Development of Probiotic Soft Cheese and Evaluation of pH and Lactic Acid Bacteria Counts Before and After Probiotic Inoculation

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Abstract. Probiotic foods have evolved from traditional fermented products into functional foods that offer health benefits beyond basic nutrition. Soft cheese is considered an effective carrier of probiotic microorganisms because of its favorable physicochemical properties. This study evaluated the effect of different concentrations of *Lactobacillus plantarum* FNCC 0026 on the pH and total lactic acid bacteria (LAB) count of probiotic soft cheese. Four formulations containing 0%, 5%, 10%, and 20% starter culture were examined. The pH and total LAB counts were measured before and after LAB addition to assess acidity and probiotic viability. Total LAB data were analyzed using Two-Way Repeated Measures ANOVA, whereas pH data were analyzed using the Wilcoxon test at a significance level of 5% ($p < 0.05$). The pH significantly decreased from 6.28–6.62 before treatment to 6.09–6.50 after LAB addition ($p < 0.05$), indicating increased lactic acid production during fermentation. Meanwhile, total LAB counts remained high, ranging from 7.60 to 8.39 log CFU/g, and met the minimum requirement for probiotic foods. No significant difference was observed in total LAB counts before and after treatment ($p > 0.05$). These findings indicate that *L. plantarum* FNCC 0026 maintained probiotic viability while significantly influencing product acidity. Therefore, the developed soft cheese has potential as a functional probiotic food with favorable microbial and physicochemical characteristics.

Keywords: Functional food, Probiotic soft cheese, Lactobacillus plantarum, pH value, total LAB.

1 Introduction

Probiotic food products have evolved from merely traditional fermented foods into scientifically supported functional foods that provide health benefits beyond fulfilling basic nutritional needs [1]. Probiotics are living microorganisms that can confer therapeutic and health-promoting effects to the host when consumed [2]. These microorgan-

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isms are commonly obtained from Lactic Acid Bacteria (LAB), especially those belonging to the genera Bifidobacterium and Lactobacillus [3]. LAB are Gram-positive bacteria capable of producing lactic acid as the main metabolite during fermentation while also functioning as bacteriocin producers and providing health benefits [4]. LAB are commonly found in fermented dairy products such as cheese [5]. A product can be categorized as a probiotic food if it contains at least $\geq 10^6$ CFU/g viable LAB at the time of consumption [6].

Cheese is one of the processed dairy products produced through fermentation [7]. Compared to yogurt and other fermented dairy products, cheese is regarded as a highly effective medium for delivering probiotic microorganisms. Its buffering properties help probiotic bacteria withstand acidic conditions in the gastrointestinal tract more effectively [5]. Cheese is available in various forms, including fresh cheese, which is considered particularly suitable as a probiotic carrier. The relatively high moisture content and pH of fresh cheese provide favorable conditions for the growth and survival of bacteria, including LAB [8].

One type of LAB commonly used in cheese production is *Lactobacillus plantarum* [9]. This bacterium is well known for its multifunctional activity, including antibacterial properties. The antibacterial activity of *Lactobacillus plantarum* is associated with the production of organic acids, hydrogen peroxide, and bacteriocins known as plantaricins [10]. *Lactobacillus plantarum* can survive under low pH conditions and produce bacteriocins capable of inhibiting the growth of pathogenic bacteria [11]. The strain *Lactobacillus plantarum* FNCC 0026 was selected due to its ability to inhibit both Gram-positive and Gram-negative bacteria, indicating its potential as an alternative to antibiotics [10].

Previous study reported that varying the concentration of Lactobacillus plantarum B1765 starter culture in soft cheese production influenced several characteristics, particularly total LAB counts and pH values [12]. pH is considered a crucial parameter because it affects the physical properties of the product [13]. In addition, pH conditions can influence microbial growth and viability. LAB strains such as Lactobacillus plantarum FNCC 0026 are known to grow within a broad pH range, from 3 to 9 [14], [15]. Furthermore, adequate LAB levels in a product are important to classify it as a probiotic food product [16]. A daily intake of approximately 10^8–10^9 CFU/g of probiotic microorganisms is considered necessary to achieve optimal health benefits for consumers [17]. Moreover, observing the pH value and total LAB counts of cheese before and after the addition of LAB is important to evaluate changes occurring during the fermentation process and to determine the viability and growth of probiotic bacteria in the product. These parameters are essential indicators for assessing the quality, safety, and probiotic potential of soft cheese products.

Based on the explanation above, this study aimed to develop probiotic soft cheese with the addition of various concentrations of Lactobacillus plantarum FNCC 0026 starter culture in each formulation. The probiotic soft cheese produced in this study was analyzed for total LAB counts and pH values to determine the best formulation and evaluate its suitability and safety as a probiotic product for consumption.

2 Methods

2.1 Preparation of Lactobacillus plantarum FNCC 0026

The probiotic strain *Lactobacillus plantarum* FNCC 0026 was obtained from Universitas Gadjah Mada. The culture was first propagated in 500 mL of MRS broth and incubated at 37 °C for 24 hours. After incubation, the bacterial population was quantified, and the culture suspension was transferred into conical centrifuge tubes (Falcon, Corning Inc., USA). Cell biomass was collected by centrifugation using an Eppendorf 5804R centrifuge at 8500 rpm for 10 minutes, followed by removal of the supernatant. The resulting pellet was resuspended in 5 mL of sterile 0.85% NaCl solution (SMARTLAB, Indonesia) and centrifuged again. The washing step with sterile 0.85% NaCl solution was performed twice to ensure cell purification.

2.2 Production of Probiotic Soft Cheese

Fresh cow's milk was supplied by the Karangploso Village Unit Producer Cooperative. A total of 2 L of milk was pasteurized at 80 °C for 15 seconds and subsequently cooled to 37 °C before further processing. The milk was distributed into glass containers and labeled according to treatment groups. Four formulations were prepared based on different concentrations of Lactobacillus plantarum FNCC 0026, namely F1 (0%), F2 (5%), F3 (10%), and F4 (20%).

For the control treatment (F1), 10 mL of vinegar was incorporated to accelerate pH reduction. In contrast, treatments F2, F3, and F4 received inoculation of Lactobacillus plantarum FNCC 0026 starter culture at the respective concentrations (v/v). All samples were covered with aluminum foil and incubated at 37 °C for approximately 6 hours until the pH reached 6.

After incubation, 0.06 g of rennet was added to each formulation and mixed evenly, followed by a resting period of approximately 1 hour to allow curd formation. During the scalding stage, the curd was cut into smaller pieces and heated at 40 °C for 30 minutes. The curd was then separated from the whey through filtration and subsequently pressed. After weighing, salt equivalent to 2% (w/w) of the curd weight was incorporated to improve flavor and extend shelf life. Finally, the cheese was homogenized and stored under refrigerated conditions at 1–4 °C.

2.3 pH Analysis

The pH value of the samples was determined using a calibrated pH meter. Prior to analysis, 10 g of sample was homogenized with 10 mL of distilled water until a uniform mixture was obtained. The pH meter was calibrated using standard buffer solutions at pH 4.01, 7.01, and 10.01 before measurement. The electrode was then immersed into the prepared sample solution, and the pH reading was recorded after approximately 2 minutes once the value had stabilized. pH measurements were conducted both before and after the addition of *Lactobacillus plantarum* FNCC 0026 to evaluate changes in acidity during the probiotic soft cheese production process.

2.4 Determination of Total Lactic Acid Bacteria (LAB)

The total population of LAB was determined using the Total Plate Count (TPC) method. Initially, the sample was diluted by mixing 1 mL of sample with 9 mL of distilled water to obtain a 10^{-1} dilution. Serial dilutions were subsequently prepared up to 10^{-7}. From each dilution level, 1 mL of sample was aseptically transferred into sterile petri dishes in triplicate and duplicated for each formulation.

Sterile MRS agar medium, previously cooled to approximately 50 °C, was poured into the petri dishes using the pour plate technique. The plates were then incubated at 37 °C for 48 hours. Following incubation, the number of visible colonies was enumerated using the Standard Plate Count (SPC) method.

The determination of total LAB was carried out before and after the addition of Lactobacillus plantarum FNCC 0026 cultures to evaluate the changes in bacterial population during probiotic soft cheese production.

2.5 Data Analysis

Statistical analysis for total *Lactobacillus plantarum* FNCC 0026 data that were normally distributed and homogeneous was performed using the Two-Way Repeated Measures ANOVA. Meanwhile, pH value data were analyzed using the non-parametric Wilcoxon test. Statistical analyses were conducted using SPSS version 25 software with the level of significance set at 5% ($p < 0.05$).

3 Results

The evaluation of pH values and total LAB counts before and after the addition of Lactobacillus plantarum FNCC 0026 was conducted to determine the effect of probiotic starter concentration on the characteristics of probiotic soft cheese. These parameters are important indicators for assessing product quality, microbial viability, and the suitability of the cheese as a probiotic food product. Changes in pH values may reflect the metabolic activity of LAB during fermentation, while total LAB counts indicate the ability of probiotic bacteria to survive and grow in the cheese matrix. The results of pH measurement and total LAB analysis before and after LAB addition are presented in Table 1 and Table 2.

Table 1. pH values of Probiotic Soft Cheese Before and After Addition of *Lactobacillus plantarum* FNCC 0026

Treatment	pH value		p-value
	Before	After	
F1	6.28 ± 0.04	6.09 ± 0.07	0.004*
F2	6.62 ± 0.08	6.45 ± 0.13	
F3	6.60 ± 0.09	6.43 ± 0.07	
F4	6.59 ± 0.15	6.50 ± 0.15	

*Significant difference at $p < 0.05$.

The pH values of probiotic soft cheese decreased after the addition of LAB in all treatments. The decrease in pH may indicate the metabolic activity of LAB during fermentation, resulting in the production of organic acids, particularly lactic acid. Statistical analysis using the Wilcoxon test demonstrated that the pH values before and after treatment were significantly different ($p < 0.05$), with a p-value of 0.004. These findings suggest that the addition of Lactobacillus plantarum FNCC 0026 influenced the acidity characteristics of the probiotic soft cheese while maintaining adequate LAB viability as a probiotic product.

The results of total LAB and pH analysis before and after the addition of *Lactobacillus plantarum* FNCC 0026 in probiotic soft cheese are presented in Table X. The total LAB values in treatments containing probiotic starter culture (F1, F2, and F3) were maintained at approximately 10^7–10^8 CFU/g after treatment, indicating that the probiotic bacteria were able to survive well in the cheese matrix. Statistical analysis using the Two-Way Repeated Measures ANOVA showed that the total LAB values before and after treatment were not significantly different ($p > 0.05$), with a p-value of 0.082 (Table 2).

Table 2. Total LAB of Probiotic Soft Cheese Before and After Addition of *Lactobacillus plantarum* FNCC 0026

Treatment	Total LAB (log CFU/g)		p-value
	Before	After	
F1	4.86 ± 2.19	2.92 ± 1.58	
F2	8.04 ± 0.16	8.39 ± 0.49	0.082*
F3	8.00 ± 0.23	7.62 ± 0.14	
F4	8.04 ± 0.24	7.60 ± 0.99	

*Significant difference at $p < 0.05$.

4 Discussion

The pH values of probiotic soft cheese decreased after the addition of LAB in all treatments, ranging from 6.28–6.62 before treatment to 6.09–6.50 after treatment. This reduction in pH was likely associated with the metabolic activity of LAB during the fermentation process. LAB are known to ferment lactose into lactic acid, resulting in the accumulation of organic acids that increase product acidity and consequently lower the pH of fermented dairy products [12]. The increase in LAB activity is generally accompanied by enhanced conversion of lactose into lactic acid, which subsequently alters the pH of the product [18].

Statistical analysis using the Wilcoxon test demonstrated a significant difference in pH values before and after treatment, with a p-value of 0.004 ($p < 0.05$). This finding indicates that the addition of Lactobacillus plantarum FNCC 0026 significantly affected the acidity characteristics of probiotic soft cheese. Nevertheless, the resulting pH values remained within the favorable growth range for Lactobacillus plantarum, which has been reported to grow optimally at pH 3–9 [14], [15].

pH is an important parameter because it directly influences the quality and safety of cheese products [19]. In addition, pH affects the types of microorganisms capable of growing in the product. Most microorganisms can grow optimally at pH values ranging from 6.0 to 8.0 and are generally unable to survive at pH levels below 2 or above 10 [20]. Therefore, maintaining an appropriate pH is essential for preserving microbial stability, ensuring product safety, and inhibiting the growth of spoilage and pathogenic microorganisms.

Furthermore, the use of coagulants such as rennet may also influence the pH characteristics of cheese. The pH value may remain relatively high if the coagulant used is insufficiently acidic or if additional ingredients with alkaline properties are incorporated into the formulation [19]. This condition may explain why the pH values obtained in the present study remained within a relatively moderate range despite the increased LAB activity during fermentation.

In contrast, the total LAB values in treatments F1, F2, and F3 remained relatively high after the addition of probiotic starter culture, ranging from 7.60 to 8.39 log CFU/g. These results indicate that Lactobacillus plantarum FNCC 0026 was able to survive and grow well in the soft cheese matrix [8]. The high viability of LAB observed in this study demonstrates that soft cheese provides a favorable environment for probiotic bacteria due to its relatively high moisture content and suitable pH conditions [21]. A product can be categorized as a probiotic food if it contains at least 10^6 CFU/g viable probiotic bacteria at the time of consumption [6]. Therefore, all probiotic soft cheese treatments in this study fulfilled the minimum requirement as probiotic products.

Statistical analysis using the Two-Way Repeated Measures ANOVA showed that there was no significant difference in total LAB values before and after treatment ($p > 0.05$). The obtained p-value of 0.082 indicates that the addition of Lactobacillus plantarum FNCC 0026 did not significantly alter the total LAB counts. This condition may be associated with the ability of LAB to adapt well to the cheese environment and maintain stable growth throughout the fermentation process. Another factor that may explain the lack of significant differences in LAB viability is their resistance to acidic and high-temperature conditions during cheese fermentation [22]. Similar findings were reported by [12], who stated that the use of Lactobacillus plantarum starter culture in soft cheese production could maintain high LAB viability.

5 Conclusion

The addition of *Lactobacillus plantarum* FNCC 0026 in probiotic soft cheese was able to maintain high total LAB counts, ranging from 7.60 to 8.39 log CFU/g, which fulfilled the minimum requirement for probiotic food products. Statistical analysis showed that the addition of LAB did not significantly affect total LAB counts before and after treatment ($p > 0.05$). However, the pH values significantly decreased after LAB addition ($p < 0.05$), indicating active fermentation and lactic acid production by probiotic bacteria.

The resulting pH values remained within the favorable growth range for *Lactobacillus plantarum*. Overall, the probiotic soft cheese produced in this study demonstrated good probiotic viability and appropriate acidity characteristics, indicating its potential as a functional probiotic food product.

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