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Probiotic Cottage Cheese Formulated with *Lactobacillus acidophilus* LA-5: Microbial Viability, Acidification Kinetics, and Consumer Acceptance

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Abstract

Cottage cheese is a promising matrix for delivering probiotic microorganisms due to its buffering capacity, high moisture content, and consumer acceptance. In this study, a probiotic cottage cheese incorporating *Lactobacillus acidophilus* LA-5 was developed and evaluated against a control formulation and commercial products. Physicochemical properties, probiotic viability, acidification kinetics, and sensory acceptance were assessed during refrigerated storage. The formulation consistently maintains viable probiotic counts above 10^8 CFU/g for 24 days, meeting the minimum threshold for health benefits. Although gradual post-acidification was observed, changes in pH (a decrease ≈ of 0.79 pH units) and titratable acidity (from 0.32 to 0.46%) did not significantly compromise probiotic survival, indicating a protective effect of the cheese matrix. Sensory evaluation, including triangular discrimination and hedonic testing, demonstrated that incorporating probiotics did not significantly affect consumer perception ($p > 0.05$), with overall acceptability scores (7.20) comparable to those of the control (7.45) and commercial cheeses (6.95-7.45). These findings highlight cottage cheese's potential as an effective probiotic carrier, delivering microbial doses while maintaining sensory and technological qualities. This study supports the development of functional dairy foods as alternatives to dietary supplements for gut health and well-being.

Keywords
Cottage cheese; probiotics; functional food; *Lactobacillus acidophilus*; sensory evaluation

1. Introduction

Probiotic foods are those that contain live microorganisms that, when consumed in sufficient quantities, confer health benefits on the host [1, 2]. Globally, consumption of probiotic foods has increased due to their health benefits, including modulation of the gut microbiota and immune function [3]. Probiotics support health, especially as populations age and disease prevention becomes vital. They help maintain microbial balance and improve gut health [4]. These probiotics can serve as starter cultures alone, in combination with traditional starters, or within fermented foods, thereby enhancing aroma, flavor, texture, and health benefits [4]. The importance of the microbiota is increasingly recognized, with evidence that probiotics in dairy products can improve several metabolic functions [5]. The dairy industry is the leading sector integrating probiotics into products such as fermented milk, cheese, yogurt, and cream. Despite scientific evidence supporting the health benefits of probiotic foods, expanding daily consumption through emerging non-dairy vehicles remains a significant challenge due to poor sensory appeal that fails to meet consumer expectations [3]. In dairy science, cheese matrices are increasingly recognized as effective carriers for probiotic cultures because of their high buffering capacity, which protects bacteria during storage and gastrointestinal transit [6-9]. Probiotic strains such as *Lactobacillus acidophilus*, *Bifidobacterium* spp., and *Lacticaseibacillus casei* can be added to develop functional cheeses [10]. Among probiotic strains, *L. acidophilus* LA-5 is widely studied for its health-promoting properties and technological compatibility with dairy products [11-13]. This strain is linked to improved gut microbiota, a stronger immune response, and a potential reduction in gastrointestinal disorders. To exert these probiotic effects, a minimum viable population of approximately 10^6-10^7 CFU/g (or CFU per serving) at the time of consumption is generally required. However, maintaining its viability at levels sufficient for functional claims (>10^7 CFU/g) throughout the shelf life of fresh cheeses remains a challenge [14, 15].

Fresh cheeses, such as cottage cheese, are characterized by high moisture content and relatively mild acidity, conditions that can favor the survival of probiotic microorganisms during refrigerated storage [16, 17]. Cottage cheese is a soft, unripened cheese with a mild acidic pH ranging from 4.75 to 5.30. It is usually made from pasteurized skim milk, where caseins coagulate near their isoelectric point due to the action of lactic acid bacteria or food-grade acids [18, 19]. The manufacturing process includes a washing step that imparts a slightly acidic, salty taste, a subtle diacetyl flavor, and a creamy aroma [8]. According to the U.S. Code of Federal Regulations, cottage cheese is defined as a "soft uncured cheese prepared by mixing cottage cheese dry curd with a creaming mixture" (21 CFR 133.128) [20]. In the process, pasteurized cream (10-20% fat) with salt and stabilizers is added and completely mixed with the curd [8, 19, 21, 22], and after a short holding period, the product is packaged and stored at 4-5°C, with a typical shelf life of 3-4 weeks. Cottage cheese, widely consumed in the US and UK and used in salads and desserts [18], makes up about 5% of global cheese production [21, 22]. Recent innovations in cottage cheese production have focused on improving its functional and nutritional qualities, including adding probiotic cultures and prebiotics, extending shelf life, developing single-serve packaging formats, fortifying with calcium, and using bacteriocin-producing cultures [22].

Growing interest in functional cottage cheese involves adding probiotic microorganisms. Previous studies have demonstrated that cottage cheese effectively supports the survival of probiotic bacteria during storage. Abadía-García et al. [12] reported that *Lactobacillus casei*, *Lactobacillus rhamnosus* GG, and commercial probiotic blends remained above 10^6 CFU/g over 28 days at 8°C, confirming their suitability as a probiotic carrier. Additionally, the presence of probiotics was associated with increased production of lactic and acetic acids, indicating metabolic activity within the cheese matrix. Evidence from other fresh, soft cheeses supports these findings and reinforces their applicability to cottage cheese systems. Probiotic strains such as *L. acidophilus* and *Bifidobacterium animalis* subsp. *lactis* have been shown to maintain viable populations (approximately 10^6-10^8 CFU/g) during storage without adversely affecting key quality parameters. However, slight increases in acidity and changes in flavor perception have been reported [23, 24]. Similarly, high probiotic viability has been observed in other cheese matrices, often exceeding the recommended therapeutic threshold of 10^7 CFU/g, highlighting the protective effect of the dairy matrix.

Although probiotic dairy foods are becoming more common, most research on probiotic cheeses concentrates on microbial counts during storage and evaluates them against thresholds for functional foods. In this context, the present study aimed to characterize probiotic cottage cheese produced with *Lactobacillus acidophilus* LA-5 by analyzing probiotic viability and pH changes during refrigerated storage, estimating the functional shelf life, evaluating the relationship between acidification and probiotic survival, and conducting sensory tests to assess product acceptability. This approach provides a comprehensive evaluation of the microbiological stability, technological features, and sensory qualities of probiotic cottage cheese as a potential functional dairy product.

2. Materials and Methods

Commercial partial-skim milk (pasteurized) and milk cream (Ingredients: 98.7% cow's milk cream, citric acid, guar gum, carrageenan, iodized salt, natamycin, cow's milk fat minimum 25%) were acquired in a local supermarket in Puebla (Mexico) and utilized. Liquid rennet, Chymosin (CUAMEX, Chr. Hansen de México S.A. de C.V., Ixtapalapa, Mexico), and white cane alcohol vinegar were used to obtain the curd.

Two commercial cottage cheese brands were characterized to establish a reference framework for developing probiotic formulations, brand A (Ingredients: pasteurized partially skimmed cow's milk, cream, skimmed milk powder, lactic cultures, iodized salt, potassium sorbate, calcium chloride, and rennet) and brand B (Ingredients: pasteurized skimmed milk, cream, milk solids, iodized salt, lactic cultures, potassium sorbate, sorbic acid, calcium chloride, carboxymethylcellulose, mono- and diglycerides, natamycin, carrageenan, and rennet).

2.1 Making Cottage Cheese

The cottage cheese was made following Kosikowski's [25] method for short-setting cottage cheese, with some adaptations. Two systems were prepared, each with 1000 mL of skimmed milk. The process is divided into the following stages:

Partial-skim pasteurized milk was used (3.1 g protein, 0.6 g fat). The milk was warmed to 32.2°C and inoculated with or without a 1% (v/v) volume of a liquid culture of the probiotic lactic acid bacterium (*L. acidophilus* LA-5, standardized to ≈10^9 CFU/mL, yielding an initial milk concentration of ≈10^7 CFU/g). Calcium chloride was added in a proportion of 0.02% to improve coagulation properties. The mixture was incubated at 36°C until the pH reached approximately 5.5. Then 0.5% rennet (w/v) was added and incubated at 36°C for 1 hour, after which 20 mL/L of vinegar (5% acetic acid) was added. The grains were left to rest for 15 minutes to allow for initial syneresis and surface hardening.

The cooking process involved gradual heating, with the temperature rising every 5 minutes during the first half hour (to reach ≈42°C), then increasing more rapidly (≈ to 50°C). During this stage, gentle, steady stirring was maintained to avoid grain clumping and ensure even heat distribution. After scalding, the whey was drained, and the curd was washed three times with purified water at decreasing temperatures (29, 16, and 4.4°C). The cheese was then drained for 30 minutes before proceeding to the creaming step. The cream was prepared following the method described by Kosikowski [25], with the following composition: 56% skimmed milk, 40% cream, and 4% salt; 330 grams of prepared cream are added for every 1000 grams of curd.

2.2 Probiotic Preparation

The probiotic *L. acidophilus* LA-5 was obtained from a pharmaceutical preparation (Winthrop Pharmaceutical de México, S.A. de C.V., Edo. de México, Mexico). Inoculations were performed using a loop into previously prepared de Man Rogosa and Sharpe broth (Difco, BD, Sparks, MD), and the cultures were incubated for 48 hours at 35°C. For use in cottage cheese production, the incubated broth was centrifuged at 8000 × g for 10 minutes at 4°C (Marathon 21k/R, Fisher Scientific, Germany) and washed twice with phosphate buffer (pH 7.0). Wet cells were used on the same day for cheesemaking.

2.3 Physicochemical Analysis of Milk

Water activity (a_w) was measured using an Aqualab Series 3TE instrument (Decagon Devices, Inc., Pullman, WA). Fat content was evaluated using the Mojonnier gravimetric method (AOAC 995.19) [26], and total crude protein content was determined by the micro-Kjeldahl method (AOAC 960.52A) [27]. Milk pH was measured by direct electrode immersion with a digital pH meter (UB-10, Denver Instrument, Bohemia, NY), previously calibrated with standard buffer solutions at pH 4.0 and 7.0. Milk titratable acidity stage was determined by titration with 0.1 N NaOH and expressed as a percentage of lactic acid. The tests were conducted in triplicate.

2.4 Physicochemical and Optical Analysis of Cottage Cheese

Physicochemical and optical evaluations of the cottage cheese samples were performed in triplicate. The moisture content was determined following the official AOAC gravimetric method 926.08 [28]. Fat content was determined by the Mojonnier method (995.19) [26], and total protein content (micro-Kjeldahl method) was determined according to AOAC Method 960.52A [27]. For pH monitoring initially and during refrigerated storage, measurements were taken by direct immersion of the calibrated electrode into the cheese matrix. Titratable acidity at the initial and during storage was determined by thoroughly homogenizing 10 g of cheese with 15 mL of distilled water, followed by titration with 0.1 N NaOH; results were expressed as a percentage of lactic acid. Water activity was measured using an Aqualab Series 3TE water activity meter (Decagon Devices, Inc., Pullman, WA). Color measurements were performed in triplicate using the Hunter scale parameters: *Lh* (lightness), *ah* (red-green), and *bh* (yellow-blue), with a Color Gard System/05 colorimeter (BYK-Gardner, Inc., Columbia, MD). The colorimeter was operated under illuminant D65 with a 10° standard observer and calibrated in reflectance mode before testing the samples. Based on the values of *Lh* and *bh*, the Hue (H) and Chroma (C) of the processed and commercial cottage cheeses were calculated, using the following equations:

$$Chroma = \sqrt{ah^2 + bh^2}$$

$$Hue = arctan\left(\frac{bh}{ah}\right)$$

2.5 Probiotic Viability - Acidity Relationship During Cheese Storage

To evaluate cheese behavior during storage, samples were packaged in opaque plastic containers, sealed with plastic film, and stored at 4°C for 24 days. Physicochemical and microbiological analyses were systematically conducted at designated storage intervals, with day 1 as the baseline, selected over immediate post-manufacturing testing to allow stabilization under cold storage conditions. The pH was measured every two days using the previously described pH meter. At the same intervals, 10 g of the cheese sample was aseptically collected, homogenized in 90 mL of sterile peptone water, and serially diluted (10-fold). Appropriate dilutions were plated on MRS agar (Difco, BD, Sparks, MD) using the pour plate method for probiotic enumeration after 24 h of incubation at 35°C.

To quantify the effect of acidification on probiotic survival during storage, an acid-viability sensitivity coefficient (AVSC) was calculated from probiotic counts and pH. It indicates the change in probiotic viability (log CFU/g) per unit pH change as:

$$AVSC = \frac{\partial(\log N)}{\partial(pH)}$$

where:
N = probiotic population (CFU/g)
$\partial(\log N)$ = change in probiotic counts (log CFU g) during storage
$\partial(pH)$ = change in pH over the same period

2.6 Sensory Evaluation

The effects of incorporating probiotic microorganisms on sensory attributes were evaluated using two triangle tests and a hedonic test with a structured scale. The samples were evaluated by a panel of 30 untrained panelists who were regular consumers of cottage cheese. For the first triangle test, they were presented with two identical samples and one different sample, comparing commercial cheese and probiotic cottage cheese. In the second triangle test, they were presented with two identical samples and one different sample of the two cheeses elaborated in this work (with and without probiotics). In the hedonic test, the attributes evaluated were flavor, color, texture, and overall acceptability. For this test, the panelists were presented with samples of the produced cottage cheeses and the two commercial ones, along with a structured questionnaire, a 9-point hedonic scale (from 1 “I dislike it extremely” to 9 “I like it extremely”).

The sensory evaluation tests adhered to ethical principles for research involving human participants. The protocol was reviewed and approved by the Research and Ethics Committee on Sensory Evaluation of Foods of the doctoral program in Food Science at the Universidad de las Américas Puebla before testing. Each panelist received an Informed Consent form detailing the study’s purpose to evaluate the sensory properties of commercially produced cottage cheese, the ingredients, and allergens, and listing the ingredients used (cow’s milk, lactic cultures, enzymes, etc.). The form also stated the participant’s right to withdraw from the panel at any time without penalty. Collected data are handled anonymously and used solely for statistical and academic purposes. Only panelists who voluntarily signed the document participated in the tests.

2.7 Statistical Analysis

The results obtained for each analyzed property and sensory attribute were analyzed with ANOVA and Tukey’s mean comparison tests ($p < 0.05$) using Minitab 20 software (Minitab LLC, PA, USA). For triangle difference sensory tests, the results were compared with the statistical tables of Roessler et al. at a significance level of $p < 0.05$. This analysis determines whether the panelists’ correct answers exceeded what would be expected by chance, thereby confirming significant differences between the cheese produced and the commercial references.

3. Results and Discussion

3.1 Characterization of Raw Materials

The physicochemical profile of the pasteurized, partially skimmed milk used for cottage cheese (Table 1) reflects typical raw material quality for acid-coagulated cheeses. The pH of 6.80 and low titratable acidity show minimal indigenous fermentation, typical of pasteurized milk [19]. Low acidity and near-neutral pH are essential for consistent acidification in cheese-making and probiotic growth, with high lactose availability [29, 30].

Table 1 Physicochemical profile of pasteurized partially skim milk used in the production of cottage cheese.

Parameter	Average value	Reference value
pH	6.80 ± 0.01	6.60-6.80 [19]
Titratable acidity (% lactic acid)	0.18 ± 0.01	0.13-0.18 [31]
Fat (%)	0.65 ± 0.04	1.0 [31]
Protein (%)	3.30 ± 0.58	≈3.0 [31]
a_w	0.992 ± 0.001	≈0.99 [32]
	Lh = 80.5 ± 0.99	76.5 [33]
Color	*ah* = -1.62 ± 0.19	-1.4 [33]
	bh = 6.87 ± 0.40	6.6 [33]

The fat content aligns with partial skimming and supports the production of cottage cheeses [21]. High water activity was expected due to the milk's high moisture content, which promotes microbial activity during processing [34, 35]. The color parameters (*Lh, ah, bh*) describe a bright, slightly warm hue, typical of fresh milk with natural pigments like riboflavin and carotenoids [19]. The prepared cream dressing had a pH of 5.53 ± 0.02 and 11.2% fat, consistent with typical creaming mixtures used to adjust texture and mouthfeel in cottage cheese [36].

3.2 Cottage Cheese Characterization

Adding cream significantly increased the yield of both cottage cheese types. For probiotic cheese, the yield went from 16.04 ± 1.12% without cream to 21.33 ± 1.37% with cream, while control cheese showed a similar pattern, rising from 15.54 ± 1.33% to 20.67 ± 1.25%. Overall, probiotic cheese consistently had slightly higher yields than the control cheese under both conditions. These results indicate that adding cream significantly increases cheese yield, likely due to improved retention of fat and total solids in the curd. Yields for cottage cheese were comparable to reported yields in the literature (~15-17%) [25, 37]. Brito et al. [38] noted a practical yield of 16.62% for cottage cheese made with homogenized cream, which is also close to the yield values observed here. The similar yields of probiotic and control cheeses indicate that the addition of *L. acidophilus* LA-5 did not adversely affect curd formation or moisture retention.

As shown in Table 2, the two commercial cottage cheeses (brands A and B) were characterized for comparison; both products exhibited high water activity (aw ≈ 0.98), consistent with their elevated moisture content and those of typical fresh cheeses [34, 35, 39, 40]. The pH values (5.01-5.27) complied with the Mexican standard NMX-F-092-1970 and fell within the expected range for cottage cheese (4.7-5.3), while titratable acidity (1.08-1.13% lactic acid) corresponded to values reported for acid-coagulated fresh cheeses and dairy matrices [12, 19]. Moisture content in commercial cheeses (~80.5%) slightly exceeded the regulatory limit but was consistent with the high-moisture nature of this cheese variety [25]. Fat content in both brands met the Codex Alimentarius [41] requirement of at least 4% for creamed cottage cheese. Protein levels (10.6-11.1%) were lower than both the label claims and the reference values in NMX-F-092-1970. This variation has been observed in other studies of commercial fresh cheeses and is likely due to differences in formulation and the amount of dairy solids included [39]. Cottage cheese's complex, biphasic structure can cause sampling issues and lead to an underestimation of crude protein. The discrepancy in protein content arises from industrial formulations and the analytical testing of moisture-rich, segmented dairy matrices.

Table 2 Comparative analysis of the parameters of the produced cottage cheeses and commercial ones.

Parameter	Cottage Cheese				Reference value
	Control	Probiotic (*Lactobacillus acidophilus* LA-5)	Brand A	Brand B	
Moisture content (%)	77.18 ± 1.50b	79.12 ± 0.30ab	80.50 ± 0.37a	80.68 ± 0.22a	78.1 [39]
Protein (%)	10.56 ± 1.03a	10.96 ± 0.69a	10.65 ± 0.45a	11.05 ± 0.75a	11.6 [39]
Fat (%)	3.94 ± 0.14b	4.10 ± 0.12b	4.49 ± 0.06a	4.17 ± 0.05b	4.2 [39]
Titratable acidity (% lactic acid)	0.96 ± 0.07b	1.54 ± 0.12a	1.08 ± 0.07b	1.13 ± 0.05b	0.6-1.0 [19]
pH	5.62 ± 0.012a	4.93 ± 0.046d	5.01 ± 0.02c	5.27 ± 0.01b	4.9-5.2 [19]
a_w	0.983 ± 0.006a	0.980 ± 0.01a	0.987 ± 0.006a	0.980 ± 0.010a	0.980-0.995 [19]
Color (*Lh)*	81.84 ± 0.11c	80.52 ± 0.02c	91.15 ± 0.73a	89.28 ± 0.62b	78.37 [33]
Color (*ah)*	-2.26 ± 0.06b	-2.35 ± 0.01b	-1.9 ± 0.04a	-2.83 ± 0.05c	0.29
Color (*bh)*	9.96 ± 0.06b	9.76 ± 0.03b	7.75 ± 0.25c	11.02 ± 0.06a	14.57
Hue (°)	102.8	103.5	103.8	104.4	
Chrome	10.2	10.0	8.0	11.4	

Different lowercase letters in each evaluated parameter indicate significant differences ($p < 0.05$) between samples.

Experimental cottage cheeses, produced without probiotic culture (control) and with *L. acidophilus* LA-5, showed physicochemical characteristics comparable to commercial products (Table 2). Both formulations complied with moisture specifications (<80%), in agreement with literature values for cottage cheese [25, 37, 38]. The absence of significant differences ($p > 0.05$) in moisture content between formulations is consistent with previous findings indicating that probiotic incorporation does not markedly affect water retention in fresh cheeses [8]. Protein contents (≈10.6-11.0%) were similar to those of commercial samples.

Fat content in the experimental cheeses was close to the Codex STAN 273-1968 minimum value; probiotic cheese (4.10%) met the requirement, whereas control cheese was marginally below (3.94%). Regarding acidification, the probiotic cheese exhibited higher ($p < 0.05$) titratable acidity (1.54 ± 0.12% lactic acid) and a lower ($p < 0.05$) pH (4.93) than the control (0.96 ± 0.07% lactic acid; pH 5.62), reflecting the metabolic activity of *L. acidophilus* LA-5. This behavior aligns with previous studies showing that probiotic lactobacilli increase lactic acid production in fresh cheeses, thereby enhancing product stability, flavor development, and probiotic viability [12, 42, 43]. Although the probiotic cheese presented a pH slightly below the minimum established by NMX-F-092-1970, it remained within the typical range reported for cottage cheese and probiotic fresh cheeses [40, 44].

Color analysis of the cheeses (Table 2) revealed subtle but statistically significant variations among the evaluated cottage cheeses (Control, Probiotic, Brand A, and Brand B). Cottage cheese is a fresh dairy product characterized by high lightness and low chroma (C), resulting in a fresh appearance [37]. The statistical color analysis showed no significant differences ($p > 0.05$) between control and probiotic cheeses, indicating that the incorporation of *L. acidophilus* LA-5 did not affect visual attributes. This is consistent with previous reports indicating that the addition of probiotics generally preserves the color characteristics of fresh cheeses, an important factor for consumer acceptance [42, 45]. Commercial samples had the highest *Lh* values (Table 2) compared with the control and probiotic batches, suggesting they use processes such as high-pressure homogenization, thickening agents, and natural hydrocolloids. These enhance light scattering from casein micelles and fat globules, boosting opacity and whiteness [46]. The *ah* parameter was negative (-1.9 to -2.83), indicating a shift towards the green spectrum typical of dairy products. The *bh* coordinate was positive, showing a yellow undertone. The hue angle was stable (102.78° to 104.40°), with only minimal changes upon adding probiotics (103.53° vs. 102.78°). This suggests probiotic fortification does not significantly alter the cheese's color, maintaining consumer-acceptable attributes [37, 46]. Overall, the results demonstrate that incorporating probiotic cultures can be achieved without major alterations to the physicochemical properties of cottage cheese, supporting its potential as a suitable carrier for probiotic delivery.

3.3 Probiotic Viability and Acidification Dynamics During Storage

In the present study, probiotic viability was monitored during refrigerated storage through periodic microbial enumeration and pH measurements (Figure 1). The initial inoculation level of *L. acidophilus* LA-5 (~10^7 CFU/g) increased rapidly to approximately 10^{10} CFU/g within the first day, indicating active adaptation to the cheese matrix during preparation steps before creaming and ensuring a high functional starting threshold from the first day of storage.

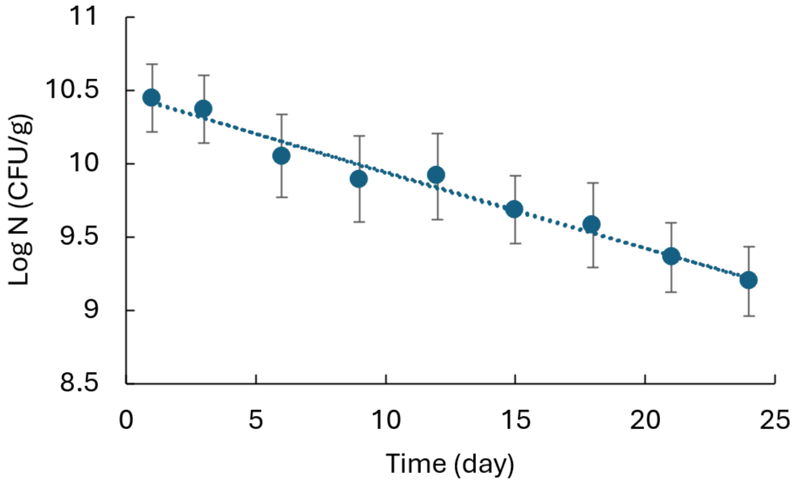


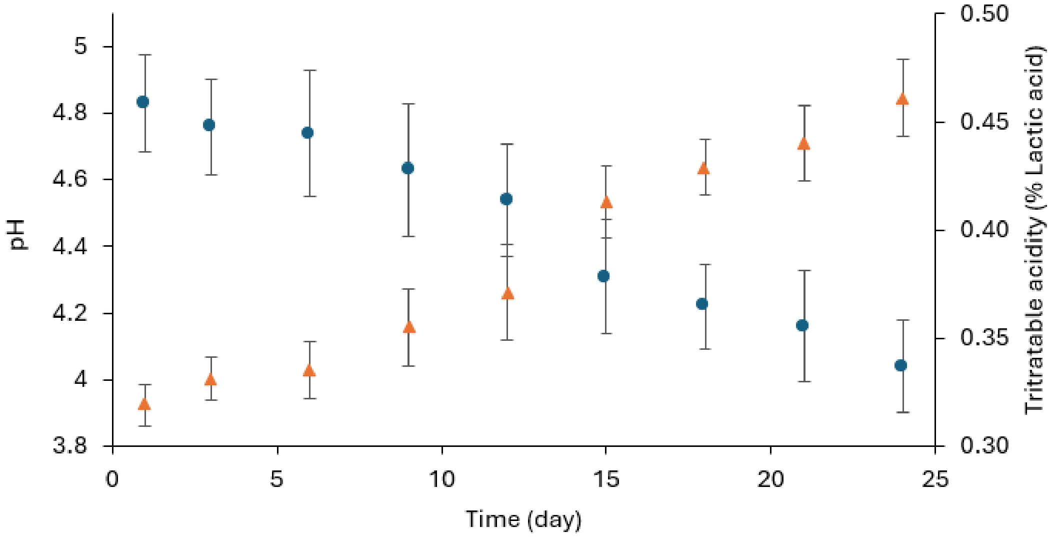
Figure 1 Probiotic viability (*Lactobacillus acidophilus* LA-5) in cottage cheese during storage at 4°C.

Viable counts remained stable until approximately day 15, then gradually declined, yet consistently exceeded 10^9 CFU/g throughout the 24-day storage period (Figure 1). These values are significantly above the minimum levels recommended for probiotic efficacy, which are generally established at ≥10^7 CFU/g at the time of consumption [2, 47, 48] and are consistent with earlier intake guidelines of 10^8-10^9 CFU/day [49]. The observed reduction in viability (~1.25 log CFU/g) followed a linear trend, consistent with previous reports of probiotic stability in cheese matrices [43, 44]. Cuffia et al. [43] reported that *L. acidophilus* and *Bifidobacterium animalis* maintained populations above therapeutic levels in probiotic cheeses during refrigerated storage. Similarly, Hernández-Figueroa et al. [44] observed sustained viability of *L. acidophilus* LA-5 (>10^8 CFU/g) in probiotic cream cheese during 30 days of storage. This stability has been attributed to the protective properties of cheese, including its protein-rich structure and buffering capacity [6, 42].

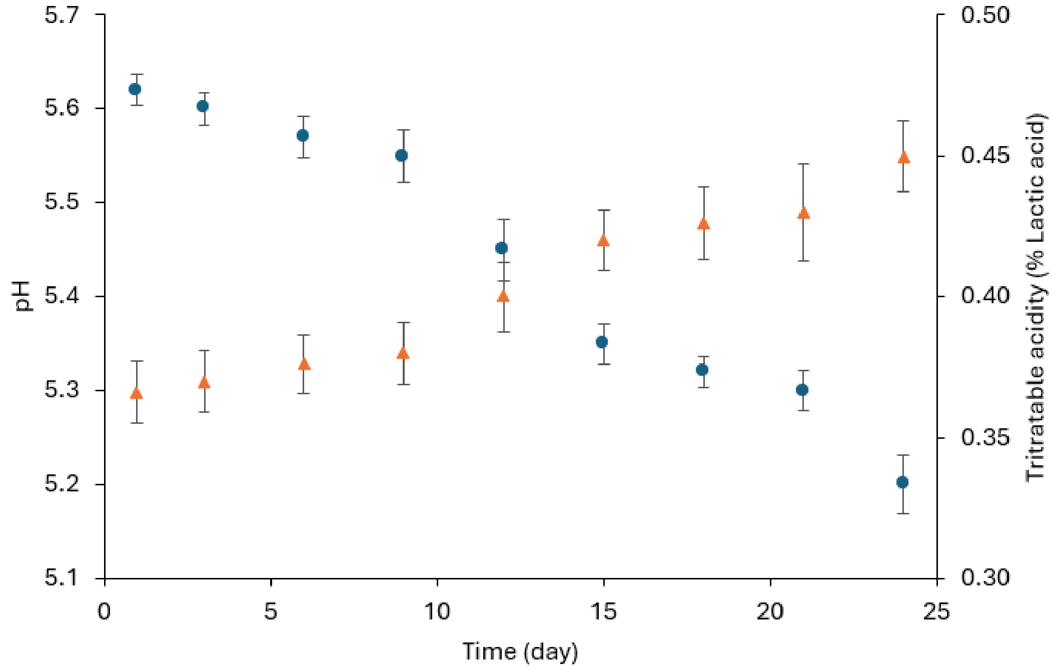
Previous studies have demonstrated the potential of cottage cheese as a probiotic delivery system. For instance, Jeon et al. [50] reported improved stability of *Lactobacillus plantarum* Lb41 in cottage cheese compared to control samples during refrigerated storage, supporting its suitability as a probiotic carrier. The incorporation of different probiotic strains and adjunct ingredients has also been widely explored. *Lactobacillus paracasei* subsp. *paracasei* LBC81 has shown probiotic potential in cottage cheese systems, with the addition of plant-based flours not significantly affecting viability, although storage time influenced microbial stability [51]. In this context, post-acidification, commonly observed in fermented dairy products, has been associated with the continued metabolic activity of LAB during refrigeration, resulting in decreased pH and increased titratable acidity.

Some studies have evaluated strategies to enhance probiotic viability in cottage cheese. Araújo et al. [52] demonstrated that inulin supplementation did not adversely affect *Lactobacillus delbrueckii* viability, while Rostami et al. [53] reported increased *Lacticaseibacillus casei* populations in cottage cheeses enriched with black cumin seeds. Ahmed et al. [54] showed that incorporating Aloe vera gel and probiotic microcapsules into cottage cheese improved probiotic stability and maintained sensory quality over 28 days of storage, with minimal loss of viability (~1 log CFU/g). Likewise, Saeed et al. [13] observed that encapsulation of *L. acidophilus* and *L. casei* in alginate-carrageenan matrices significantly enhanced survival in cottage cheese compared to free cells and improved sensory attributes. Obando et al. [15] reported that probiotic populations in cottage cheese, including *L. acidophilus* LA-5, remained above 10^6 CFU/g during refrigerated storage, with minimal impact on key sensory properties.

Acidification kinetics showed a gradual decline in pH during storage (Figure 2), with an overall decrease of about 0.79 units, along with an increase in titratable acidity from 0.32% to 0.46% lactic acid. The control cheese without *L. acidophilus* maintained greater pH stability during storage, while the probiotic cheese experienced steady acidification starting around day 6, with pH dropping from 4.8 to 4.04 by day 24. This pattern further supports the role of probiotic metabolic activity in driving acidification dynamics. Post-acidification is commonly observed in probiotic dairy products due to the continued fermentation of residual lactose during storage [42, 55, 56]. Similar pH reductions have been reported in lactobacilli-containing probiotic ricotta and fresh cheeses, where metabolic activity persists at low temperatures but at reduced rates [23, 24, 57].



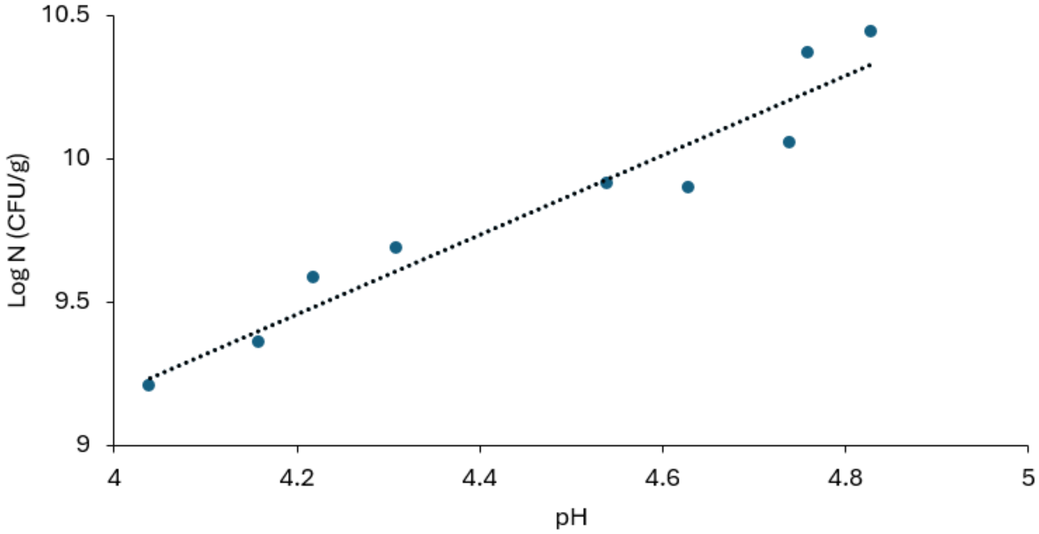
(a)



(b)

Figure 2 Comparative changes in pH (blue circles) and titratable acidity (expressed as % lactic acid, orange triangles) during refrigerated storage at 4°C. (a) control cottage cheese and (b) probiotic cottage cheese formulated with *Lactobacillus acidophilus* LA-5. Plotted points represent experimental means, and vertical bars denote the standard deviation.

A strong positive correlation between pH and probiotic viability (Figure 3) was found, indicating that acidification influenced microbial survival, although the decline was not sufficient to compromise functionality. The acid-viability sensitivity coefficient (AVSC = 1.58) indicates moderate probiotic sensitivity to pH changes, as limited acidification (<1 pH unit) maintained high counts during storage. Compared with probiotic strains in fluid systems such as fermented milk drinks or yogurt [58-60], which often have AVSC > 2.50, indicating greater sensitivity; lower AVSC indicates greater tolerance, such as in other cheeses with dense structures that exhibit low AVSC due to physical shielding [6, 8]. The intermediate AVSC of 1.58 indicates cottage cheese offers a protective, balanced microenvironment. These results confirm that the cheese matrix supports probiotic stability and microbial delivery [6, 42].



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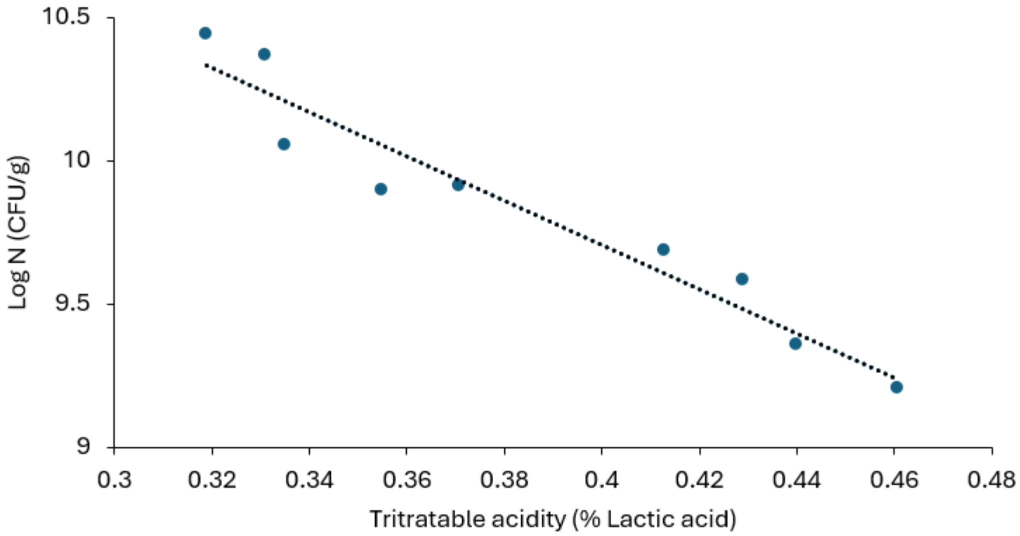

(b)

Figure 3 Linear regression and sensitivity analysis showing the relationship between matrix pH (a) or titratable acidity (b), and the viability of *Lactobacillus acidophilus* LA-5 in cottage cheese during storage. Individual data points represent the mean experimental values.

Lactic acid bacteria activity decreases pH and increases titratable acidity during refrigerated storage, a phenomenon known as post-acidification, which can cause acid stress and affect microbial viability [61, 62]. However, in cottage cheese, this impact is reduced due to its composition: it maintains a pH above 4.0 and contains protein and mineral salts that help buffer the effects of acidity [8]. Despite increased acidity, probiotic counts stay above 10^9 CFU/g, ensuring potential benefits. The results confirm that the cottage cheese matrix can protect microorganisms through its network of protein micelles, which can trap probiotic cells and reduce their exposure to oxygen and oxidative stress [7, 8]. The cream can form a film that acts as a barrier against proton diffusion and osmotic shock, and the protein concentration and moderate pH also act as buffers. This type of matrix (solid or semi-solid) offers greater protection than liquid matrices [7].

3.4 Sensory Evaluation

The sensory properties of the cottage cheeses produced (probiotic and control cheese) were assessed using two complementary methodologies: a triangular discrimination test and a nine-point hedonic evaluation of flavor, color, texture, and overall acceptability. This combined approach enables both the detection of perceptible differences and the quantification of consumer preference [63]. The triangular test revealed that only 20% of panelists correctly identified the different samples when comparing probiotic cheeses with a control one. According to standard statistical tables, this proportion is insufficient to establish a significant difference at the 95% confidence level, indicating that the incorporation of *L. acidophilus* LA-5 did not produce perceptible sensory changes relative to the control formulation. In contrast, when probiotic cheese was compared with a commercial reference, 85% of panelists correctly identified the different sample, demonstrating a significant difference ($p < 0.05$). These findings suggest that while probiotic incorporation did not substantially modify the sensory profile of the experimental cottage cheese, differences between laboratory-produced and commercial products were perceptible, likely due to variations in formulation, processing conditions, or ingredient standardization.

These results are consistent with previous studies reporting minimal sensory impact from incorporating probiotics into fresh cheeses. For example, Abadía-García et al. [12] found no significant sensory differences between probiotic and control cottage cheese, while Felicio et al. [64] reported that probiotic Minas cheese maintained comparable sensory characteristics to conventional formulations. Similar observations have been reported in other fresh cheese systems, where probiotic cultures did not adversely affect sensory perception [42, 65].

A more detailed evaluation using a nine-point hedonic scale (1 = “dislike very much”; 9 = “like very much”) showed (Table 3) that all samples achieved favorable acceptance, with mean scores ranging from 6 to 8 and clustering around 7 (“like moderately”).

Table 3 Sensory scores of attributes of different cottage cheeses.

	Type of cottage cheese			
Attribute	Brand A	Brand B	Control	Probiotic (*Lactobacillus acidophilus* LA-5)
Texture	7.60 ± 1.05a	6.40 ± 1.19a	6.80 ± 1.54a	7.15 ± 1.46a
Color	8.00 ± 1.21a	7.85 ± 0.81a	7.55 ± 1.10a	7.10 ± 1.48a
Flavor	7.30 ± 1.13a	6.85 ± 1.39a	7.15 ± 0.99a	7.35 ± 1.18a
Acceptability	7.45 ± 1.28a	6.95 ± 1.43a	7.45 ± 1.00a	7.20 ± 1.40a

For the same attribute, the same letter indicates no significant difference ($p > 0.05$) between the samples.

These values indicate positive consumer perception across all formulations. For flavor, the highest scores were observed for the A-brand commercial cheese and the probiotic cheese, with the latter averaging 7.35. The B-brand cheese obtained the lowest flavor score, although all samples remained within the acceptable range and were not significantly different ($p > 0.05$). Color scores were slightly higher for commercial cheeses, likely reflecting their more standardized appearance. However, experimental cheeses showed comparable values ($p > 0.05$), with control (7.55) and probiotic (7.10) remaining within the same acceptance level. Texture evaluation followed a similar trend, with the A-brand cheese receiving the highest score, followed by the probiotic (7.15) and control (6.80) cheeses. Despite minor differences, all values indicated satisfactory texture perception.

Overall acceptability indicated that the control cheese achieved a score comparable to the A-brand product (7.45), whereas the probiotic formulation showed a slightly lower score (7.20). Statistical analysis indicated no significant differences ($p > 0.05$) among samples for most attributes, supporting the sensory equivalence of the probiotic formulation. These preliminary results, from a panel of 30 untrained consumers, suggest that including *L. acidophilus* LA-5 likely doesn't affect quality, but larger sensory studies are needed to confirm market acceptance. The observed sensory acceptability aligns with previous findings on probiotic cheese products. Hernández-Figueroa et al. [44] reported high consumer acceptance of probiotic cream cheese containing *L. acidophilus* LA-5, with no significant differences compared to conventional products. Likewise, Granato et al. [66] and Karimi et al. [6] highlighted that cheese matrices are particularly suitable for probiotic incorporation due to their mild flavor and buffering capacity, which help maintain sensory quality. More recent studies have also emphasized that fresh cheeses can effectively deliver probiotics without compromising sensory attributes, even during storage [8, 67].

Probiotic foods should deliver at least 10^7 CFU/g or per serving [2, 68]. In this study, *L. acidophilus* LA-5 (>10^9 CFU/g) stayed viable during storage, ensuring a relevant dose. This supports health benefits and confirms cottage cheese as an effective probiotic carrier. Probiotic cottage cheese links nutrition and health, offering an alternative to supplements. Adding *L. acidophilus* LA-5 boosts its benefits without affecting taste, making fresh cheeses effective probiotic carriers.

4. Conclusion

This study demonstrates that cottage cheese is an alternative functional vehicle for delivering *L. acidophilus* LA-5, effectively overcoming the common bottleneck of sensory loss in functional dairy products. This matrix maintained a high probiotic concentration (>10^8 CFU/g) throughout a 24-day refrigeration period, exceeding the minimum threshold required for health benefits. While the natural post-acidification process caused a gradual decrease in pH and a simultaneous increase in titratable acidity, the probiotic's viability remained almost unaffected. Additionally, sensory profiling revealed no significant differences between the probiotic formulation and the control cottage cheese. It was accompanied by positive consumer acceptance, demonstrating that enhancing functional value does not compromise sensory quality. Future research should build upon these results by exploring multi-strain systems to optimize probiotic performance and investigating microencapsulation strategies further to extend structural and microbial stability during longer storage cycles.

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Author Contributions

R. H. Hernández-Figueroa: Conceptualization, Methodology, Data curation, Formal analysis, Writing- Original draft preparation, Writing- Reviewing and Editing. E. Mani-López: Methodology, Formal analysis, Data curation, Writing- Original draft preparation, Writing- Reviewing and Editing. A. López-Malo: Conceptualization, Data curation, Resources, Writing- Reviewing and Editing.

Competing Interests

The authors declare that they have no conflicts of interest related to the work described in this manuscript.

Data Availability Statement

The datasets used during the current study are available from the corresponding author upon reasonable request.

AI-Assisted Technologies Statement

Artificial intelligence (AI) tools were used solely for basic grammar correction and language refinement in preparing this manuscript. Specifically, OpenAI's ChatGPT and Grammarly were used to improve the readability and linguistic clarity of the English text. All scientific content, data interpretation, and conclusions were developed independently by the author. The authors have thoroughly reviewed and edited the AI-assisted text to ensure its accuracy and accept full responsibility for the manuscript's content.

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