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Total lactic acid bacteria, microstructure and texture analysis of probiotic soft cheese with rennet-induced protein gelation

Analisis total bakteri asam laktat, mikrostruktur dan tekstur keju lunak probiotik dengan gelasi protein terinduksi rennet

Lola Ayu Istifiani[1*], Rahma Micho Widyanto[2], Iva Tsalissavrina[3], Windi Jinni Erlita[4], Bella Agustina Putri Handayani[5]

[1] Department of Nutrition, Faculty of Health Sciences, Universitas Brawijaya, Malang, East Java, Indonesia.
E-mail: istifiani@ub.ac.id
[2] Department of Nutrition, Faculty of Health Sciences, Universitas Brawijaya, Malang, East Java, Indonesia.
E-mail: micho@ub.ac.id
[3] Department of Nutrition, Faculty of Health Sciences, Universitas Brawijaya, Malang, East Java, Indonesia.
E-mail: ivats@ub.ac.id
[4] Department of Nutrition, Faculty of Health Sciences, Universitas Brawijaya, Malang, East Java, Indonesia.
E-mail: windyjn@student.ub.ac.id
[5] Department of Nutrition, Faculty of Health Sciences, Universitas Brawijaya, Malang, East Java, Indonesia.
E-mail: bellagustina292@student.ub.ac.id

***Correspondence Author:**
Department of Nutrition, Faculty of Health Sciences, Universitas Brawijaya, Malang, East Java, Indonesia.
E-mail: istifiani@ub.ac.id

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Abstract

Consumers are becoming increasingly aware of the importance of choosing healthy probiotic-rich functional foods, such as cheese. Total lactic acid bacteria (LAB) is a crucial measure of success in the development of probiotic soft cheese. Furthermore, it is important to analyze the microstructure and texture of products to determine consumer acceptance. In this study, a randomized block design (RBD) was used with four concentrations of *Lactobacillus plantarum* FNCC 0026 (0%, 5%, 10%, and 20%). Total LAB was quantified using the total plate count (TPC) method. The microstructure and texture were analyzed using field-emission scanning electron microscopy (FESEM) and a texture profile analyzer (TPA), respectively. In this study, 5% concentration yielded the highest LAB count of 8.054 ± 0.54 log CFU/g (2.0 × 10^8 CFU/g) and optimal texture attributes. The texture values included hardness (32.41–48.89 N), adhesiveness (0.03–2.65 Nmm), and springiness (0.78–0.83). Furthermore, FESEM analysis revealed no differences in the protein and fat distribution in the microstructure of probiotic soft cheese. These results substantiate the potential of probiotic soft cheese as functional foods. A concentration of 5% *Lactobacillus plantarum* FNCC 0026 yielded optimal results for total LAB count and texture profile attributes, including hardness, adhesiveness, and springiness.

Keywords: Probiotic cheese, *Lactobacillus plantarum*, Lactic Acid Bacteria, Scanning Electron Microscope, Texture Profile Analyzer

Abstrak

Konsumen semakin menyadari pentingnya memilih makanan fungsional yang sehat dan kaya probiotik seperti keju. Pada pengembangan keju lunak probiotik, total bakteri asam laktat (BAL) menjadi salah satu indikator penentu keberhasilan produk. Selain itu, mikrostruktur dan tekstur dari suatu produk juga penting untuk dianalisis untuk mengetahui penerimaan dari konsumen. Pada penelitian ini, rancangan acak kelompok (RAK) digunakan dengan empat konsentrasi *Lactobacillus plantarum* FNCC 0026 (0%, 5%, 10%, dan 20%). Total BAL dikuantifikasi menggunakan metode *Total Plate Count* (TPC). Mikrostruktur dan tekstur dianalisis menggunakan *field emission scanning electron microscope* (FESEM) dan penganalisis profil tekstur (TPA). Penelitian ini menggunakan konsentrasi 5% dari *Lactobacillus plantarum* FNCC 0026 dan menghasilkan total LAB tertinggi yaitu 8.054 ± 0.54 log CFU/g (2.0 x 10^8 CFU/g) dan atribut tekstur yang optimal. Nilai tekstur meliputi *hardness* (32.41–48.89 N), *adhesiveness* (0.03–2.65 Nmm), dan *springiness* (0.78–0.83). Analisis FESEM tidak menunjukkan perbedaan dalam distribusi protein dan lemak dalam mikrostruktur keju lunak probiotik. Penelitian ini menunjukkan bahwa keju lunak probiotik memenuhi syarat sebagai makanan fungsional dengan konsentrasi 5% *Lactobacillus plantarum* FNCC 0026 menghasilkan hasil optimal dalam jumlah total BAL dan atribut profil tekstur, termasuk *hardness, adhesiveness and springiness.*

Kata Kunci: Keju Probiotik, *Lactobacillus plantarum*, Bakteri Asam Laktat, *Scanning Electron Microscope, Texture Profile Analyzer*

Introduction

As knowledge of nutrition expands and the prevalence of chronic and non-communicable diseases (NCDs) increases, consumer awareness of purchasing healthy foods, particularly functional foods, is growing (Ali & Rahut, 2019; Firoozzare et al., 2024). This is evidenced by the growing demand for functional foods in developing countries (Ali & Rahut, 2019). Health issues in Indonesia are currently receiving significant attention owing to the rise in NCDs attributed to the unhealthy lifestyles of many individuals, especially those with hypercholesterolemia (Amirus et al., 2024). Hypercholesterolemia is characterized by total blood cholesterol levels > 200 mg/dL (Anggraini & Hasni, 2021). According to the 2023 Indonesian Health Survey, 27.8% of Indonesians exhibit slightly elevated cholesterol levels, while 11.7% have high cholesterol levels (Kemenkes, 2024).

The administration of probiotics as functional foods can facilitate the management of hypercholesterolemia. Probiotics can reduce blood cholesterol levels by decreasing low-density lipoprotein (LDL) levels and increasing high-density lipoprotein (HDL) levels in the bloodstream (Andriani et al., 2020; Felix et al., 2024). Administration of the probiotic *Lactobacillus plantarum* at a dosage of 2.0 x 10^9 CFU/g for 12 weeks may serve as a natural, user-friendly, and well-tolerated cholesterol-lowering agent for individuals with hypercholesterolemia (Costabile et al., 2017). Probiotics are live microorganisms that enhance the balance of intestinal microbes, thereby providing health benefits to individuals who consume them (Momin et al., 2023). Several probiotic products are found in functional or fermented foods, including yogurt, pickles, buttermilk, and cheese (Momin et al., 2023; Sharafi et al., 2021). The development of probiotic soft cheese holds significant promise as a functional food because the cheese matrix can effectively protect microorganisms while traversing the digestive tract and during storage (Rolim et al., 2020). Additionally, cheese can produce a buffering effect that counteracts the highly acidic environment of the gastrointestinal tract, thereby creating optimal conditions for probiotic resistance during the journey to the stomach (Homayouni et al., 2018).

The development of probiotic soft cheese in this study involved the addition of lactic acid bacteria (LAB), specifically *Lactobacillus plantarum* FNCC 0026, to the manufacturing process. *Lactobacillus plantarum* FNCC 0026 is a non-pathogenic LAB that inhibits the growth of both gram-positive and gram-negative pathogenic bacteria (Aini et al., 2021; Marwah et al., 2023). *Lactobacillus plantarum* contributes to fat reduction by producing bile salt hydrolase (BSH), lowering the pH, and enhancing short-chain fatty acid (SCFA) levels (Krismiyanto et al., 2021). The minimum standard for bacteria present in probiotic food products is 10^6-10^7 CFU/g (Trimudita & Djaenudin, 2021). Therefore, the microbiological quality of probiotic soft cheese requires further evaluation.

In addition, to achieve food quality standards that consumers prefer, physical quality tests on food products are performed as parameters of quality to distinguish between various types (Zheng et al., 2016). The physical quality test parameters applicable to probiotic soft cheese include the microstructure and texture analysis of cheese products. The chemical composition at the macro- and micro-levels, such as protein and fat content, as well as the addition of probiotics, can affect the texture of cheese (Musra et al., 2021). This study introduces a novel approach using the concentration of lactic acid bacteria along with a comprehensive examination of their impact on the microstructure and texture of probiotic soft cheese produced with *Lactobacillus plantarum* FNCC 0026 through rennet gelation. Therefore, the purpose of this study was to: 1) analyze whether the total LAB was in accordance with the minimum standards for probiotic foods; 2) analyze the microstructure of probiotic soft cheese using field emission scanning electron microscopy (FESEM); and 3) analyze the texture of cheese (hardness, adhesiveness, and springiness) using a texture profile analyzer

(TPA) as a physical quality parameter of the product.

Methods

This study was included in true experimental research used a Randomized Block Design (RBD) with 12 experimental units, consisting of one control and three treatment groups, each replicated three times in the application of probiotic soft cheese. The formulation and concentration of the probiotic bacteria *Lactobacillus plantarum* FNCC 0026 were determined based on the modification of the results of previous research, namely F0 (0%); F1 (5%); F2 (10%); and F3 (20%).

Preparation

Cow's milk was supplied by Koperasi Podusen Unit Desa Karangploso, rennet was provided by Danisco (USA), MRS broth and MRS agar were obtained from Himedia (India), and NaCl was purchased from Merck (Germany).

Preparation of *Lactobacillus plantarum* FNCC 0026 Culture

Lactobacillus plantarum FNCC 0026 was inoculated into 500 mL of MRS broth and incubated for 24 h at 37°C. After incubation, the bacterial concentration in the culture that had developed was counted and transferred to a Falcon tube.

The culture was separated by centrifugation for 10 min at 8500 rpm. The supernatant was discarded, and the residue was suspended in 5 mL of sterile 0.85% NaCl solution and centrifuged again. This washing process was carried out 2 times using a sterile 0.85% NaCl solution.

Manufacture of Probiotic Soft Cheese

Two liters of fresh cow's milk were pasteurized at 80°C for 15 s and subsequently cooled to 37°C. The milk was subsequently transferred to glass bottles and labeled according to the treatment. In the control treatment (F0), 10 ml of vinegar was added to the milk to quickly lower its pH. In F1, F2, and F3, the *Lactobacillus plantarum* FNCC 0026 starter was administered according to the respective concentrations (v/v). The glass bottle containing milk was covered with aluminum foil and incubated at 37°C for approximately six hours until the pH reached 6.

Furthermore, 0.06 g of rennet was added to each fermented milk sample. The mixture was stirred and left until curd was formed for approximately 1 h. During scalding, the curd was cut into small fragments and heated at 40°C for 30 min. After heating, filtration was performed to segregate the curd from the whey, followed by pressing the curd. The curd was weighed, and 2% (w/w) salt was added to improve the taste and durability of cheese. The mixture was then stirred until smooth and stored in a refrigerator at a temperature–1-4°C.

Determination of Total LAB

Total LAB was quantified using the total plate count (TPC) method. The sample was dissolved in 9 ml of distilled water (10^{-1} dilution) and then diluted to 10^{-7}. A 1 ml sample was taken from each dilution, replicated three times, and duplicated in each formulation.

Sterile MRS agar medium was cooled to 50°C and poured into a Petri dish using the pour plate method, followed by incubation at 37°C for 48 h. The colonies that grew were recorded and counted using a standard plate count (SPC).

FESEM Procedure

An Al specimen stub was prepared using acetone and dried with a lint-free tissue. Carbon double-sided tape was then attached to the Al specimen stub. Once ready, the sample was attached to a carbon double-sided tape.

The samples were coated with gold (Au) using a Quorum Q150R S Plus instrument (Quorum Technologies Ltd. England) for 35 s at a current of 20 mA to produce an Au layer with a thickness of ± 30 nm. The sample was then inserted into the chamber of a field-emission scanning electron microscope (FESEM QUANTA FEG 650, Field Electron and Ion Company, USA). The sample was observed using a low large-field detector (LFD) under low vacuum.

TPA Procedure

The texture of the probiotic soft cheese was analyzed using the TPA method with a texture analyzer (Ametek type Lloyd TA1, USA). Probiotic soft cheese samples were cut into cubes and uniformly pressed twice with a probe 2 times.

The probe speed was 1 mm/2 with a sample pressure of up to 50% of the initial height of the sample. The parameters observed were hardness, adhesiveness, and springiness values were measured using a texture analyzer software. The selection of texture parameters, including hardness, adhesiveness, and springiness, was based on their critical role in determining the sensory quality, consumer acceptability, and structural integrity of cheese, particularly in probiotic-containing products.

Statistical analysis

All data are presented as mean ± SD of three replicates. The results of statistical tests that were normally and homogeneously distributed, such as total LAB, hardness, and elasticity, were analyzed using univariate ANOVA, followed by Bonferroni post-hoc analysis. For data that were normally distributed but not homogeneous, the ANOVA Welch test was applied to measure the adhesiveness of the probiotic soft cheese. Statistical analysis was performed using SPSS 25 software, with the level of significance set at 5% (p<0.05).

Result and Discussion

Total LAB of Probiotic Soft Cheese

Figure 1 indicates a significant difference between the F0 and F1, F2, and F3 treatments. However, there was no significant difference (p>0.05) between the treatment groups (Figure 1). The F1 group exhibited the highest average total LAB at 8.054 ± 0.54 log CFU/g (2.0×10^8 CFU/g). The second highest average total LAB count was observed in the F3 group at 7.939 ± 0.64 log CFU/g (1.7×10^8 CFU/g), followed by the F2 group at 7.617 ± 0.14 log CFU/g (4.3×10^7 CFU/g), with the lowest recorded in the F0 group at 2.919 ± 1.57 log CFU/g (1.8×10^3 CFU/g). As shown in Figure 1, increasing the concentration of *Lactobacillus plantarum* FNCC 0026 did not increase the number of LAB in groups F1, F2, and F3.

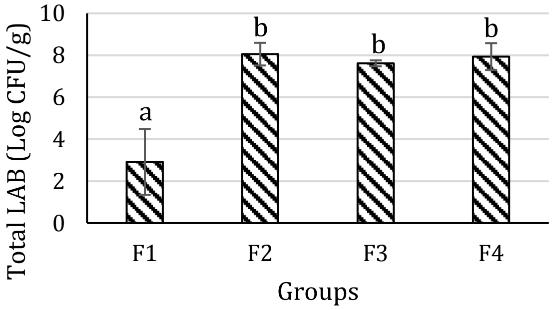

Figure 1. Total Lactic Acid Bacteria of Probiotic Soft Cheese

In the manufacture of probiotic soft cheese, determining total LAB is a crucial parameter that is influenced by several factors, such as raw materials, probiotic bacterial strains, pH, salt, and manufacturing process (temperature and incubation time) (Feeney et al., 2021; Meghzili et al., 2024). The total LAB count in the F0 treatment tended to be lower because it only relied on natural LAB from raw product materials, and the type of microflora that contributed was unknown (Sionek et al., 2023).

Raw milk and natural whey cultures are the main sources of non-starter LAB in cheeses. LAB groups that may be involved include *Lactobacillus lactis, Lactobacillus paracasei, Lactobacillus plantarum, Enterococcus faecalis,* and *Pediococcus acidilactici* (Sujaya et al., 2016). Total BAL did not differ significantly between the treatment groups. These findings demonstrated that variations in the concentration of the *Lactobacillus plantarum* FNCC 0026 starter did not significantly affect the total BAL in the treatment group. The total BAL is likely affected not only by the *Lactobacillus plantarum* FNCC 0026 starter culture but also by environmental factors that influence the final outcomes. Nutritional support and environmental conditions affect bacterial growth and development (Possas et al., 2021).

Environmental factors that can affect bacterial growth include incubation time and temperature (Mardalena, 2016). The longer the incubation time, the higher the total number of LAB (Mulyani et al., 2021). Conversely, a rapid incubation time that is too fast can inhibit optimal LAB growth (Yunus & Zubaidah, 2015). In line with another study, the total LAB count increased by approximately ±1 log CFU/g as the incubation time increased from the 0th, 3rd, and 6th hours (Emmawati et al., 2021). This is because LAB can adapt by utilizing nutrients and the environment for survival and reproduction (Mulyani et al., 2021).

Temperature can also affect the growth of microorganisms. LAB have an optimal temperature range of 37 – 42°C. In addition, the temperature during storage affects the total LAB (Prahardhini et al., 2020). Previous research has shown that products stored at room temperature (25°C) experience a faster decrease in LAB compared to those stored at cold temperatures (4 – 5°C) (Kasmiyetti et al., 2022). In this study, the total number of LAB may have decreased when the samples were stored at room temperature (25°C).

Furthermore, an increase in the concentration of *Lactobacillus plantarum* FNCC 0026 may lead to a more rapid decline in pH, owing to accelerated acidification. Excessive accumulation of organic acids subsequently inhibits the growth of LAB cells (Shu et al., 2014). In pasta filata cheese, higher concentrations of *Lactobacillus* spp. have been shown to elevate organic acid production and reduce the pH during cheese production. This condition negatively impacts probiotic viability, particularly at elevated LAB levels, where acidification progresses faster than the capacity of the cells to adapt physiologically (Reale et al., 2019).

As shown in Figure 1, the total LAB count in the treatment group in this study met the criteria for the selection of probiotic products that have been set. This is evidenced by the total LAB count reaching ≥ 106 CFU/g, according to the quality requirements of ISO 19344:2015. The total LAB count also met the criteria for categorizing it as a functional food, which is a minimum of 106 CFU/g (Diza et al., 2016).

Microstructure of Probiotic Soft Cheese

The microstructures of the probiotic soft cheese obtained using FE-SEM are shown in Figure 2. Microstructural images of the probiotic soft cheese were obtained at 400x magnification. Observing fat and protein globules, as reported in previous studies, is challenging.

This is because probiotic soft cheese was observed using FESEM under dry conditions. Probiotic soft cheese is a dairy food product that contains large amounts of water. Based on previous research, it has been stated that proteins form a continuous field where fat is trapped as separate cavities (granules) or elongated clusters and has a smooth appearance. Fat globules appear as lumps in probiotic soft cheeses. Based on Figure 2, the protein formed is indicated by a thick white arrow, whereas the fat globules are indicated by black arrows.

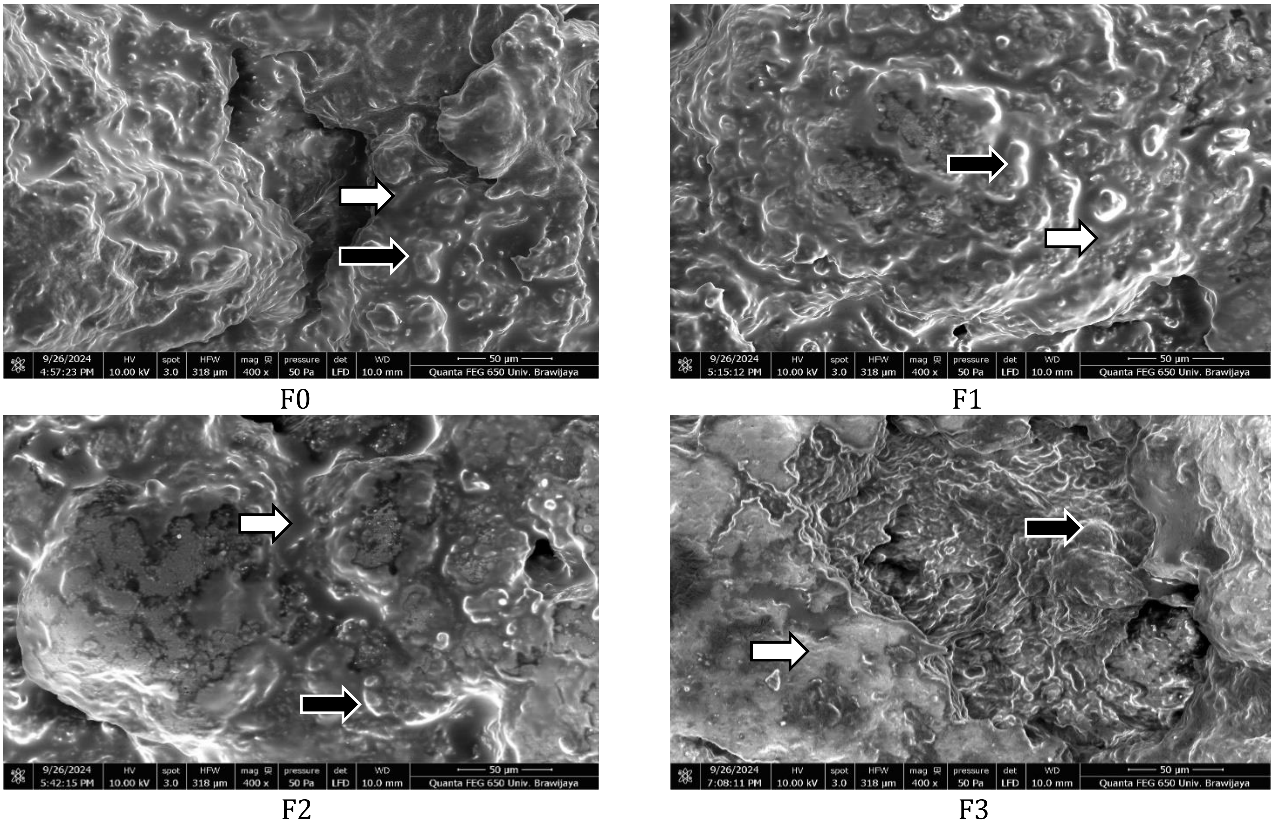

Figure 2. Microstructure (400x) of probiotic soft cheese with different concentrations of *Lactobacillus plantarum* FNCC 0026 (F0 = 0%, F1 = 5%, F2 = 10%, and F3 = 20%). Bar Indicates 50µm

The structure of cheese is complex. This complex structure results from manufacturing processes such as acidification, coagulation, dehydration, forming, and salting (Mehta, 2018). Microstructural analysis revealed no significant differences among F0, F1, F2, and F3. FESEM pictures at 400x magnification revealed that the control (F0) and treatment groups (F1, F2, and F3) had more compact, dense, and smooth protein structures, with regions of fat globules spread between areas of protein. This finding indicates a disruption in the uniform distribution of fat globules. Alterations in the fat and protein matrices resulting from diverse deliveries or ingredient variations lead to modifications in textural properties (Meghzili et al., 2024).

In this study, lipids aggregated into globular spaces, possibly due to the hydrolysis of the casein matrix during the storage of probiotic soft cheese that holds fat globules (Feeney et al., 2021). In all treatments, the microstructure of the protein matrix became denser and smoother. An increase in the density of the protein matrix indicates a more interconnected protein network (Meghzili et al., 2024).

The increase in protein density can be caused by temperature, pH, addition of rennet and calcium chloride, and the initial treatment of milk (Mehta, 2018; Salvador et al., 2022; Vigneux et al., 2022). The protein microstructure in cheese is established through coagulation or gelation of milk following the introduction of rennet enzymes (Salvador et al., 2022). The coagulation process occurs because of the interaction between temperature (10–40 °C) and acidity with casein micelle proteins consisting of αs1-, αs2-, β-, and κ-casein (Deshwal et al., 2023; Takagi et al., 2022). Throughout this process, casein micelles aggregate to create a solid matrix containing fat globules, free fat, soluble and casein-bound minerals such as calcium, water molecules, and sodium chloride (Mehta, 2018).

Texture of Probiotic Soft Cheese

Statistical tests showed that the texture of probiotic soft cheese, including hardness, adhesiveness, and springiness, was not significantly different (Table 1).

Although the differences in hardness, adhesiveness, and springiness were not statistically significant, the results suggest that probiotic addition did not substantially affect the textural properties of soft cheese. The hardness of probiotic soft cheese ranged from 32.41 to 48.89 N. The hardness values were as follows: F0 (40.18 N), F1 (36.01 N), F2 (48.89 N), and F3 (32.41 N). The highest hardness value was obtained for F2 (48.89 N ± 16.1). The lowest hardness value was obtained for F3 (32.41 N ± 19.9). The higher the hardness value, the firmer is the texture of the cheese. From a sensory perspective, the hardness value measured by TPA represents the initial resistance to mastication, describing how firm or hard the cheese is perceived during the first bite (Cinar et al., 2024). Accordingly, F3 was considered to have the softest texture among the samples, as supported by the data. Furthermore, the adhesiveness value of the probiotic soft cheese varied from 0.03 to 2.65 Nmm. The adhesiveness values were F0 (0.13 Nmm), F1 (2.65 Nmm), F2 (0.51 Nmm), and F3 (0.03 Nmm).

Based on the data in Table 1, the adhesiveness of probiotic soft cheese with the addition of *Lactobacillus plantarum* FNCC 0026 bacterial starter (F1, F2, and F3) tended to be higher than that of the control. Subsequently, the springiness value of the probiotic soft cheese ranged from 0.78-0.83. The elevated springiness value of the probiotic soft cheese indicated an increase in its elasticity.

Table 1. Texture of probiotic soft cheese by using texture profile analyzer

Variables	Groups				p-value
	F0	F1	F2	F3	
Hardness (N)	40.18 ± 1.81	36.01 ± 18.4	48.89 ± 16.1	32.41 ± 19.9	0.313
Adhesiveness (Nmm)	0.13 ± 1.16	2.65 ± 2.99	0.51 ± 0.57	0.03 ± 0.07	0.478
Springiness	0.78 ± 0.03	0.82 ± 0.06	0.83 ± 0.04	0.82 ± 0.06	0.472

The p-value is a statistical test with a significance of $p<0.05$

The textural attributes of probiotic soft cheeses, including hardness, adhesiveness, and springiness, may be influenced by several factors. The diminished hardness of F3 may have been affected by the fat content of the cheese (Sulistyo et al., 2018). The fat content of cheese is inversely proportional to its hardness. If the fat content in cheese is low, its hardness value of cheese increases. The presence of fat in cheese can produce a softer texture because fat globules are evenly distributed in casein proteins (Juniawati et al., 2015). The addition of salt to cheese and its water content can also affect the hardness of cheese (Sulistyo et al., 2018). Salt added to cheese can influence the syneresis process, such that the water activity in cheese decreases more quickly. A decrease in the water activity of cheese results in reduced water content, leading to a firmer texture (Musra et al., 2021). Therefore, cheese hardness can be reduced by reducing the concentration of salt or NaCl during cheesemaking (Zakariah et al., 2022).

In a similar study that produced soft cheese with *Lactobacillus plantarum*, the hardness value of probiotic soft cheese was significantly higher than those reported in other similar studies. The hardness value of the resulting cheese was 47.75 gf, equivalent to 0.47 N in a previous study (Setiawardani et al., 2016). Previous studies have reported cheese hardness values ranging from 0.14 to 0.49 N (Amar et al., 2024). In comparison, the probiotic soft cheese in this study showed a hardness of 32.41–48.89 N, indicating a significantly higher firmness than that of cheeses reported in similar studies. Based on this range, it can be concluded that the hardness of the probiotic soft cheese was approximately 100 times higher than that reported in similar studies. In a study on the production of Cheddar cheese (hard cheese), the hardness produced ranged from 30-50 to N (Reale et al., 2022). Based on this range, the hardness of probiotic soft cheese was comparable to that of hard cheese. This could be attributed to the duration of scalding or whey separation during cheese production. A study on Sheep Halloumi cheese demonstrated that the duration of scalding significantly affects both the moisture content and hardness. A scalding time of 20 min resulted in optimal hardness and chewiness, whereas extending the scalding duration to 30 min reduced the softness and sensory acceptability (Kaminarides et al., 2015). Post-gelation heating at temperatures ranging from 55 to 75 °C for a few seconds to several minutes has been shown to increase cheese firmness, and this phenomenon is relevant to the scalding process in soft cheeses (Gao et al., 2023).

The texture of adhesiveness contrasts with that of hardness. For soft cheese, the optimal texture exhibits a low hardness value, coupled with a high adhesiveness value. The adhesiveness of cheese can be influenced by several factors, such as casein and fat, which play a role in the formation of the cheese matrix and the storage duration of the cheese (Setiawardani et al., 2016; Zheng et al., 2016). The longer the storage time, the higher the adhesiveness of cheese (Mohammed et al., 2019; Zheng et al., 2016).

The high adhesiveness of F1 may be influenced by its fat and protein content. The protein matrix that forms the cheese curd contains fat clumps. Fat inhibits the formation of casein cross-links and acts as a plasticizer (increasing the plastic properties of cheese) (Zheng et al., 2016). The higher the fat content, the higher was the adhesiveness of the cheese. Conversely, the lower the fat content in cheese, the lower the adhesiveness. However, adhesiveness was negatively correlated with the protein content. In a similar study, the adhesiveness value of the resulting cheese ranged from 0.3 to 0.6 mJ, or the equivalent of 0.3 to 0.6 Nmm (Singh et al., 2023). In other studies, the adhesiveness of the resulting cheese ranged from 1.47 to 3.2 (Koranteng et al., 2021). Therefore, the range of adhesiveness values in similar studies is 0.3–3.2 Nmm. Based on this, it can be concluded that the adhesiveness texture of probiotic soft cheese still falls within the range of adhesiveness textures for soft cheese in similar studies but tends to be lower in F1 (0.13 Nmm ± 1.16) and P3 (0.03 Nmm ± 0.07).

High springiness indicates that food has a greater ability to return to its original shape after pressure application. The springiness of cheese is influenced by its fat content and moisture level (Astuti et al., 2021; Parra-Ocampo et al., 2020). Cheese with low or minimal fat content tends to have a denser or harder texture but is more elastic and has higher springiness (Singh et al., 2023). A high springiness value can also occur when the moisture content in cheese is low, making the cheese texture denser, as springiness is directly proportional to the hardness.

The hardness and springiness values of probiotic soft cheese align with studies stating that hardness is directly proportional to springiness. The highest hardness and springiness values were found in the probiotic soft cheese with the same treatment, namely, the probiotic soft cheese with the addition of *10% Lactobacillus plantarum FNCC 0026 starter* (F2). The hardness and springiness values of F2 were 48.89 N ± 16.13, and the springiness value of F2 was 0.83 ± 0.04. The decrease in hardness also contributes to the reduction in springiness because this texture reflects the effort required to chew food to break it down to a texture suitable for swallowing (Parra-Ocampo et al., 2020). In other studies, the springiness value of the resulting cheese ranged from 0.48 to 0.91 (Amar et al., 2024). The average springiness value of the resulting cheese was 0.78 ± 0.10 in a previous study (Astuti et al., 2021). Therefore, the range of springiness values in similar studies was 0.48-0.91. Based on the evaluation of total LAB counts and textural properties, the optimal formulation of probiotic soft cheese was determined using the Multiple Attribute Zeleny Method. This analysis identified the addition of *Lactobacillus plantarum* FNCC 0026 at a concentration of 5% as the most effective treatment, providing a balanced outcome in terms of microbial viability and desirable textural characteristics.

Conclusion

The findings of this study indicate that probiotic soft cheese can be successfully produced. Probiotic soft cheese made with 5% *Lactobacillus plantarum* FNCC 0026 had the highest concentration of LAB and optimum textural characteristics, fulfilling the functional food standard of at least 10^6 CFU/g. In addition, based on the FESEM results, all treatments had high protein densities.

The production of cheese with the addition of probiotic bacteria has been widely studied. However, the best manufacturing process to produce probiotic soft cheese with total LAB, texture, and taste that meets quality standards for consumers still needs to be determined. Further investigation is required to determine the viability and sensory characteristics of probiotic soft cheese stored under varying pH conditions.

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