The Effect of Cacl₂ Addition on Milk Coagulation Using Papaya Latex on the Moisture Content, Firmness, and Meltability of Fresh Cheese

Alfilail Nurrana Adista Hamka*[1], Wendry Setiyadi Putranto[2], Lilis Suryaningsih[2]

[1]Student at the Faculty of Animal Husbandry, Departement of Livestock Product Technology, Universitas Padjadjaran

[2]Faculty of Animal Husbandry, Departement of Livestock Product Technology, Universitas Padjadjaran, Sumedang, Indonesia

***Corresponding Author**

DOI: https://dx.doi.org/10.47772/IJRISS.2026.10100076

Received: 31 December 2025; Accepted: 05 January 2026; Published: 22 January 2026

ABSTRACT

The use of plant-derived coagulants such as papaya latex has emerged as an alternative to animal rennet in fresh cheese production; however, mineral optimization is required to obtain desirable physical and functional properties. This study aimed to evaluate the effect of calcium chloride ($CaCl_2$) addition on moisture content, firmness, and meltability of fresh cheese coagulated using papaya latex. The experiment was arranged in a completely randomized design with five $CaCl_2$ concentrations (0.01%, 0.03%, 0.05%, 0.08%, and 0.10%), each with four replications. Fresh cheese was produced using 0.35% papain extract as the coagulant. Moisture content was determined by the oven-drying method, firmness was measured using a texture analyzer, and meltability was evaluated using a modified Schreiber melt test. The results showed that $CaCl_2$ addition did not significantly affect moisture content ($P > 0.05$), although a decreasing trend was observed with increasing $CaCl_2$ concentration. In contrast, $CaCl_2$ significantly increased firmness and reduced meltability of fresh cheese ($P \leq 0.05$). Higher $CaCl_2$ concentrations promoted a more compact protein matrix with greater structural rigidity and heat stability. Intermediate $CaCl_2$ concentrations (0.03–0.05%) exhibited a more balanced combination of moisture retention, moderate firmness, and acceptable meltability, which are desirable characteristics of fresh cheese. Therefore, appropriate $CaCl_2$ addition is essential to optimize the quality of fresh cheese produced using papaya latex.

Keywords: fresh cheese, calcium chloride, moisture content, firmness, meltability

INTRODUCTION

Milk and dairy products play an essential role in meeting nutritional needs while supporting the growth of the food industry. Southeast Asia has been identified as a low milk supply region, with consumption levels below 30 kg per capita per year (Food and Agriculture Organization, 2023). In Indonesia, per capita milk consumption is only 16.27 kg per year, while domestic milk production reaches approximately 808.352,84 tons, resulting in a continued reliance on imports (Badan Pusat Statistik, 2024). These conditions indicate the need for the development and utilization of more diverse, value-added dairy products that align with current trends and consumer preferences in order to increase milk consumption and improve nutritional adequacy.

One of the most popular dairy products is cheese. Fresh cheese is a type of cheese with strong development potential because it can be produced without a ripening process, allowing production at various scales, including household-level industries (Fox et al., 2017). The primary step in cheese making is the coagulation of casein using milk-clotting enzymes such as rennet (Nindyasari et al., 2022). These enzymes are typically derived from the stomachs of ruminant animals, making them expensive and limited in availability.

An alternative and more economical milk coagulant can be obtained from plant-derived enzymes, such as papaya latex. Papaya (Carica papaya) is a tropical plant that is widely available in Indonesia. The latex of unripe papaya contains papain, a proteolytic enzyme capable of hydrolyzing milk casein and inducing coagulation (Zahra et al., 2023). However, the use of papain as a coagulant requires optimization because its relatively non-specific proteolytic activity may affect curd formation and the physical quality of the resulting cheese. The physical quality of fresh cheese is not only determined by successful coagulation but also by the balance of key physical properties, namely moisture content, firmness, and meltability.

Moisture content plays an important role in determining the texture and structural stability of fresh cheese. Excessively high moisture content can result in a soft and unstable product, while excessively low moisture content may produce a cheese that is too firm and inconsistent with fresh cheese characteristics (Juniawati et al., 2015). Firmness reflects the compactness of the protein matrix; low firmness indicates a weak structure, whereas excessively high firmness indicates an overly rigid structure (Nugroho P et al., 2018). Meltability is a functional property describing the ability of cheese to soften and spread when heated, and an appropriate level of meltability is required to maintain structural integrity during heating (Atik & Huppertz, 2023). These physical and functional characteristics are strongly influenced by coagulation conditions and the presence of calcium ions in the milk protein system.

Calcium chloride ($CaCl_2$) is commonly added during cheese making to increase the availability of free calcium ions, thereby strengthening interactions between casein micelles. $CaCl_2$ can accelerate coagulation, enhance curd firmness, and influence water retention within the protein matrix, directly affecting moisture content, firmness, and meltability of fresh cheese (Pawlos et al., 2023). Therefore, this study aimed to further investigate the effect of $CaCl_2$ addition during milk coagulation using papaya latex on the moisture content, firmness, and meltability of fresh cheese.

MATERIAL AND METHODS

Research on the effect of adding $CaCl_2$ to the milk coagulation process using papaya latex on moisture content, firmness, and meltability was conducted from August to October 2025 at the Animal Product Processing Laboratory and the Biotechnology Research and Testing Laboratory of the Faculty of Animal Husbandry, Universitas Padjadjaran

Tools and Materials

The materials used in this research consisted of 20 liters of fresh cow's milk, calcium chloride ($CaCl_2$), latex extracted from unripe papaya fruit, and sodium chloride (NaCl). The equipment used for fresh cheese production included a digital balance (Taffware I2000; accuracy 0.01 g), thermometer (accuracy 0.1°C), stove, stirrer, cooking pot, cheesecloth, curd mold, and aluminum foil. Equipment for moisture content analysis included porcelain crucibles, oven, aluminum trays, analytical balance (accuracy 0.001 g), and desiccator. Firmness was measured using a Texture Analyzer TMS-Pro (Food Technology Corporation, USA) equipped with a 500 N load cell and a 35-mm cylindrical probe. Meltability analysis utilized aluminum foil, a ruler, and an oven.

Preparation of Papain Enzyme

Papain enzyme was prepared from papaya latex using a modified method based on Hidayat et al. (2020). Unripe papaya fruits were prepared, and shallow incisions (1 – 2 mm) were made on the fruit surface using a knife to collect the latex. The collected latex was measured and diluted with distilled water at ratio of 1:10, then stirred until homogeneous. The resulting papain solution was used as the milk coagulant.

Fresh Cheese Production

Fresh cheese was produced using a modified method described by Raisanti et al. (2022). One liter (1000 ml) of fresh cow's milk was prepared and $CaCl_2$ was added at five different concentrations. The milk was heated to 85°C, followed by the addition of papain enzyme at a concentration of 0.35%, and stirred until homogeneous. The milk was then incubated for 30 minutes to allow protein coagulation and curd formation. The papain

ISSN No. 2454-6186 | DOI: 10.47772/IJRISS | Volume X Issue I January 2026

concentration was selected based on preliminary trials and previous studies reporting effective milk coagulation without excessive proteolysis.

The curd was cut using a knife, and whey separation was performed by filtering the curd through a fine cheesecloth with a small surface area, after curd formation, the curd was weighed and NaCl was added 2% (w/w) of the curd weight. The curd was molded into a 10 × 10 cm mold and pressed with a 1 kg weight for 60 minutes to reduce moisture content and increase yield. The pressed curd was wrapped in aluminum foil and stored in a chiller at 4°C for 12 hours to obtain fresh cheese.

Moisture Content Determination

Moisture content analysis was conducted following AOAC (2005). Empty porcelain crucibles were dried in an oven at 105°C for 1 hour, cooled in a desiccator, and weighed. Approximately 5 g of fresh cheese sample was placed in the crucible and dried in the oven at 105°C for 5–7 hours. The crucible was then cooled in a desiccator and weighed. Moisture content was calculated using the following equation :

$$\text{Moisture content \%} = \frac{B - C}{B - A} \times 100$$

where :

A = weight of empty crucible (g)

B = weight of crucible with sample (g)

C = weight of crucible with dried sample (g)

Firmness Measurement

Firmness was measured using a Texture Analyzer TMS-Pro (Food Technology Corporation, USA) equipped with a 500 N load cell and a 35-mm cylindrical probe in compression mode. Cheese samples were cut into cubes (3 × 3 × 3 cm) and placed at the center of a flat plate. The test was conducted at a crosshead speed of 1 mm/s to a depth of 50% of the sample height at room temperature. The maximum force (Fmax, N) recorded during compression was used as the firmness value.

Meltability Measurement

Meltability was expressed as the percentage increase in sample dimensions after controlled heating, measured using a modified Schreiber melt test following the principles described by Helgetun and Jensen (2023). Rectangular cheese samples were placed on an aluminum tray and heated in an oven at 200°C for 5 minutes. After cooling to room temperature, the length and width of the samples were measured before heating ($P_0 \times L_0$) and after heating ($P_1 \times L_1$). Meltability was calculated using the following formula:

$$\text{Meltability \%} = \frac{(P1 \times L1) - (P0 \times L0)}{P0 \times L0} \times 100$$

where:

P_0 = length before heating

L_0 = width before heating

P_1 = length after heating

L_1 = width after heating

Statistical Analysis

The experiment was conducted using a Completely Randomized Design with five treatments: $CaCl_2$ addition at 0.01% (T1), 0.03% (T2), 0.05% (T3), 0.08% (T4), and 0.10% (T5). Each treatment was replicated four times, resulting in 20 experimental units. Data were analyzed using analysis of variance (ANOVA), and significant differences were further tested using Duncan's Multiple Range Test.

RESULT AND DISCUSSION

Table 1. Result of Effect of $CaCl_2$ Addition on Milk Coagulation Using Papaya Latex on the Moisture Content, Firmness, and Meltability of Fresh Cheese

Parameter	Treatment				
	T1	T2	T3	T4	T5
Moisture content (%)	68.06 ±2.24^{a}	67.94±1.58^{a}	67.40±2.90^{a}	66.22±4.17^{a}	65.07±3.10^{a}
Firmness (N)	2.70±0.56^{a}	3.39±0.36^{b}	3.74±0.25^{b}	3.82±0.37^{b}	4.71±0.36^{c}
Meltability (%)	93.30±4.05^{c}	87.73±2.94^{bc}	84.47±3.24^{b}	83.39±4.14^{b}	76.86±5.70^{a}

Data are presented as mean± SD. Different superscripts indicate a significant difference ($P \leq 0.05$), while the same superscripts indicate no significant difference ($P > 0.05$). All treatments used 0.35% papain extract with $CaCl_2$ addition at 0.01% (T1), 0.03% (T2), 0.05% (T3), 0.08% (T4), and 0.10% (T5).

Moisture Content

The results showed that $CaCl_2$ addition did not significantly affect the moisture content of fresh cheese ($P > 0.05$), although a decreasing tendency was observed with increasing $CaCl_2$ concentration. As shown in Table 1, the moisture content ranged from 68.06% in T1 to 65.07% in T5, with all treatments sharing the same superscript. This indicates that within the concentration range used in this research $CaCl_2$ was not the primary factor controlling water retention in the cheese matrix.

According to Walstra et al. (2005), moisture content in fresh cheese is more strongly influenced by curd syneresis dynamics, cutting intensity, pressing force, and salt addition than by calcium concentration itself. When the processing variables are standardized, variations in ionic calcium may not produce statistically significant differences in moisture content. Calcium primarily modifies protein interactions rather than expelling serum, particularly in unripened cheeses with short pressing times (Everett & Auty, 2008). This explains why $CaCl_2$ addition mainly altered textural attributes without markedly reducing moisture in cheese. Furthermore, the use of papain as coagulant may have contributed to the similar moisture levels observed among treatments. The absences may also be attributed to the addition of sodium chloride (NaCl) at a constant level.

Firmness

Calcium chloride addition had a significant effect on the firmness of fresh cheese ($P \leq 0.05$) as it shown in Table 1. Firmness values increased progressively from T1 (2.70 N) to T5 (4.71 N), indicating that higher $CaCl_2$ concentrations produced a firmer cheese structure. The lowest firmness was observed in T1, while T5 showed the highest firmness and differed significantly from all other treatments.

The increase in firmness can be attributed to the role of Ca^{2+} ions in promoting stronger protein interactions with the casein network. Recent studies have shown that calcium supplementation enhances calcium mediated cross-linking between casein particles, resulting in a denser and more cohesive protein matrix. Pawlos et al (2023) reported that increasing calcium concentration significantly increased hardness in acid – rennet cheese by strengthening intermolecular bonds within the protein network. This mechanism is consistent with the observed increase in firmness in the present study as $CaCl_2$ concentration increased.

The higher ionic calcium levels reduce micellar mobility and promote closer packing of casein strands, leading to increased resistance to deformation (Soodam et al., 2015). As firmness is defined as the maximum force required to compress the cheese structure, the formation of a compact protein matrix directly contributes to higher firmness values observed in treatments with higher $CaCl_2$ concentrations. Papain also exhibits broad proteolytic activity, which can partially hydrolyze casein chains and weaken curd structure if not adequately controlled so the calcium addition can compensate for excessive proteolysis by stabilizing the protein network and improving textural strength in cheeses produced with non – rennet coagulants (Ong et al., 2013). Excessive calcium – induced cross - linking can reduce matrix flexibility, which, although beneficial for curd strength, may negatively affect functional properties such as meltability. This indicates that while $CaCl_2$ improves firmness, its concentration must be carefully optimized to avoid excessive rigidity in fresh cheese.

Meltability

The results showed that meltability decreased significantly with increasing $CaCl_2$ concentration ($P \leq 0.05$). Fresh cheese produced with the lowest $CaCl_2$ concentration (T1) exhibited the highest meltability, while higher $CaCl_2$ levels resulted in progressively lower meltability values (T5). This indicates that calcium ions played a direct role in modifying the thermal behavior of the cheese matrix during heating.

Meltability in fresh cheese is primarily governed by the ability of the protein matrix to soften and rearrange under heat. Increasing calcium content shifts calcium equilibria toward enhanced protein cross-linked, thereby reducing matrix flexibility and limiting structural flow during heating. As a results, cheese with higher calcium levels exhibit restricted melting behavior even when moisture content remains relatively similar. Calcium can independently reduce meltability by stabilizing protein interactions during thermal treatment. Excessive calcium content increases heat stability of casein networks by reinforcing calcium- mediated bonds, which limits protein unfolding and fusion during heating (Lucey et al., 2017). This condition restricts fat and serum phase redistribution, leading to lower meltability values.

CONCLUSION

The results indicate that calcium chloride ($CaCl_2$) concentration plays a key role in determining the structural and functional properties of fresh cheese produced using papaya latex. Although $CaCl_2$ addition did not significantly affect moisture content ($P > 0.05$), increasing $CaCl_2$ concentration significantly increased firmness and reduced meltability ($P \leq 0.05$). Among the tested treatments, intermediate $CaCl_2$ concentrations (0.03–0.05%) exhibited a more balanced combination of moisture retention, moderate firmness, and acceptable meltability, which are desirable characteristics of fresh cheese. In contrast, excessive $CaCl_2$ addition produced overly rigid structures with reduced melting performance. Therefore, moderate $CaCl_2$ levels are recommended to achieve an optimal balance between texture and functional properties in fresh cheese. Further studies are recommended to include sensory evaluation and consumerr acceptance to access the practical applicability of the product.

ACKNOWLEDGEMENT

The authors would like to express their sincere gratitude to Dr. Wendry Setiyadi Putranto, S.Pt., M.Si., for funding this research and granting permission to use the Research and Biotechnology Testing Laboratory. Appreciation is also extended to Dr. Ir. Lilis Suryaningsih, M.Si., Head of the Laboratory of Animal Product Processing Technology, for the permission to use the laboratory facilities, which enabled the successful completion of this research.

REFERENCES

1. (AOAC) Association of Official Analytical Chemyst. 2005. Official Method of Analysis of The Association of Official Analytical of Chemist. Arlington, Virginia, USA: Association of Official Analytical Chemist, Inc.
2. Atik, D. S., & Huppertz, T. (2023). Melting of natural cheese: A review. International Dairy Journal, 142, 105648. https://doi.org/10.1016/j.idairyj.2023.105648
3. (BPS) Badan Pusat Statistik. (2023). Produksi susu segar menurut provinsi [Tabel statistik].
4. Everett, D. W., & Auty, M. A. (2008). Cheese structure and current methods of analysis. International Dairy Journal, 18(7), 759-773. https://doi.org/10.1016/j.idairyj.2008.03.012
5. (FAO) Food and Agriculture Organization of the United Nations. (2023). Milk and milk products.
6. Fox, P. F., Guinee, T. P., Cogan, T. M., & McSweeney, P. L. H. (2017). Fundamentals of cheese science (2nd ed.). In Springer. https://doi.org/10.1002/9781118534168.ch22
7. Helgetun, K., & Jensen, I.-J. (2023). Comparison of dairy mozzarella and plant-based alternatives under pizza baking conditions, with focus on sensorial-, nutritional-, and rheological properties.
8. Hidayat, S. F., Mas'udah, & Santosa, S. (2020). Pengaruh Jenis Pelarut dan Waktu Pengeringan dalam Pembuatan Enzim Papain dari Getah Buah Pepaya. 8(9), 14.
9. Juniawati, Usmiati, S., & Damayanthi, E. (2015). Karakter/sifat fisik kimia keju rendah lemak dari berbagai bahan baku susu modifikasi. Jurnal Penelitian Pascapanen Pertanian, 12(2), 28–36.
10. Lucey, J. A., Johnson, M. E., & Horne, D. S. (2003). Invited review: Perspectives on the basis of the rheology and texture properties of cheese. Journal of dairy science, 86(9), 2725-2743.https://doi.org/10.3168/jds.S0022-0302(03)73869-7
11. Nindyasari, K. D., Moentamaria, D., Kimia, J. T., Malang, P. N., Soekarno, J., & No, H. (2022). Untuk Pembuatan Keju Mozarella. 8(9), 133–140.
12. Nugroho P, Dwiloka B, & Rizqiati H. (2018). Rendemen, Nilai pH, Tekstur, dan Aktivitas Antioksidan Keju Segar denganBahan Pengasam Ekstrak Bunga Rosella Ungu. Jurnal Teknologi Pangan, 2(1), 33–39.
13. Ong, L., Dagastine, R. R., Kentish, S. E., & Gras, S. L. (2013). The effect of calcium chloride addition on the microstructure and composition of Cheddar cheese. International dairy journal, 33(2), 135-141. https://doi.org/10.1016/j.idairyj.2013.03.002
14. Pawlos, M., Znamirowska-Piotrowska, A., Kowalczyk, M., Zaguła, G., & Szajnar, K. (2023). Possibility of Using Different Calcium Compounds for the Manufacture of Fresh Acid Rennet Cheese from Goat's Milk. Foods, 12(19), 3703.https://doi.org/10.3390/foods12193703
15. 1Raisanti, I. A. M., Putranto, W. S., & Badruzzaman, D. Z. (2022). Pengaruh Penambahan Monosodium Fosfat pada Pembuatan Processed Cheese dengan Koagulan Sari Nanas terhadap Kadar Air, Rendemen, dan Akseptabilitas. Jurnal Teknologi Hasil Peternakan, 3(1), 1. https://doi.org/10.24198/jthp.v3i1.39078
16. Soodam, K., Ong, L., Powell, I. B., Kentish, S. E., & Gras, S. L. (2015). Effect of calcium chloride addition and draining pH on the microstructure and texture of full fat Cheddar cheese during ripening. Food Chemistry, 181, 111-118. https://doi.org/10.1016/j.foodchem.2015.01.135
17. Walstra, P., Walstra, P., Wouters, J.T.M., & Geurts, T.J. (2005). Dairy Science and Technology (2nd ed.). CRC Press. https://doi.org/10.1201/9781420028010
18. Zahra, N., Nugraha, D., & Wahlanto, P. (2023). Uji Iritasi Dermal Sediaan Salep Kulit Dari Getah Buah Pepaya California (Carica papaya L.) Pada Hewan Uji Kelinci. Pharmacy Genius, 2(2), 95–103. https://doi.org/10.56359/pharmgen.v2i2.274