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Evaluation the effect of different concentrations of liquid rennet on total acid, viability of lactic acid bacteria, physicochemical and organoleptic properties of goat milk mozzarella cheese

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ABSTRACT

Background: Goat milk has been recognized as a potential substitute for cow milk due to its high digestibility and rich nutritional composition. However, its short shelf life limits its utilization. Transforming goat milk into mozzarella cheese offers an alternative to extend its stability while enhancing its functional properties. This study aimed to evaluate the effect of different liquid rennet concentrations on the total acidity, lactic acid bacteria (LAB) viability, physicochemical, and organoleptic characteristics of goat milk mozzarella cheese.

Methods: The experiment used a Completely Randomized Design (CRD) with three rennet concentrations (P1: 120 ppm, P2: 180 ppm, and P3: 240 ppm) and six replications. The parameters analyzed included total acid, LAB count, yield, pH, protein content, elongation, texture, and sensory attributes (color, aroma, texture, taste). Data were analyzed by ANOVA followed by Duncan's Multiple Range Test (DMRT) for significant differences ($P < 0.05$).

Results: Increasing liquid rennet concentration significantly enhanced total acid (0.185–0.275%), LAB viability (6.30×10^4–21.00×10^4 CFU/g), yield (7.423–8.777%), elongation (35.83–39.17 cm), protein content (32.33–35.74%), and texture. Sensory evaluation showed that texture and taste improved significantly at higher rennet levels, while color and aroma remained unaffected. The highest overall quality was achieved at 240 ppm liquid rennet.

Conclusion: Liquid rennet concentration plays a crucial role in determining the physicochemical and sensory properties of goat milk mozzarella cheese. A concentration of 240 ppm produced the best balance between microbial viability, composition, and sensory acceptance, suggesting its suitability for commercial cheese production.

KEYWORDS

Goat milk, liquid rennet, mozzarella cheese, fermentation, functional food

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INTRODUCTION

Goat milk has attracted increasing attention as a functional alternative to cow milk due to its superior digestibility, smaller fat globules, and higher proportions of short- and medium-chain fatty acids. It also contains lower levels of αs1-casein, which reduces allergenicity and makes it suitable for individuals with mild dairy sensitivities[1]. However, despite these nutritional advantages, goat milk is highly perishable and susceptible to microbial spoilage because of its rich nutrient profile. This short shelf life limits its economic potential and availability in regions with limited cold-chain infrastructure. Therefore, transforming goat milk into value-added products with extended shelf life and improved functionality is essential to enhance its utilization and market competitiveness[2,3].

Among various goat milk derivatives such as yogurt, kefir, and fermented drinks, cheese offers distinctive advantages, including higher nutrient retention, protein concentration, better preservation stability, and broader culinary applications[4,5]. Particularly, mozzarella cheese is a fresh, high-moisture, non-aged cheese with unique stretchability, mild flavor, and excellent consumer acceptance, making it highly demanded by the modern food industry[6–8]. The development of goat milk mozzarella not only enhances the commercial value of goat milk but also supports small-scale dairy enterprises by reducing milk waste and increasing product diversity[9].

The rennet-induced coagulation process plays a crucial role in determining the structure, yield, and textural attributes of cheese[10]. Variations in enzyme concentration, type, and activity directly affect curd formation, moisture retention, and microbial growth[11]. While previous studies have examined the use of different types of rennet (animal, plant, or microbial) in cheese manufacturing, most have focused on cow milk[6]. Research on goat milk mozzarella remains limited, especially regarding the effect of varying liquid rennet concentrations on microbiological (LAB viability), physicochemical (yield, protein, moisture, elongation), and sensory properties[12].

This limitation presents a significant research gap: the optimal concentration of liquid rennet for goat milk mozzarella production has not been well established. Existing studies tend to emphasize rennet source or pH treatment, while the influence of rennet dosage on balancing microbial viability, acidity, and sensory quality in goat milk cheese is underexplored[13]. Addressing this gap is urgent, not only to improve cheese-making efficiency and product standardization for artisanal producers but also to support halal-certified, safe, and sustainable dairy innovation.

Therefore, this study aims to evaluate the effect of different liquid rennet concentrations on the total acidity, lactic acid bacteria (LAB) viability, physicochemical characteristics (yield, pH, protein, moisture, elongation, texture), and organoleptic properties (color, aroma, texture, and taste) of goat milk mozzarella cheese. The results are expected to provide practical guidance for optimizing rennet concentration to enhance cheese quality, functionality, and consumer acceptability in the goat milk dairy sector.

METHODS

Design, Place and Time

This experimental study employed a Completely Randomized Design (CRD) with three treatments and six replications per treatment. Treatments were defined by liquid rennet concentration: P1 = 120 ppm, P2 = 180 ppm, and P3 = 240 ppm. Each experimental unit (replicate) consisted of a single batch of mozzarella prepared from goat milk under identical processing conditions.

The study was carried out at the Livestock Production Laboratory Faculty of Agriculture Universitas Lampung, Indonesia. All laboratory analyses were performed according to validated standard methods (AOAC, SNI) as cited below.

Ingredients and Equipment

The materials used in this study included fresh goat milk obtained from farm, food-grade citric acid as an acidulant, plant-based liquid rennet at varying concentrations, food-grade sodium chloride, and analytical reagents for titration, protein, and microbiological assays. The primary equipment consisted of a pasteurization unit capable of maintaining 65°C, pH meter, hot plate with a built-in thermometer, cheese moulds, analytical balance, oven, Kjeldahl apparatus, texture analyzer (TMS-Pro), ruler or calliper for elongation testing, and standard microbiological instruments such as incubators and sterilization tools.

Sample Preparation

Before making goat milk mozzarella cheese, pre-research activities were carried out two times using goat milk, with the aim that there would be no errors or failures when the main research was carried out. The first thing to do is to sterilize the tools that will be used with hot water. After all the tools are ensured to be clean and sterile, the next step is the pasteurization of goat milk at a temperature of 65°C for 30 minutes. Then the temperature is lowered to 33°C. The next step is to add citric acid solution, then liquid rennet according to the treatment (P1: 120 ppm, P2: 180 ppm, P3: 240 ppm). Approximately 20 minutes after adding liquid rennet, curd will form. After the curd is formed, the curd and whey are separated by filtering. The last process is pasteurizing the curd until it is cooked and then stretching the cheese to a temperature of 80°C.

Parameter Analysis

The parameters analyzed included microbiological, physicochemical, and sensory properties. Total acidity was measured by titration and expressed as a percentage of lactic acid, while lactic acid bacteria (LAB) viability was determined using the pour plate method in accordance with SNI 2981:2009[14]. pH was measured using a calibrated pH meter, and yield was calculated as the ratio of cheese weight to milk weight multiplied by 100%. Protein content was analyzed using the Kjeldahl method, and moisture content was determined through oven drying following AOAC (2005)[15]. Elongation was assessed using the method described by measuring the maximum stretch length (cm) before breakage, and texture strength (N) was evaluated using a TMS-Pro texture analyzer[16]. The organoleptic characteristics—including color, aroma, texture, and taste—were assessed by 30 untrained panelists using a five-point hedonic scale under controlled sensory evaluation conditions.

Data Analysis

All data were collected in triplicate and expressed as mean ± standard deviation. Statistical analysis was performed using Analysis of Variance (ANOVA) to identify significant differences among treatments. When significant effects were detected ($P < 0.05$), Duncan's Multiple Range Test (DMRT) was applied to determine pairwise differences. Prior to analysis, assumptions of normality and homogeneity of variance were verified using the Shapiro–Wilk and Levene's tests, respectively. In cases where assumptions were not met, data were appropriately transformed (e.g., log10 for microbial counts). Statistical analyses were conducted using SPSS version 22, and non-parametric tests such as Kruskal–Wallis were applied for sensory data when necessary.

RESULTS

Total Acid Testing, LAB Viability, Physicochemical Properties and Organoleptic Test of Goat Mozarella Cheese

The results presented in Table 1 show that increasing liquid rennet concentration significantly affected the physicochemical and microbiological properties of goat milk mozzarella cheese. The total acidity increased progressively from 0.185% in treatment P1 (120 ppm) to 0.275% in P3 (240 ppm), indicating enhanced acid formation with higher enzyme concentration. This pattern correlates with the increased activity of lactic acid bacteria (LAB), where the viability rose from 6.30×10^4 CFU/g in P1 to 21.00×10^4 CFU/g in P3. The higher LAB count in samples with greater rennet concentration suggests that more favorable microenvironments for microbial growth were achieved due to more efficient curd formation and nutrient retention.

The yield of mozzarella also increased from 7.42% to 8.78% across treatments, demonstrating that a higher concentration of liquid rennet enhanced curd compaction and reduced fat and casein loss into the whey. Similarly, the elongation values ranged from 35.83 cm (P1) to 39.17 cm (P3), confirming that a denser protein network and balanced acidity at higher rennet levels improve stretchability — a desirable property in mozzarella cheese. Protein content also showed a positive trend (32.33–35.74%), suggesting more efficient coagulation and protein retention. Conversely, the moisture content decreased slightly (55.47% to 54.11%) as higher enzyme concentrations produced firmer curds with reduced water-holding capacity. Texture strength increased correspondingly (7.47 N to 7.94 N), reflecting a tighter protein matrix and reduced softness in high-rennet samples. The pH values (5.62–5.68) showed no significant difference among treatments, consistent with the controlled acidification method using citric acid. This stability implies that rennet concentration primarily affected curd structure and enzyme activity rather than altering the pH equilibrium of the system.

Table 2 summarizes the organoleptic evaluation results, which revealed that the addition of higher liquid rennet concentrations significantly improved the texture and taste attributes ($P < 0.05$), while color and aroma remained unaffected. The color scores ranged from 3.80 to 4.17, and aroma scores from 3.23 to 3.77, showing that the increase in enzyme

Table 1. Results of Total Acid Testing, LAB Viability, and Physicochemical Properties

Parameter	Treatment		
	P1	P2	P3
Total Acid (%)	0.185 ± 0.007^c	0.240 ± 0.000^b	$0.275 \pm 0,007^a$
LAB Viability (Cfu/g)	$6.30 \times 10^4 \pm 0.84^b$	$10.90 \times 10^4 \pm 1.55^b$	$21.00 \times 10^4 \pm 2.83^a$
pH value	5.620 ± 0.028	5.670 ± 0.028	5.680 ± 0,014
Yield (%)	$7.423\pm 0,333^{b}$	$8.460 \pm 0,628^{a}$	8.777 ± 0.751^{a}
Elongation (cm)	35.833 ± 1.835^{b}	37.833 ± 0.753^{a}	39.1667 ± 0.983^{a}
Protein (%)	32.330 ± 2.753	34.197 ± 6.026	35.742 ± 5.996
Moisture (%)	55.47 ± 0.74^a	54.68 ± 0.69^{ab}	54.11 ± 0.52^b
Texture (N)	7.47 ± 0.17^b	7.62 ± 0.29^{ab}	7.94 ± 0.40^a

P1 (treatment 1 liquid rennet 120 ppm), P2 (treatment 2 liquid rennet 180 ppm), P3 (treatment 3 liquid rennet 240 ppm). Notations a and b in different columns indicate significant effect (P<0.05).

Table 2. Organoleptic Test Results of Goat's Milk Mozzarella Cheese

Parameter	Treatment		
	P1	P2	P3
Colour	3.80 ± 0.80	3.83 ± 0.79	4.17 ± 0.82
Aroma	3.23 ± 1.00	3.67 ± 1.17	3.77 ± 1.19
Texture	2.46 ± 0.93^{b}	3.60 ± 0.96^{a}	3.96 ± 1.29^{a}

P1 (treatment 1 liquid rennet 120 ppm), P2 (treatment 2 liquid rennet 180 ppm), P3 (treatment 3 liquid rennet 240 ppm). Notations a and b in different columns indicate significant effect (P<0.05).

concentration did not cause notable sensory variation in visual or olfactory aspects. Texture scores increased substantially from 2.46 (P1) to 3.96 (P3), consistent with instrumental measurements, while taste scores followed the same pattern. These findings suggest that consumers preferred the firmer, more elastic texture and balanced flavor associated with higher rennet concentrations.

Overall, the results indicate that increasing liquid rennet concentration enhances curd formation efficiency, protein retention, and structural integrity, leading to higher yield, better elongation, and improved sensory quality. The treatment with 240 ppm liquid rennet (P3) provided the most desirable combination of physicochemical and sensory properties, making it the optimal concentration for goat milk mozzarella production.

DISCUSSION

The results of this study demonstrated that increasing the concentration of liquid rennet from 120 ppm to 240 ppm significantly influenced the physicochemical, microbiological, and sensory characteristics of goat milk mozzarella cheese. A higher enzyme concentration enhanced total acidity, lactic acid bacteria (LAB) viability, yield, elongation, protein content, and textural firmness, while slightly reducing moisture content. These findings align with the fundamental enzymatic role of rennet in promoting casein micelle coagulation through κ-casein cleavage, leading to stronger gel networks and higher curd recovery[13]. The increase in total acidity observed with higher rennet levels reflects enhanced enzymatic and microbial activity, as greater coagulation efficiency reduces whey loss and creates a microenvironment conducive to LAB growth and acid production. Similar results were reported by Pawlos et al. (2023), who found that higher rennet dosages in goat milk cheese improved curd compactness and acid retention without significantly altering pH stability[17].

Although total acidity increased, the pH of the mozzarella cheese remained relatively stable (5.62–5.68). This phenomenon can be attributed to the buffering capacity of the casein-lactate system and the standardized acidification step using citric acid prior to rennet addition. The titratable acidity thus reflects localized lactic acid formation rather than a bulk pH change. Li et al. (2023) explained that the pre-acidification level of milk strongly modulates rennet coagulation dynamics but that once the buffering threshold of milk proteins is reached, small variations in acid production do not substantially alter pH[18]. Furthermore, stable pH ensures optimal proteolytic activity during the renneting phase, facilitating balanced gel strength and elasticity, both of which are key to the stretchability of mozzarella[19].

The positive correlation between rennet concentration and yield indicates more efficient protein and fat retention in the curd. Higher enzyme levels accelerate casein micelle aggregation, producing a denser curd matrix that traps more solids and reduces nutrient losses through whey drainage. This agrees with the findings of Abrahamsen et al. (2021), who reported that optimizing coagulant concentration enhances cheese yield and curd firmness by promoting casein cross-linking and reducing fat expulsion. Likewise, the protein content increased proportionally with rennet concentration, likely due to more effective curd formation and minimized casein solubilization[20]. The observed decrease in moisture content at higher enzyme levels supports this interpretation, as stronger curd networks tend to retain less free water, resulting in firmer textures[7,8].

Goat milk mozzarella cheese provides substantial nutritional benefits due to its rich composition of high-quality protein, bioavailable minerals, and beneficial fatty acids, making it a functional food for diverse consumers. It is particularly high in easily digestible proteins such as β-casein and αs2-casein, while having lower αs1-casein, which reduces allergenicity and improves gastrointestinal tolerance compared to cow milk[1,2]. The cheese also retains essential micronutrients—such as calcium, phosphorus, zinc, and vitamin A—critical for bone and immune health[3]. Furthermore, the fermentation process enhances the bioavailability of nutrients and supports the growth of lactic acid bacteria, which contribute to gut health and potential probiotic effects[21]. From a consumer perspective, goat milk mozzarella offers convenience as a ready-to-eat, high-protein dairy product suitable for individuals seeking nutritious and easily digestible alternatives to traditional cow milk cheese. Its soft texture, mild flavor, and excellent meltability make it appealing for both children and adults, while its production in small-scale settings supports local food systems and sustainable dairy diversification[9].

Texture and elongation were significantly improved with increasing rennet concentration, reaching the highest values in the 240 ppm treatment. These improvements are closely related to curd structure and proteolysis degree. In mozzarella cheese, the balance between the cohesive forces among casein molecules and the rearrangement of those molecules under heating determines stretchability and meltability[22]. The denser protein network formed at higher enzyme concentrations enhances structural integrity, allowing the cheese to stretch without breaking. Hars et al. (2024) emphasized that appropriate proteolysis during renneting contributes to improved elasticity and plasticity in pasta-filata cheeses, consistent with the results obtained here[23].

The microbiological results showed that higher rennet concentrations increased LAB viability, with the highest count (21.00×10^4 CFU/g) observed in the 240 ppm treatment. This may be attributed to a more favorable curd microstructure and nutrient retention, which provide a protective and nutritive environment for microbial survival. LAB growth is essential in developing flavor precursors, improving acidity, and enhancing overall cheese quality. A recent study by Kaushik et al. (2023) confirmed that coagulant concentration can indirectly influence microbial population dynamics by modifying curd density, which determines oxygen diffusion and nutrient accessibility[21]. These findings indicate a strong interaction between rennet-induced physicochemical modifications and microbial ecology during cheese manufacture.

The sensory analysis revealed that increasing liquid rennet concentration significantly enhanced the texture and taste of the mozzarella, while color and aroma remained unaffected. The stable color scores (3.8–4.2) can be attributed to the low β-carotene content of goat milk, resulting in naturally white cheese regardless of processing conditions. The unchanged aroma profile suggests that the mild enzymatic reactions at different rennet levels did not generate volatile compounds capable of altering the characteristic flavor of goat milk cheese. These results are in line with Siddikey et al. (2022), who found that varying rennet levels had minimal influence on aroma but could improve texture and palatability through better protein structuring[24]. The enhanced sensory acceptance in this study highlights the importance of optimizing enzyme dosage for balancing functional and consumer-perceived quality.

Comparing these findings with previous works, most studies have focused on rennet *type* rather than *concentration*. For instance, Manuelian et al. (2020) reported that vegetable rennet produced higher protein and elongation values than animal rennet in cow milk mozzarella, but the role of concentration was not explored[25]. Our results demonstrate that concentration itself, regardless of rennet origin, plays a decisive role in defining curd compactness, stretchability, and microbial viability. This confirms recent conclusions by Hovjecki et al. (2022) and Falih et al. (2024), who emphasized that both dosage and enzyme source are critical factors controlling cheese yield, microstructure, and textural behavior[8,13].

From a practical perspective, using a 240 ppm rennet concentration appears optimal for goat milk mozzarella production under the present experimental conditions. It provides a balance between yield, microbial viability, textural integrity, and consumer preference. The improvement in LAB viability may also contribute to functional benefits if probiotic strains are present, though further characterization would be required. These results have industrial implications for small-scale goat milk processors aiming to standardize their products and improve profitability through better rennet utilization.

However, this study has several limitations. First, it used goat milk from a single source and lactation period; variations in composition across breeds, seasons, or feeding systems may affect generalizability. Second, the concentration range tested (120–240 ppm) was limited; intermediate or higher levels might reveal non-linear effects or plateau responses. Third, proteolysis and volatile compound analyses were not conducted, which could have provided insights into flavor development mechanisms. Fourth, the sensory panel consisted of 30 untrained participants, limiting the statistical robustness of consumer acceptability conclusions. Finally, shelf-life stability and microbial dynamics during refrigerated storage were not evaluated; such analyses are essential to assess the long-term quality and safety of goat milk mozzarella.

CONLUSION

This study revealed that the concentration of liquid rennet significantly influenced the physicochemical, microbiological, and sensory qualities of goat milk mozzarella cheese. Increasing rennet concentration from 120 ppm to 240 ppm enhanced total acidity, LAB viability, yield, protein content, elongation, and texture, while slightly decreasing moisture content. The optimal formulation at 240 ppm produced cheese with superior structural integrity, elasticity, and sensory acceptance without altering color or aroma. These findings imply that optimizing rennet dosage is crucial for improving curd formation, protein retention, and consumer-preferred texture in goat milk cheese. Practically, a 240 ppm concentration is recommended for small-scale producers to achieve higher yield and consistent quality, while theoretically it contributes to understanding enzyme–substrate interactions in non-bovine milk systems. Future studies should explore broader rennet ranges, proteolytic profiles, and storage stability to develop standardized, functional, and market-ready goat milk mozzarella products.

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