro M, Saraiva JA, Franco D. Main groups of microorganisms of relevance for food safety and stability: General aspects and overall description. In: Barba FJ, Santana AS, Orlien V, Koubaa M, editors. Innovative technologies for food preservation. London (UK): Academic Press; 2018 [cited 20 Dec 2025]. p. 53–107. Available in: https://goo.su/H0F4

[10] Yeşilova Y, Sula B, Yavuz E, Uçmak D. Probiyotikler [Probiotics]. J. Kartal TR. 2010; 21(1):49–56. https://goo.su/EsJ6t

[11] Tomasello F, Valero A, Serraino A, Possas A. Methods of inoculation and quantification for collecting data on microbial responses in foods. In: Alvarenga VO, editor. Basic protocols in predictive food microbiology. New York (USA): Springer; 2023. p. 31–46. doi: https://doi.org/qx7z

[12] Tekinşen OC, Atasever M, Keleş A, Tekinşen KK. Süt, Yoğurt, Tereyağı, Peynir: Üretim control [Milk, Yogurt, Butter, Cheese: Production control]. Konya (Türkiye): Selçuk University Press; 2002. 91 p. Turkish.

[13] Kılınç GE, Keser A, Özer HB. Determination of nutritional value, antioxidant activities, microbiological and sensory properties of almond, soy and oat based fermented beverages. J. Culin. Sci. Technol. [Internet]. 2026; 24(1):16–32. doi: https://doi.org/qx75

[14] Fatima SM, Hekmat S. Microbial and sensory analysis of soy and dairy milk-based yogurt as a probiotic matrix for *Lactobacillus rhamnosus* GR–1. Fermentation [Internet]. 2020; 6(3):74. doi: https://doi.org/qx76

[15] Bouarab-Chibane L, Degraeve P, Ferhout H, Bouajila J, Oulahal N. Plant antimicrobial polyphenols as potential natural food preservatives. J. Sci. Food Agric. [Internet]. 2019; 99(4):1457–1474. doi: https://doi.org/gkf5jt

[16] Nitbani FO, Tjitda PJP, Nitti F, Jumina J, Detha AIR. Antimicrobial properties of lauric acid and monolaurin in virgin coconut oil: a review. ChemBioEng. Rev. [Internet]. 2022; 9(5):442–461. doi: https://doi.org/qx78

[17] Mandalari G, Bisignano C, D'Arrigo M, Ginestra G, Arena A, Tomaino A, Wickham MSJ. Antimicrobial potential of polyphenols extracted from almond skins. Lett. Appl. Microbiol. [Internet]. 2010; 51(1):83–89. doi: https://doi.org/d3kk9r

[18] Eyres L, Eyres MF, Chisholm A, Brown RC. Coconut oil consumption and cardiovascular risk factors in humans. Nutr. Rev. [Internet]. 2016; 74(4):267–280. doi: https://doi.org/f8jfvd

[19] Nasir NAMM, Abllah Z, Jalaludin AA, Shahdan IA, Abd-Manan WNHW. Virgin coconut oil and its antimicrobial properties against pathogenic microorganisms: a review. Adv. Health Sci. Res. [Internet]. 2018 [cited 13 Oct 2025]; 192–199. Available in: https://goo.su/ByKb

[20] Shori AB, Al Zahrani AJ. Non-dairy plant-based milk products as alternatives to conventional dairy products for delivering probiotics. Food Sci. Technol. [Internet]. 2022; 42:e101321. doi: https://doi.org/qx8c

[21] Jogi S, Adhao A, Pachori RR. Exploration of probiotic bacteria from plant based milk. Int. J. Res. Anal. Rev. [Internet]. 2024 [cited 22 Dec 2025]; 11(1):157–163. Available in: https://goo.su/xuUSjt

[22] Genet BML, Xiao H, Christensen LF, Laforce IN, Mohammadifar MA, Bang-Berthelsen CH, Hansen EB. Selection of proteolytic LAB starter cultures for acidification of soy based dairy alternatives. LWT [Internet]. 2023; 184:115082. doi: https://doi.org/qx8h

[23] Montemurro M, Pontonio E, Coda R, Rizzello CG. Plant-based alternatives to yogurt: state-of-the-art and perspectives of new biotechnological challenges. Foods. [Internet]. 2021; 10(2):316. doi: https://doi.org/gt57q2

[24] Yuyama KT, Rohde M, Molinari G, Stadler M, Abraham WR. Unsaturated fatty acids control biofilm formation of *Staphylococcus aureus* and other gram-positive bacteria. Antibiotics 2020; 9(11):788. doi: https://doi.org/qx8k

[25] Kusumah D, Wakui M, Murakami M, Xie X, Yukihito K, Maeda I. Linoleic acid, α–linolenic acid, and monolinolenins as antibacterial substances in the heat-processed soybean fermented with *Rhizopus oligosporus*. Biosci. Biotechnol. Biochem. [Internet]. 2020; 84(6):1285–1290. doi: https://doi.org/qx8m

[26] Demircan B. Probiyotik laktik asit bakterileri kullanılarak bitkisel süt ikamelerinden fermente içecek tasarımı [Production of novel vegetal based beverages fermented by probiotic lactic acid bacteria]. [master's thesis on the Internet]. İstanbul (Türkiye): Istanbul Technical University; 2017 [cited 20 Jan 2026]. 99 p. Turkish. Available in: https://goo.su/Cd2kx

[27] Deziderio MA, de Souza HF, Kamimura ES, Petrus RR. Plant-based fermented beverages: Development and characterization. Foods [Internet]. 2023; 12(22):4128. doi: https://doi.org/qx8n

[28] Erik S, Ergenekon FS. Lor Peynirlerine İlave Edilen Probiyotik Kültürlerin Bazı Patojen Mikroorganizmaların Yaşam Süreleri Üzerine Etkisinin Araştırılması [Investigation of the effect of probiotic cultures added in lor cheese on the life span of some pathogenic microorganisms]. Akademik Et ve Süt Kurumu Derg. [Internet]. 2024 [cited 20 Dec 2025]; (7):4–14. Turkish. Available in: https://goo.su/ETJn3A8

[29] Okur HH. Probiyotik Lactobacillus rhamnosus GG (ATCC 53103) suşu kullanarak fermente süt ürünü üretimi [Production of fermented milk product by using probiotic Lactobacillus rhamnosus GG (ATCC 53103) strain]. [master's thesis on the Internet]. İzmir (Türkiye): Ege University; 2022 [Cited 20 Jan 2026]. 114 p. Turkish. Available in: https://goo.su/XnFVno8

[30] Wang X, Kong X, Zhang C, Hua Y, Chen Y, Li X. Comparison of physicochemical properties and volatile flavor compounds of plant-based yoghurt and dairy yoghurt. Food Res. Int. [Internet]. 2023; 164:112375. doi: https://doi.org/grs87k

[31] Masia C, Geppel A, Jensen PE, Buldo P. Effect of *Lactobacillus rhamnosus* on Physicochemical Properties of Fermented Plant-Based Raw Materials. Foods [Internet]. 2021; 10(3):573. doi: https://doi.org/gjp38f

[32] Yiğit AA. Animal and plant-based milk and their antioxidant properties. MAEU Vet. Fak. Derg. [Internet]. 2019; 4(2):113–122. doi: https://doi.org/qx83