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ORIGINAL ARTICLE

# Physicochemical, textural properties, and lactic acid bacteria counts of Peruvian fresh cheese with added probiotic cultures

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## Abstract

The present study aims to evaluate the impact of incorporating commercial probiotic lactic acid bacteria cultures on the physicochemical and textural characteristics of fresh cheese, as well as their viability. The probiotic strains *Lactobacillus acidophilus* and *Lacticaseibacillus rhamnosus* were assessed at two different incorporation times — before the renneting and salting stages — using a factorial treatment design. Over a refrigerated storage period of 21 days (4 °C to 5 °C), the total lactic acid bacteria count, acidity, pH, and syneresis were monitored at intervals of 0, 1, 7, 14, and 21 days. Texture profile analysis was conducted at the 21-day mark to assess instrumental texture parameters of the cheese treatments. After the storage period, fresh cheeses supplemented with *L. rhamnosus* added before the renneting process exhibited similar acidity values (0.18% lactic acid), pH (5.36), and percentage of syneresis (4.71%), when compared to the control cheeses without probiotics. Except for the adhesiveness, no significant differences were observed regarding the texture profile parameters of the evaluated cheeses. Furthermore, the fresh cheese supplemented with *L. rhamnosus* added before renneting maintained a microorganism count exceeding 6 logarithmic cycles per gram throughout the storage period. The findings of this study suggest that Peruvian fresh cheese presents a promising matrix for delivering probiotic microorganisms, particularly the *L. rhamnosus*.

**Keywords:** Functional food; Syneresis; *Lactobacillus acidophilus*; *Lacticaseibacillus rhamnosus*; Probiotics; Dairy products.

## Highlights

- The incorporation of probiotics did not alter the quality characteristics of Peruvian fresh cheese
- The viability of the *L. rhamnosus* remained satisfactory within the fresh cheese matrix throughout 21 days of storage

## 1 Introduction

The demand for functional probiotic foods is on the rise, as consumers increasingly recognize the positive impact of these products on health (Blaiotta et al., 2017). Consequently, companies are actively working to create innovative probiotic goods that offer health benefits to consumers. These functional foods not only provide essential nutrition but also contribute to enhancing the immune system, promoting digestive health, and improving overall well-being, thus reducing the risk of certain diseases in individuals (Coelho et al., 2022; Yerlikaya & Ozer, 2014).

Moreover, cheese, whether fresh or matured, solid or semi-solid, has been identified as an excellent matrix for delivering probiotic microorganisms and transforming the product into a functional food. Its solid protein structure, fat, calcium, moisture, and near-neutral pH are conducive to the development and viability of microorganisms, presenting a better buffering capacity and a lower oxygen content, which can protect microbial cells through the digestive system (Andrade et al., 2023; Rolim et al., 2020). Several studies have highlighted that the probiotic viability in a given food product depends on factors such as storage conditions, probiotic strains and food matrix characteristics, including pH, organic acids, fat, as well as moisture content (Castro et al., 2015; Karimi et al., 2011; Ningtyas et al., 2019). These attributes allow cheese to be a promising vehicle for delivering probiotics and conferring health benefits to consumers.

Fresh cheese is a cultural symbol in Latin America and is closely associated with the gastronomy of each country. Peruvian fresh cheese, which is commonly manufactured without starter cultures, typically has high moisture levels (> 55%), a pH value close to neutrality, low salt content, and a soft albeit firm texture, which could be a great alternative to transport probiotics to the consumer benefit. Certainly, various studies have investigated the incorporation of probiotic bacteria into different fresh cheeses such as Minas *frescal* (fresh) cheese (Verruck et al., 2015), whey cheese (Madureira et al., 2013), cottage cheese (Abadía-García et al., 2013), cream cheese (Ningtyas et al., 2019; Speranza et al., 2018), and ricotta cheese (Meira et al., 2015; Rubel et al., 2022). In this sense, Buriti et al. (2005) reported that fresh Minas cheese (F*rescal*) containing *L. acidophilus* was a suitable food system for the delivery of this probiotic microorganism, with a viability greater than or equal to 6 log CFU $g^{-1}$ during storage for 21 days at 5 °C. However, the viability of *L. acidophilus* incorporated into cream cheese showed an accentuated decrease, registering counts around 3 log CFU $g^{-1}$ at the end of refrigerated storage (Alves et al., 2013).

There is a universal consensus that probiotic cultures in food products must remain viable above the threshold level of $10^6$ CFU $g^{-1}$, which is recommended to confer probiotic benefits (Castro et al., 2015; Karimi et al., 2011). On the other hand, *L. rhamnosus* is a commonly studied probiotic due to its multiple beneficial properties, such as a greater resistance to bile, the potential to modulate the immune system response, as well as the prevention and treatment of allergic inflammation (Basturk et al., 2020). This microorganism also presents the greatest ability to colonize and exist more stably in dairy products (Niu et al., 2023). In fact, it has been proven that it remained viable (> 6.6 log CFU $g^{-1}$) after 5 weeks of storage in reduced-fat cream cheese, both when encapsulated and non-encapsulated (Ningtyas et al., 2019). With this in mind, the present study focused on the incorporation of the commercial microorganisms *L. acidophilus* La3 and *L. rhamnosus* R into Peruvian fresh cheese.

Having said that, the addition of probiotics in fresh cheeses can face many challenges, including the preservation of their sensory features as well as a suitable probiotic viability throughout the storage period (Karimi et al., 2011). Probiotic bacteria may cause alterations due to their metabolism, leading to destabilization in the cheese structure during storage. The quality attributes of probiotic cheeses can likewise be affected in cases where a high level of supplementation is used (Castro et al., 2015). Gomes et al. (2011) reported lower pH values and a greater production of organic acids in probiotic Minas *Frescal* cheese when high counts of *L. acidophilus* (>9 log CFU $g^{-1}$) were present throughout shelf-life, which resulted in alterations in its appearance, aroma, and texture. Therefore, the objective of the present work was to add probiotic microorganisms into Peruvian fresh cheese and evaluate their effect on the physicochemical and textural characteristics of the dairy product assessed herein, as well as the viability of Lactic Acid Bacteria (LAB) during 21 days of refrigerated storage.

## 2 Materials and methods

### 2.1 Materials

Raw cow milk was provided by the Department of Animal Science at the Universidad Nacional Agraria La Molina (Lima, Peru). Samples of the raw milk were collected and transported to the laboratory for routine testing. This milk, standardized to 3% fat content (acidity as lactic acid: 0.15 ± 0.01%; total solids: 12.06 ± 0.03%; fat: 3.02 ± 0.02%; protein: 3.54 ± 0.01%; pH: 6.71 ± 0.08; and density: 1.03 ± 0.01 g/mL), was utilized in the cheese production. Additionally, two lyophilized commercial probiotic strains, L. acidophilus (Lyofast LA 3) and *L. rhamnosus* (Lyofast LR B), obtained from Sacco System (Cadorago, Italy), were used in this study.

### 2.2 Probiotic fresh cheese production

Fresh cheese is a type of dairy product that is meant to be consumed shortly after it is manufactured, without aging or ripening, being is characterized by its white and soft appearance. Peruvian fresh cheese, in particular, can be categorized within the group of semi-hard cheeses, since the milk curds are typically heated up to 38 °C during its production process. Peruvian fresh cheese production was carried out according to the process described in Figure 1.

Four treatments (T1 through T4) and a control of fresh cheeses were performed in triplicate (three repetitions of each trial were produced on different days). Following the traditional Peruvian fresh cheese manufacturing process, this procedure did not consider the addition of any starter culture. For each treatment as well as the control, twelve liters of milk were pasteurized at 72 °C for 15 s and cooled to 37 °C. Then, calcium chloride (0.2 g/L milk) was added and, at this stage, activated probiotic microorganisms were separately incorporated into treatments T1 (*L. acidophilus*) and T3 (*L. rhamnosus*) at a minimum of $10^7$ CFU/mL milk, following the supplier's instructions (Sacco S.R.L., Cadorago, Italy) as well as the universal recommendation on the minimum viable quantities of probiotic cultures required in a cheese for it to be recognized as probiotic (Castro et al., 2015; Karimi et al., 2011). Subsequently, chymosin (CHY–MAX®, Chr. Hansen, Spain), given its average coagulation rate of 2235 IMCU/g, was added at a 2 g/100 L ratio for milk coagulation for 45 min. When coagulated, the curd was cut into 1-cm cubes and left to rest for 5 min. Then, the curd cubes were agitated for 5 min, followed by a first draining, in which a third of the volume of whey was removed.

Following this procedure, water at 70 °C was added until a temperature of 38 °C was reached and a second stronger stirring was performed for 20 min, followed by a second draining until the level of the grains. Curd washing is a technological process intended to reduce the lactose level, which shapes both the texture and the taste that are typical of this traditional Peruvian cheese. As previously described, the activated probiotic bacteria were incorporated into the corresponding treatments T2 (*L. acidophilus*) and T4 *(L. rhamnosus*) before salting. Subsequently, a dry salting was performed in the cheese curd at a proportion of 0.4 g salt/100 g, followed by a thorough agitation and resting for an additional 5 min. Cheese curd pieces were then placed into molds to drain off the whey and to cool down. Thereafter, the cheeses were packed in polyethylene bags and stored at refrigeration temperature (4 °C to 5 °C) for 21 days. Analyses were carried out in triplicate during this storage period at 0, 1, 7, 14, and 21 days. For comparison purposes, a control batch was prepared following the same process without the probiotic addition.

### 2.3 Physicochemical analysis

Milk samples were evaluated for density (AOAC 925.22) (Association of Official Analytical Chemists, 2016), acidity expressed as lactic acid (w/v%) (IDF, 150:1991) (International Dairy Federation, 1991), pH using a potentiometer from Hanna Instrument, fat (IDF 13C:1987) (International Dairy Federation, 1987), protein (IDF standard 20B) (International Dairy Federation, 1993), and total solids content (IDF 21B:1987) (International Dairy Federation, 1987). Additionally, cheese samples were subjected to acidity analysis (AOAC, 16.267), pH measurement and a syneresis assessment (%) based on the methodology outlined by

Pearse & Mackinlay (1989), which relies on the weight loss due to whey released during storage. All analyses were conducted in triplicate at 0, 1, 7, 14 and 21 days of refrigerated storage (4 °C to 5 °C).

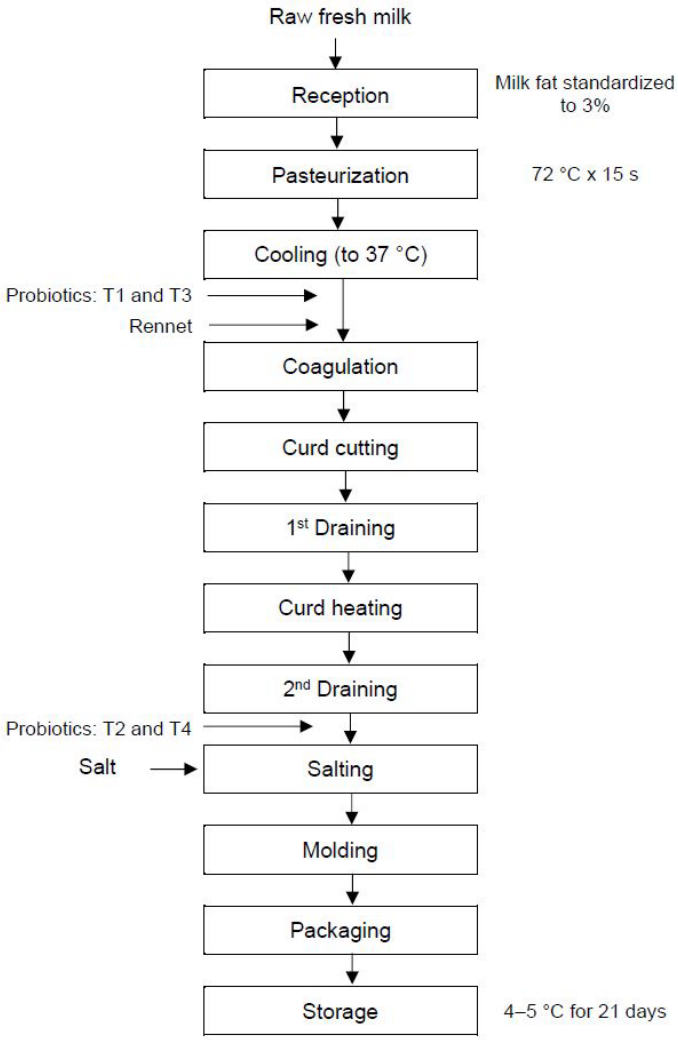


**Figure 1.** Schematic diagram of fresh cheese production (T1: *L. acidophilus* added before renneting; T2: *L. acidophilus* added before salting; T3*: L. rhamnosus* added before renneting; T4: *L. rhamnosus* added before salting).

## 2.4 Texture Profile Analysis (TPA)

The cheese samples were evaluated for five TPA parameters: hardness (N), springiness, gumminess (N), cohesiveness and adhesiveness (N-mm). These textural traits were assessed following the method recommended by Tunick & Van Hekken (2010), utilizing the QTS-25 texture analyzer (Brookfield Viscometer, Massachusetts, US). Test samples, 15 mm in thickness each, were compressed to 75% of their initial height at a constant speed of 100 mm/min. This comprehensive analysis was conducted in triplicate following a 21-day storage period at 4° C to 5 °C.

## 2.5 Lactic acid bacteria count

### 2.5.1 Medium preparation

The Man-Rogosa-Sharpe (MRS) agar medium (Sigma-Aldrich) was prepared following the guidelines provided by Salfinger & Tortorello (2015). The pH was adjusted to 5.7 using 1N HCl, followed by the addition of sorbic acid dissolved in NaOH to achieve a 0.1% concentration (v/v). Furthermore, 0.1% (w/v)

cysteine hydrochloride was added. It is important to emphasize that the inclusion of cysteine hydrochloride and sorbic acid as supplements in the MRS medium was performed to increase its selectivity and promote the growth of the evaluated probiotic bacteria, as suggested by Salfinger & Tortorello (2015). The resulting mixture was sterilized in a Fravill AV DA 030 autoclave (Equipamientos Ciencia S.A.C, Lima, Peru) at 121 °C for 15 minutes.

#### 2.5.2 Sample preparation and dilutions

A 10-gram cheese sample was aseptically selected and homogenized with 90 mL of sterile peptone saline solution. The homogenized cheese was then subjected to serial decimal dilutions using the same solution and plated on MRS agar (Sigma-Aldrich), with 1 mL in each dish. Subsequently, the plates were incubated at 37 °C for 48 hours in an Incucell V-MMM Group incubator located in Munich, Germany (Blaiotta et al., 2017; Yerlikaya & Ozer, 2014). All microbiological analyses were conducted in triplicate on days 0, 1, 7, 14 and 21 of storage. Sterility controls were included as blanks in each test. The results were expressed as log CFU $g^{-1}$ of cheese.

### 2.6 Statistical analysis

A completely randomized design with factorial arrangement was employed in the present study. The factors included the type of probiotic bacteria (L. acidophilus and *L. rhamnosus*), the time of incorporation into the mixture (before the renneting and salting processes), and the day of storage (0, 1, 7, 14, and 21). The response variables encompassed microbiological and physicochemical parameter values. Following the analysis of variance, the treatment means were subjected to multiple comparisons using Tukey's test at a 95% confidence level. All statistical analyses were conducted using the Statgraphics software (Version 19).

## 3 Results and discussion

### 3.1 Viability of lactic acid bacteria (LAB) during storage

Table 1 shows the viability of lactic acid bacteria in fresh cheeses for 21 days of storage at 4 °C. There was an interaction ($p < 0.05$) between the time of incorporation and the type of probiotic bacteria. When comparing the population of T1 and T2 (*L. acidophilus*) *versus* T3 and T4 (*L. rhamnosus*), evident differences ($p < 0.05$) were observed between these microorganisms when they were added before renneting. However, there were no differences ($p > 0.05$) when they were incorporated before salting. Similar results were found by Blaiotta et al. (2017) when adding 7 log CFU $g^{-1}$ of *L. acidophilus* and *L. rhamnosus* during the conditioning stage of Italian cheese production with an increase of 0.51–0.86 logarithmic cycles during the first days of storage, which could be attributed to the incorporation time. Probiotic microorganisms added into the fresh cheese treatments T1 and T3 had a longer time to adapt to the medium than the probiotic bacteria in T2 and T4. Moreover, the T3 treatment had the highest probiotic counts (> 5.6 log CFU $g^{-1}$) compared to the other treatments throughout the storage time. These results suggest that the *L. rhamnosus* adapted better to the food matrix than the *L. acidophilus*. Indeed, Phillips et al. (2006) likewise reported that the *L. rhamnosus* was found to be more stable than the *L. acidophilus* in cheddar cheese at levels above the suggested minimum limit of $10^6$–$10^7$ log CFU $g^{-1}$ after 32 weeks. In the present study, cheeses containing *L. rhamnosus* (T3 and T4) exhibited a greater ($p < 0.05$) average count (5.44 log CFU $g^{-1}$) in comparison with samples containing *L. acidophilus* (T1 and T2; 4.67 log CFU $g^{-1}$) under storage conditions.

The cheese treatments containing *L. rhamnosus* reached their peak microbial population on the 7th day ($p < 0.05$), maintaining consistency throughout the storage period. This behaviour is consistent with the findings of Yerlikaya & Ozer (2014), who observed that fresh white cheeses supplemented with *L. rhamnosus* experienced maximal growth on the 7th day, reaching a population of 9.37 log CFU $g^{-1}$, followed by a sustained level above 8 log CFU $g^{-1}$ during 28 days of refrigerated storage. Conversely, in the same fresh

white cheeses, viable counts of *L. acidophilus* bacteria consistently declined from 10.23 log CFU $g^{-1}$ on day 1 to 7.24 log CFU $g^{-1}$ on day 28 of storage, indicating that the *L. rhamnosus* exhibited a greater stability than the L. acidophilus, a trend also observed in the present study. In contrast, Abadía-García et al. (2013) found that incorporating *L. casei* and *L. rhamnosus* GG as probiotic cultures into cottage cheese resulted in a probiotic population consistently above $10^6$ CFU $g^{-1}$ during 28 days of storage at 8 °C.

**Table 1.** Viability of lactic acid bacteria in fresh cheeses during storage at 4 °C ($log_{10}$ CFU $g^{-1}$).

| Storage (d) | *L. acidophilus* | | *L. rhamnosus* | | *Control* |
|---|---|---|---|---|---|
| | T1 | T2 | T3 | T4 | |
| 0 | $4.66 \pm 0.09^{b}$ | $4.56 \pm 0.18^{b}$ | $5.63 \pm 0.07^{aZ}$ | $4.48 \pm 0.41^{bY}$ | $3.26 \pm 0.17^{c}$ |
| 1 | $4.79 \pm 0.01^{b}$ | $4.40 \pm 0.26^{b}$ | $6.01 \pm 0.26^{aYZ}$ | $4.41 \pm 0.35^{bY}$ | $2.86 \pm 0.13^{c}$ |
| 7 | $4.93 \pm 0.05^{b}$ | $4.86 \pm 0.27^{b}$ | $6.60 \pm 0.20^{aXY}$ | $5.08 \pm 0.28^{bX}$ | $3.31 \pm 0.51^{c}$ |
| 14 | $4.73 \pm 0.12^{b}$ | $4.75 \pm 0.20^{b}$ | $6.63 \pm 0.04^{aX}$ | $4.78 \pm 0.22^{bXY}$ | $3.96 \pm 0.88^{c}$ |
| 21 | $4.38 \pm 0.04^{b}$ | $4.63 \pm 0.34^{b}$ | $6.25 \pm 0.07^{aXYZ}$ | $4.50 \pm 0.05^{bXY}$ | $3.26 \pm 0.17^{c}$ |

$^{a, b,c}$ Means within a row followed by different superscripts differ significantly ($p < 0.05$). $^{X, Y, Z}$ Means within a column followed by different superscripts, differ significantly ($p < 0.05$). T1: Added before renneting; T2: Added before salting; T3: Added before renneting; T4: Added before salting.

The T3 samples consistently maintained a microbial population exceeding 6 log CFU $g^{-1}$ from day 1 through the entire 21-day storage period, demonstrating superior counts when compared to the other treatments ($p < 0.05$). This meets the threshold of at least $10^6$ CFU $g^{-1}$ established for probiotic activity by most national legislations (Castro et al., 2015; Karimi et al., 2011; Yerlikaya & Ozer, 2014), which was achieved herein with the Peruvian fresh cheese containing *L. rhamnosus* at the end of its shelf life (> 6 log CFU $g^{-1}$).

This behavior may be attributed to the fact that *L. rhamnosus* are facultative anaerobic heterofermentative bacteria, which is less demanding in its nutritional requirements than other bacteria in the genus. By the same token, Ningtyas et al. (2019) concluded that *L. rhamnosus* microorganisms incorporated into functional reduced-fat cream cheese, whether non-encapsulated or encapsulated, remained viable ($10^6$–$10^8$ CFU $g^{-1}$) after 5 weeks of refrigerated storage. Similarly, microbial counts exceeding 7 log CFU $g^{-1}$ were observed in Wagashi cheese containing *L. rhamnosus* among other probiotics during refrigerated vacuum storage at 4 °C for 30 days (Anihouvi & Kesenkaş, 2022). In this context, Silva et al. (2015) demonstrated that Latin-style fresh cheese (*Frescal*) can significantly enhance the survival of LAB strains against gastrointestinal juices, possibly due to the cheese's buffering capacity (pH around 6.5), dense matrix, and fat content. Consequently, these authors suggest that all LAB strains incorporated into such cheeses could be considered resistant to gastric acidity.

### 3.2 Acidity

Titratable acidity and pH are crucial factors influencing the stability of probiotic bacteria, with high pH and low acidity values being desirable, common characteristics in cheeses. However, the use of probiotics could potentially disrupt this balance (Karimi et al., 2011). Figure 2 indicates the acidity levels of fresh cheeses during storage for 21 days at 4 °C. No interactions ($p > 0.05$) were observed among the type of LAB, time of incorporation, and day of evaluation, indicating that one factor did not affect the outcome of the other during the acidity assessment.

Cheese treatments with microorganisms exhibited higher acidity levels than the control, with the highest acidity being registered in T1 and T3. LAB added before the renneting demonstrated a longer activity when compared to those incorporated before salting, the penultimate operation in the cheese-making process. When assessing whether the variable related to the microorganism type influenced acidity values in fresh cheese, it

was evident that the control samples differed significantly ($p < 0.05$) from the samples with *L. acidophilus* (T1 and T2) and *L. rhamnosus* (T3 and T4). The lowest acidity (0.08% lactic acid) was observed in the control, compared to the probiotic cheese treatments on day 0. By day 21 of storage, the highest acidity was observed in T1 and T3 treatments with an average value of 0.18% lactic acid, while the control had the lowest acidity (0.16% lactic acid). *L. rhamnosus* presented a high fermentative activity on lactose in the initial storage hours. In line with this, the lactic acid profile of Wagashi cheese containing *L. rhamnosus* increased progressively during 30 days of storage at 4 °C (Anihouvi & Kesenkaş, 2022).

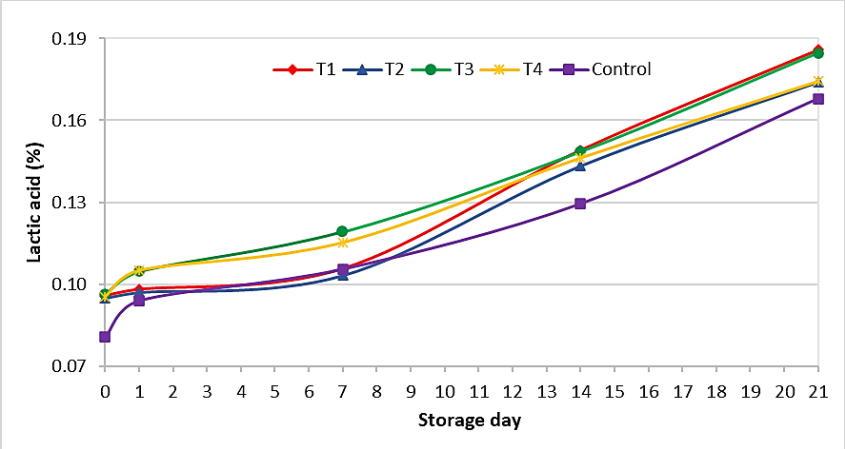


**Figure 2.** Acidity (lactic acid, %) of fresh cheeses during storage at 4 °C. T1: *L. acidophilus* added before renneting; T2: *L. acidophilus* added before salting; T3*: L. rhamnosus* added before renneting; T4: *L. rhamnosus* added before salting.

The cheese components may remain unaffected by the incorporation of probiotic microorganisms; nevertheless, these bacteria could induce acidity alterations due to their metabolism in this dairy product, when compared to cheeses without lactic fermentation (Yerlikaya & Ozer, 2014). In the present study, all samples, including the control, exhibited a progressive increase in acidity during storage. Similar outcomes were reported by Buriti et al. (2005), who observed that titratable acidity increased in Minas *frescal* cheeses with *L. acidophilus* bacteria during 21 days of storage at 5 °C. This behaviour can be explained by the action of bacteria on simple carbohydrates such as glucose and lactose, which, through fermentation, result in a higher concentration of lactic acid (Chandan & Kilara, 2013; Jyoti et al., 2003).

### 3.3 pH

Figure 3 shows the pH values of fresh cheeses during storage at 4 °C. The pH values for all treatments, including the control, tended to decrease over time. However, cheeses containing probiotic microorganisms (T1 through T4) had significantly lower pH values than the control after the 7th day of storage. These differences in pH values can be attributed to the action of the added probiotic bacteria. Similarly, Buriti et al. (2005) reported that Minas *frescal* cheese containing *L. acidophilus* exhibited a progressive pH reduction (from 6.72 to 5.37 on day 1 and day 21, respectively) with an increase in titratable acidity (from 0.12% to 0.89% on day 1 and day 21, respectively) during storage at 5 °C.

Treatments with higher LAB counts (T1 and T3) presented lower pH values (5.40 and 5.37, respectively). Among the probiotic Peruvian fresh cheeses, the T3 treatment consistently had the lowest pH values, especially after the 7th day of storage. Nonetheless, Prezzi et al. (2020) reported that the addition of *L. rhamnosus* did not affect the physicochemical (pH, moisture, fat, protein) and textural characteristics of Minas *frescal* cheeses.

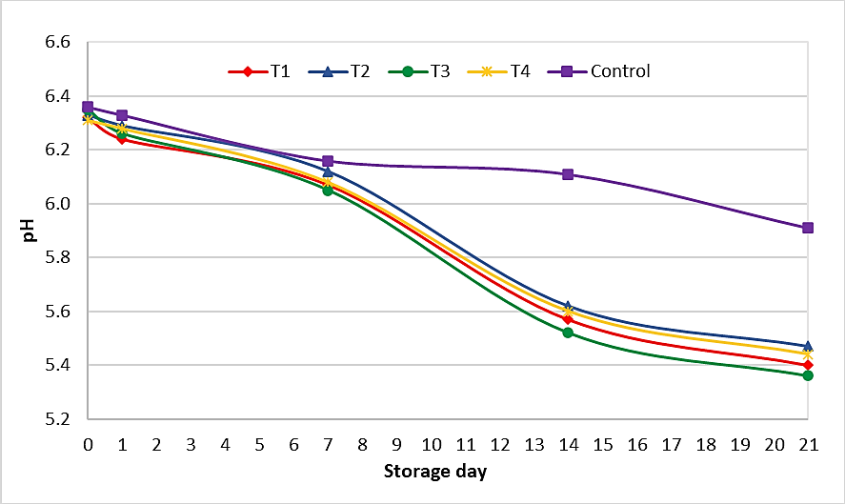


**Figure 3.** pH of fresh cheeses during storage at 4 °C. T1: *L. acidophilus* added before renneting; T2: *L. acidophilus* added before salting; T3*: L. rhamnosus* added before renneting; T4: *L. rhamnosus* added before salting.

### 3.4 Syneresis

A significant interaction ($p < 0.05$) was observed among all the factors concerning the syneresis percentage. As depicted in Figure 4, the control exhibited a lower percentage of syneresis compared to the other treatments during storage. Furthermore, when the microorganism was incorporated before renneting (T1 and T3), the syneresis level was significantly higher ($p < 0.05$) than that achieved when the probiotic bacterium was added before salting (T2 and T4). Additionally, it was observed that the control had the lowest syneresis percentage (3.55%) after 21 days of storage, while the highest values were registered for T1 and T3 (4.72% and 4.71%, respectively), which also exhibited the highest acidity levels (0.19% and 0.18% lactic acid, respectively). These findings align with previous research suggesting that an increase in syneresis is directly associated with acidity and inversely correlated with pH (Meira et al., 2015).

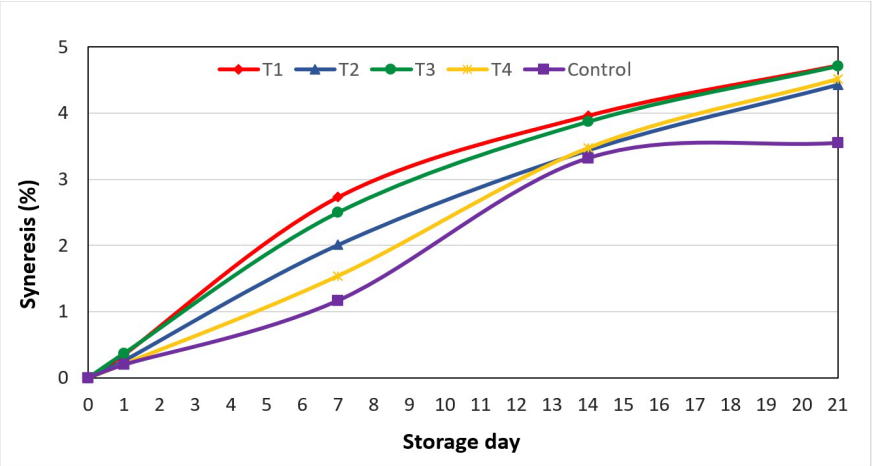


**Figure 4.** Syneresis (%) of fresh cheeses during storage (21 days) at 4 °C. T1: *L. acidophilus* added before renneting; T2: *L. acidophilus* added before salting; T3*: L. rhamnosus* added before renneting; T4: *L. rhamnosus* added before salting.

A noticeable increase in syneresis levels, hence, an increase in acidity and reduction in pH were observed in probiotic Peruvian fresh cheeses, particularly staring at the 7th day of storage. These findings are consistent with those reported by Meira et al. (2015), who found that ricotta cheeses containing *L. acidophillus* La-05 or *B. lactis* Bb-12 presented a greater syneresis level than the cheese samples without probiotics.

Concurrently, Verruck et al. (2015) found that Minas *frescal* cheeses made with buffalo milk and produced with *Bifidobacterium* Bb-12 showed an increase in syneresis values, resulting in a cheese with a more closed structure after 30 days of storage.

Overall, syneresis is affected by various factors, which can produce variations in the draining process, such as the presence of microorganisms and the acidity influence in the medium, thus producing higher syneresis percentages. Because of this, several studies have also reported that probiotic fresh cheeses presented a progressive whey drainage throughout refrigerated storage increasing syneresis levels (Buriti et al., 2005; Oliveira et al., 2023). Despite this fact, Rubel et al. (2022) found that the incorporation of probiotic bacteria in spreadable ricotta cheese did not affect the stability of this dairy product during storage (i.e., there were no differences in the syneresis values compared to the control).

### 3.5 TPA

Table 2 shows the texture profile parameters of fresh cheeses after 21 days of storage at 4 °C. Except for adhesiveness, no significant differences ($p > 0.05$) were observed in the texture profile parameters between the treatments and the control. These findings suggest that the metabolism of the added microorganisms did not impact the textural characteristics of Peruvian fresh cheeses. While some cheeses containing Lactic Acid Bacteria (LAB) may exhibit a softer texture, previous studies have reported the stability of the texture profile parameters during the storage of fresh and ripened cheeses containing probiotics (Buriti et al., 2005; Prezzi et al., 2020; Tomar, 2019). Generally, the influence of quality factors such as pH on cheese texture may be contingent upon the degree of pH alteration during the manufacturing process or storage (Pastorino et al., 2003; Tunick & Van Hekken, 2010). In the present study, despite the lower pH values of the cheeses containing probiotics compared to the control at the end of storage, this pH difference did not affect their hardness, cohesiveness, springiness or gumminess.

**Table 2.** Texture profile parameters of fresh cheeses at 21 days of storage (4 °C).

| Treatment | Hardness (N) | Adhesiveness (N-mm) | Cohesiveness | Springiness | Gumminess (N) |
|---|---|---|---|---|---|
| T1 | 14.02 ± 2.06 | 56.94 ± 0.07[a] | 0.23 ± 0.09 | 0.78 ± 0.09 | 3.31 ± 1.12 |
| T2 | 13.46 ± 1.82 | 185.32 ± 0.07[e] | 0.22 ± 0.12 | 0.77 ± 0.06 | 4.52 ± 1.84 |
| T3 | 12.48 ± 3.19 | 69.36 ± 0.08[b] | 0.24 ± 0.07 | 0.77 ± 0.07 | 4.26 ± 1.03 |
| T4 | 13.33 ± 1.29 | 108.48 ± 0.08[d] | 0.21 ± 0.03 | 0.77 ± 0.10 | 4.24 ± 1.52 |
| Control | 16.62 ± 1.45 | 80.51 ± 0.05[c] | 0.24 ± 0.05 | 0.82 ± 0.04 | 6.36 ± 0.70 |

[a, b, c, d, e] Means ± SD within a column followed by different superscripts differ significantly ($p < 0.05$). T1: *L. acidophilus* added before renneting; T2: *L. acidophilus* added before salting; T3*: L. rhamnosus* added before renneting; T4: *L. rhamnosus* added before salting.

The cheeses T2 and T4 exhibited higher adhesiveness values (185.32 and 108.48 N-mm, respectively) than cheeses T1 and T3 (56.94 and 69.36 N-mm, respectively). This suggests that the incorporation of microorganisms before salting may have contributed to an increased adhesiveness in the cheeses. However, no significant differences ($p > 0.05$) were observed in cohesiveness values among the samples.

The control cheese may have maintained a better protein structure, thus preserving the product's shape for a longer period. This could be attributed to a stronger interaction between the intact proteins of the cheese, resulting in a greater hardness (Pastorino et al., 2003). Moreover, the cohesiveness value obtained from the control cheese (0.24) was comparable to those reported by other authors who conducted texture profile analysis in fresh cheeses, such as Tunick & Van Hekken (2010), as well as Tomar (2019). Finally, although there were no significant differences among treatments, the cheeses containing probiotic microorganisms exhibited lower cohesiveness. This might be due to the relationship among texture, moisture content, and

syneresis (Buriti et al., 2005; Pastorino et al., 2003). It's worth noting that all treatments presented a certain syneresis level, which could be attributed to a lower force exerted by the internal bonds of the cheese. This may help explain why some probiotic cheeses can present lower cohesiveness values (Tomar, 2019).

The control cheese exhibited a springiness and gumminess of 0.82 and 6.36 N, respectively, which was comparable to other fresh cheeses with a commercial starter culture (Oliveira et al., 2023). In contrast, the probiotic cheese treatments showed an average of 0.77 and 4.08 N, respectively. The elasticity of the cheese is positively correlated with the structure of the cheese protein, while the decrease in elasticity is attributed to the hydrolysis of calcium paracaseinates (Pastorino et al., 2003; Tomar, 2019). The increased acidity and lower pH, resulting from the presence of a higher number of bacteria, could impact the dynamic equilibrium between the calcium and inorganic phosphate concentrations in the paracasein network, leading to the production of soluble calcium in the cheese structure (Pastorino et al., 2003). Nevertheless, there was no evidence of alterations in the instrumental texture characteristics of cheeses containing probiotic cultures compared to the control at the end of the refrigerated storage. This suggests that Peruvian fresh cheese would be a suitable food matrix to manufacture new functional fresh cheeses.

## 4 Conclusions

The findings of the present study indicate that Peruvian fresh cheese has the potential to serve as a suitable food matrix for the preservation of probiotic microorganisms during a 21-day storage period at temperatures ranging from 4 °C to 5 °C. However, the viability of the probiotic strain and the timing of its incorporation play crucial roles, given that only the *L. rhamnosus* added before the renneting process resulted in satisfactory survival rates during refrigerated storage. Apart from adhesiveness, the texture profile analysis revealed consistent results across all treatments, with the cheese containing *L. rhamnosus* added before renneting displaying characteristics comparable to those of traditional fresh cheeses. Notably, after the storage period, this particular sample exhibited acidity values (0.18% lactic acid), pH (5.36), and syneresis percentage (4.71%) similar to those of cheeses without probiotics. Furthermore, the fresh cheeses containing *L. rhamnosus* added before the renneting process maintained a microorganism count exceeding 6 logarithmic cycles/g throughout the storage period, suggesting that Peruvian fresh cheese could indeed be a favorable food matrix for the viability of probiotic bacteria. Subsequent research endeavors could focus on optimizing the incorporation of these probiotics into fresh cheese formulations for industrial applications.

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