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Physicochemical and Organoleptic Properties of Fresh Cheese Coagulated with Thiol Protease from *Plumeria obtusa*

Propiedades fisicoquímicas y organolépticas del queso fresco coagulado con tiol proteasa de *Plumeria obtusa*

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ABSTRACT

Fresh cheese is made from fresh cow's milk from Friesian Holstein (FH) without aging and is pasteurized at 75 °C for 15 seconds using a coagulant. An effective and renewable coagulant that may serve as a potential substitute for commercial animal rennet is the thiol protease enzyme extracted from frangipani (*Plumeria obtusa*) stems. This study aims to determine the physicochemical properties (yield, protein content, and water content) and organoleptic properties (taste, color, aroma, and texture) of fresh cheese produced using of thiol protease from frangipani stems compared to commercial animal rennet (0.02%). The results of the study showed that physicochemical tests on fresh cheese with different concentrations of thiol protease from frangipani stems did not significantly affect protein content (P>0.05). However, they significantly affected yield, water content, and fat content (P<0.05). The organoleptic test showed no significant effect on aroma, but there were significant effects on color, flavor, texture, and preference. Based on the results, P3 (0.06%) was the best treatment based on the physicochemical and organoleptic qualities of fresh cheese. Frangipani thiol protease may represent a sustainable, plant-based coagulant candidate for the dairy industry, with potential to support local cheese production and reduce dependence on imported rennet.

Palabras clave:
Alimento funcional
Cuajo local
Enzimas de coagulación de la leche
Coagulantes vegetales
Plumeria obtusa

RESUMEN

El queso fresco se elabora a partir de leche fresca de vaca Friesian Holstein (FH) sin maduración y se pasteuriza a 75 °C durante 15 segundos utilizando un coagulante. Un coagulante eficaz y renovable que puede sustituir al cuajo animal comercial es la enzima tiol proteasa extraída de los tallos de frangipani (*Plumeria obtusa*). Este estudio tiene como objetivo determinar las propiedades fisicoquímicas (rendimiento y contenidos de proteínas y agua) y organolépticas (sabor, color, aroma y textura) del queso fresco producido con tiol proteasa de tallos de frangipani en comparación con el cuajo animal comercial (0,02%). Los resultados del estudio mostraron que las pruebas fisicoquímicas en queso fresco con diferentes concentraciones de tiol proteasa de tallos de frangipani no afectaron significativamente el contenido proteico (P>0,05). Sin embargo, afectaron significativamente el rendimiento, el contenido de agua y el contenido de grasa (P<0,05). La prueba organoléptica no mostró un efecto significativo en el aroma, hubo efectos significativos en el color, el sabor, la textura y la preferencia. Según los resultados, P3 (0,06%) fue el mejor tratamiento en función de las cualidades fisicoquímicas y organolépticas del queso fresco. El tiol proteasa de frangipani podría representar un coagulante vegetal sostenible para la industria láctea, con potencial para apoyar la producción local de queso y reducir la dependencia del cuajo importado.

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Cheese is a processed product derived from cow's milk, which has a high nutritional content. Based on its density, cheese is divided into three types, namely, hard cheese, semi-soft cheese, and soft cheese (Ningrum et al. 2024). Hard cheese is cheese that goes through a fermentation process, for example Edam cheese and Cheddar. Semi-soft cheese has a semi-firm texture, such as Picodon, Le Brin, and blue cheese. Soft cheeses are produced by a faster process with little or no ripening, for example, fresh cheese, cheese spread, cottage cheese and mozzarella that can be consumed directly, which are commonly used as spreads on bread. Fresh cheeses are also widely produced and consumed in Latin America, including cheeses such as queso fresco, hight-moisture cheeses used in a variety of traditional dishes. Fresh cheese is generally used as a complementary ingredient to foods such as pizza, pies, burgers, desserts, and salads.

Fresh cheese is made from fresh cow's milk that is pasteurized at 75 °C for 15 seconds, processed without a second fermentation process (ripening), and the addition of rennet enzymes and the addition of acids (vinegar, lactic acid, and lemon) (Wangi et al. 2021). Fresh cheese is a type of cheese without a ripening process made from cow's milk with the addition of rennet enzymes and is usually used as an ingredient in making cakes. Fresh cheese has a short shelf life, has a soft, smooth, creamy texture, and is white. Rennet is a coagulant containing protease enzymes and is usually used as a coagulant, which functions to separate the solid (curd) and liquid (whey) parts in cheese making (Wangi et al. 2021).

Commercial rennet is generally animal rennet. Animal rennet, traditionally derived from calf gastric tissue, is widely used in cheese production due to its high specificity and efficient coagulation properties. However, its use raises several concerns, including religious and vegetarian restrictions, ethical issues related to animal slaughter, and dependence on a limited and often imported supply. Vegetable-based rennets, such as microbial or plant-derived proteases, offer a potential alternative that can address these limitations while supporting more sustainable and locally adapted cheese production. Nevertheless, many vegetable coagulants still face challenges in terms of consistency, proteolytic activity, and flavor development compared to animal rennet. Therefore, exploring new plant-based coagulants, such as thiol proteases derived from frangipani stems, is important for expanding the range of viable coagulants available for industrial and artisanal cheese production.

Cheese production can utilize efficient and economical alternative coagulants, one of which is vegetable rennet, a thiol protease enzyme. Vegetable rennet, derived from plants, has coagulation properties similar to commercial rennet. Several plants and fruits that can be used as alternatives to commercial rennet include moringa leaves, noni fruit, pineapple, and mango, which have coagulation properties (Vejayan et al. 2025). Furthermore, there is a plant whose stem is still underutilized for its protease content, namely the frangipani plant. Plumeria plants have small and large stems, sparse but slightly thick leaves, and flowers with four to six petals. Plumeria plants are commonly used as medicine; and contain chemical compounds throughout the plant, especially the stems (Bihani et al. 2021). Plumeria flower extract contains active compounds including phenols, tannins, steroids, saponins, flavonoids, alkaloids, and glycosides (Candra et al. 2024). Plumeria stems contain agoniadin, plumierid, plumeric acid, lipeol, and serotinic acid (Zulkifli et al. 2022). Vegetable rennet from plumeria stems (*Plumeria obtusa*) can be used as a coagulant in fresh cheese, producing high-quality, halal cheese with low production costs (Rahma and Issustiarani 2025). Extraction and testing of protease activity from frangipani plants have been conducted in previous studies (Laksanawati et al. 2022).

However, this study did not apply pure frangipani stem thiol protease extract as a coagulant in fresh cheese production. Leulmi et al. (2023) investigated the extraction of coagulants *Pergularia tomentosa* latex leaves as a substitute for animal rennet and proved to be a new alternative vegetable coagulant that has the potential to replace rennet. Research by Aktayeva et al. (2018) was related to the exploration of proteolytic enzymes in cheese making derived from animal, vegetable, and microbial sources. Animal proteases such as chymosin form calf rennet are traditionally employed, whereas plant proteases (for example papain, bromelin, ficin, and other cysteine proteases) and microbial proteases from bacteria and fungi (example *Bacillus* spp.) have been explored

as alternative coagulants and processing aids in the dairy industry. Meanwhile, research by Rahma and Issustiarani (2025) investigated the development of halal, consistent, and safe coagulants from vegetable rennet. However, no studies have specifically evaluated purified *Plumeria obtusa* stem thiol protease performance in fresh cheese production, particularly its impact on physicochemical properties and consumer sensory acceptance, key determinants for industrial adoption.

This study evaluated the use of *Plumeria obtusa* stem thiol protease as an alternative coagulant for fresh cheese production. The effects of different enzyme concentrations on physicochemical properties (yield, protein, moisture, and fat contents) and sensory attributes (color, taste, aroma, texture, and overall acceptability) were assessed and compared with those obtained using commercial rennet. It was hypothesized that the enzyme concentration would significantly affect cheese yield, composition, and consumer acceptance, with intermediate concentrations providing the most favorable technological and sensory performance.

MATERIALS AND METHODS

The raw materials used in this study were frangipani stems (*Plumeria obtusa*), fresh cow milk from Friesian Holstein (FH), acetic acid, distilled water, phosphate buffer (disodium hydrogen phosphate (Na_2HPO_4) and sodium dihydrogen phosphate (NaH_2PO_4)), ammonium sulfate ($(NH_4)_2SO_4$), ethanol (96%), rennet, salt (NaCl), dialysis tube (cut-off 12-14 kDa).

Experimental design

This research design used a non-factorial Completely Randomized Design with four treatments and three replications, resulting in 12 experimental units for the physicochemical test and for the organoleptic test, a non-factorial Randomized Block Design was used, including (taste, color, aroma, texture, and preference). The data obtained were tabulated and analyzed using analysis of variance (ANOVA). If significant differences are found (P<0.05), further analysis is performed using Duncan's New Multiple Range Test (DMRT) using SPSS version 26 (IBM). The research design is presented (Table 1).

Table 1. Treatments for the development of fresh cheese with thiol protease from frangipani stem.

Treatments	Description (%)
PK	0.02 animal rennet
P1	0.02 thiol protease
P2	0.04 thiol protease
P3	0.06 thiol protease

Process extraction of thiol protease from frangipani stems

Frangipani stems (100 g) were cut into 1 cm pieces, washed with ethanol 96% at 1:1 (w/v) ratio for 30 minutes, rinsed, and then blended for 2 minutes. The resulting homogenate was extracted using 0.1 M phosphate buffer (pH=7.0) at 1:4 (w/v) ratio and centrifuged at 3,000 rpm for 10 minutes at 4 °C. The supernatant was then precipitated with 60% ($(NH_4)_2SO_4$ 60%, 100 mL) at 55 °C with continuous stirring for 30 minutes and stored at 4 °C for 24 hours, following the procedure described by Laksanawati et al. (2023). The resulting partially purified thiol protease was then separated from the supernatant by adding 16 mL of 0.05 M phosphate buffer, pH=7.5. Next, dialysis was performed using a cellophane tube with a 10 kDa cut-off membrane. Dialysis was performed with 0.05 M phosphate buffer, pH=7.5, and the buffer was periodically replaced with a lower concentration (Laksanawati et al. 2023). The protease extract was then washed with distilled water and stored. The extraction process of thiol protease from frangipani stems is illustrated (Figure 1).

Process making fresh cheese

The second stage, the fresh cheese production process begins with pasteurization of fresh cow's milk at 70 °C for 30 seconds, cooled to 37 °C. The milk was then added with thiol protease for P1 (0.02%), P2 (0.04%), P3 (0.06%), and PK (control treatment) using commercial animal rennet at 0.02%. The incubation process was then carried out for 7-8 hours at 37 °C (Sari et al. 2023). After incubation, the curd was separated using cheesecloth,

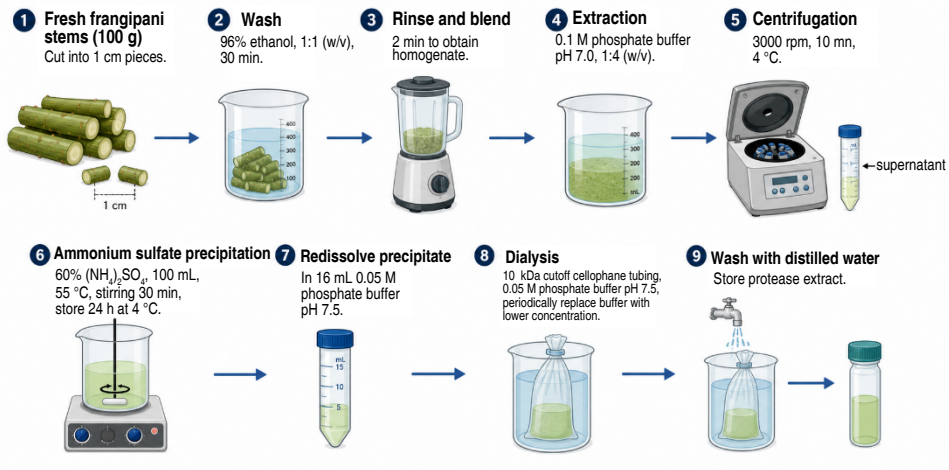


Figure 1. Process extraction of thiol protease from frangipani stems.

salted with 2% (w/w) NaCl, and weighed to calculate the cheese yield. The resulting cheese was subsequently analyzed for protein, moisture, and fat content, and was evaluated organoleptically for color, taste, aroma, texture, and overall preference. The process of making fresh cheese is shown (Figure 2).

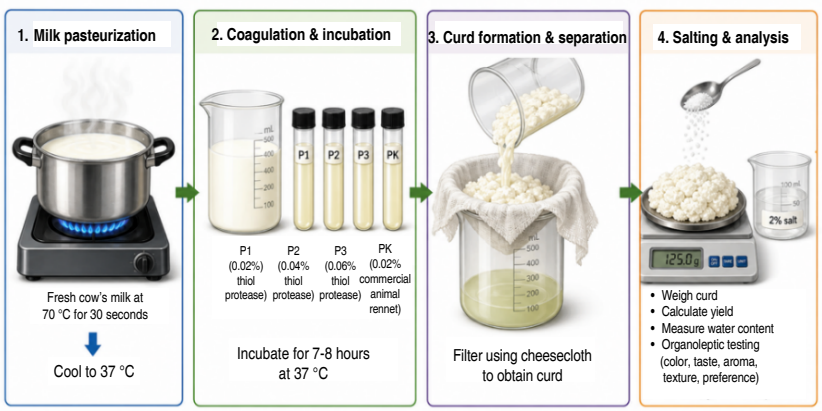


Figure 2. Process making fresh cheese with thiol protease from frangipani stem.

Physicochemical and organoleptic analysis

The yield (%) is obtained by comparing the weight of the curd formed with the weight of the milk used (Bonfatti et al. 2019). The method used in determining this moisture content (%) is the Oven method, by calculating the weight loss of the sample after oven-baking. The first step is to weigh 2 g of the sample and put it into a covered porcelain cup whose weight has been previously known. Next, dry the sample using a porcelain cup using an oven at 105 °C for 3 hours, then cool the dried sample in a desiccator, then weigh the sample until a constant weight is obtained (Chen et al. 2020). The process of determining protein content (%) uses the Kjeldahl method (Al-Mentafji 2006). Fat content (%) testing was carried out by the Soxhlet method (Al-Mentafji 2006). Organoleptic testing conducted in the fresh cheese research included color, taste, aroma, and texture carried out by 30 untrained panelists (Arziyah et al. 2022).

Prior to testing, panelists were instructed to avoid consuming strongly flavored foods or beverages at least 30 minutes before the evaluation. The sensory analysis was performed in a clean, odor-free, and well-lit room under controlled conditions. Cheese samples were cut

into uniform sizes (approximately 10–15 g) and served at chilled temperature (10–12 °C). Each sample was labeled with random three-digit codes to minimize bias. Samples were presented randomly to each panelist. Drinking water was provided for rinsing the mouth between samples. The organoleptic testing scale for fresh cheese is as follows: (1) color by looking at the color of the fresh cheese, (2) taste produced by the fresh cheese, (3) aroma produced by the fresh cheese, (4) touching or holding the fresh cheese to observe its texture, (5) overall preference for the resulting fresh cheese. Information on the organoleptic test quality scale can be seen in Table 2.

Table 2. Organoleptic Quality Atributes fresh cheese.

Scale	Quality attributes				
	Color	Taste	Aroma	Tekstur	Overall preference
1	Very yellow	Not salty, very bitter	Very Non-Milky	Very not soft	Dislike very much
2	Yellow	Slightly salty, bitter	Non-Milky	Not soft	Dislike
3	Slightly white	Slightly salty, slightly bitter	slightly milky	Bit soft	Slightly like
4	White	Salty, slightly bitter	distinct milky	Soft	Like
5	Bright white	Very salty, not bitter	Very milky	Very soft	Like very much

RESULTS AND DISCUSSION

The physicochemical characteristics of fresh cheese using *Plumeria obtusa* (thiol protease) as a coagulant at different concentrations are shown in Table 1, and the organoleptic properties are shown in Table 3.

Yield

The analysis results showed that different thiol protease concentrations significantly affected yield (P<0.05). The lowest average yield was found in treatment P1, at 22.8%, although not significantly different from PK. While the highest yield was found in treatment P3, at 26.0%, not significantly different from P2. This is due to the increased concentration of *Plumeria obtusa* thiol protease, which effectively coagulates milk, thus increasing yield. Thiol protease from *Plumeria obtusa* cleaves peptide bonds in milk casein, leading to proteolysis and modification of the casein network. At higher enzyme concentrations, more extensive proteolysis may occur, which can influence curd formation, curd yield, and the water-holding capacity of the curd through changes in the structure of the protein matrix (Troncoso et al. 2022). The increased yield may be due to the proteolytic activity of the thiol protease in the frangipani stem, which breaks down milk proteins.

Table 3. Physicochemical test of fresh cheese using thiol protease from frangipani stem.

Parameters (%)	Treatment			
	PK	P1	P2	P3
Yields	24.3±1.16^{bc}	22.8±0.75^{c}	25.0±0.52^{ab}	26.0±0.70^{a}
Protein Content	12.9±0.77^{a}	13.1±0.66^{a}	13.6±0.67^{a}	13.6±0.47^{a}
Moisture Content	67.2±0.86^{b}	67.0±1.01^{b}	69.4±0.96^{a}	70.7±1.23^{a}
Fat Content	15.0±0.94^{b}	16.7±0.96^{a}	14.5±0.67^{b}	12.2±0.01^{c}

Note: PK (control, commercial rennet 0.02%), P1 (thiol protease 0.02%), P2 (thiol protease 0.04%), P3 (thiol protease 0.06%). Different ^{abc} notations on the same line show a noticeably different influence (P<0.05).

The frangipani stem protease belongs to the hydrolase group of enzymes, capable of breaking down proteins into simpler molecules, such as short oligopeptides or amino acids, by hydrolyzing peptide bonds (Troncoso et al. 2022). The high yield is due to enzymatic hydrolysis, which involves the hydrolysis of casein protein in milk, followed by agglomeration (Gruppi et al. 2022). The higher the concentration of frangipani stem thiol protease, the higher the yield, and the greater the agglomeration of casein micelles following protein hydrolysis.

Increasing the concentration of frangipani stem thiol protease results in a higher yield, while conversely, a lower

concentration of frangipani stem thiol protease results in a lower yield of fresh cheese. These two processes cause physical changes in the milk, namely, coagulation or clumping. The higher the concentration of protease enzymes used, the more optimal the coagulation process in the milk used (Junior et al. 2018). The yield produced in fresh cheese production ranges from 10–13% (Lanur et al. 2025). The high yield is also influenced by the composition of the curd that coagulates, which consists of fat, salt, and water. The cheese yield obtained in this study indicated that the treatments influenced curd formation efficiency and moisture retention. Compared with the commercial cheese sample (PK), several treatments produced comparable yields, suggesting that the enzymatic treatment was effective in retaining milk solids during cheese production. Higher yield values may also be associated with increased water-holding capacity within the protein matrix.

Protein content

The results of the analysis of variance (ANOVA) showed that differences in thiol protease concentrations had no effect (P>0.05) on the protein content of fresh cheese. Based on the PK treatment, P1, P2, and P3 met the protein standards for fresh cheese. The standard protein content for fresh cheese is expected to be around 12-16%. Factors that did not affect the protein content may have been the addition of too low concentration of thiol protease, resulting in less-than-optimal protein content and no significant effect. This suggests that the addition of commercial rennet and the addition of plumeria stem thiol protease produced equally optimal protein levels. This is consistent with the observation that increasing enzyme concentration increases the rate of enzymatic reactions, or that the rate of enzymatic reactions is directly proportional to enzyme concentration (Rodrigues et al. 2012). This means that the addition of commercial enzymes and plumeria stem thiol protease to fresh cheese has the same ability to coagulate milk effectively. Treatment P3 showed the highest protein content, possibly due to enhanced protein retention during coagulation and reduced protein loss in whey. The protein content of the cheese samples was relatively higher than the initial milk composition, indicating successful protein concentration during coagulation, which is consistent with the characteristics of fresh cheese described in the Colombian Technical Standard NTC 5894.

Moisture content

ANOVA analysis results showed that differences in thiol protease concentrations significantly affected water content (P<0.05). The lowest average water content was found in treatment P1 at 67.0%, while the highest average value was found in treatment P3 at 70.7%. Treatments PK through P3 showed an increase in water content. Increasing the concentration of thiol protease in the plumeria stem increased water content.

Thiol protease from *Plumeria obtusa* hydrolyzes peptide bonds in casein, leading to proteolysis and modification of the protein network formed during curd formation. At higher enzyme concentrations, more extensive proteolysis can occur, which tends to produce a softer and more open curd structure, thereby increasing the amount of water retained within the curd matrix. Protease enzymes catalyze the hydrolysis of peptide bonds in proteins, breaking them down into smaller peptides and amino acids, and are therefore classified as hydrolases that require water to carry out the reaction (Andoyo et al. 2021). In this study, the increase in thiol protease concentration was associated with higher moisture content, suggesting that enzyme-induced changes in the casein network enhanced the water-holding capacity of the curd.

The lower the moisture content of fresh cheese, the longer it its shelf life, whereas higher moisture levels generally lead to faster spoilage because they facilitate microbial growth and physicochemical changes (Karwacka et al. 2024). The relatively high moisture content of fresh cheese is characteristic of products that have not undergone ripening and contributes to a softer texture and smoother mouthfeel perceived by panelists (Monasterio et al. 2024). Higher moisture content contributed to a softer cheese texture and improved mouthfeel perceived by the panelists. Moisture content is an important quality parameter in fresh cheese because it affects texture, softness, and shelf life. The moisture values obtained in this study were still consistent with the general characteristics of fresh cheese according to the Colombian Technical Standard NTC 5894.

Fat Content

The results of the analysis of variance (ANOVA) in Table 1 show that differences in the concentration of thiol protease

from the frangipani stem had a significant effect (P<0.05) on the fat content of fresh cheese. The lowest average fat content was found in treatment P3 with a value of 12.2%, while the highest average value was found in treatment P1 with a value of 16.7%. Based on the results of the fat content test, the higher the concentration of thiol protease from the frangipani stem added to fresh cheese, the lower the fat content. This trend may be related to changes in the curd structure and in the distribution of fat between the curd and whey phases during manufacture. Proteolysis of casein can modify the protein network, influencing how effectively fat globules are entrapped or retained within the curd, so that a more open or less cohesive matrix facilitates fat loss into the whey. Large fat globules are more easily removed from the curd during the filtering process. There are two possible outcomes in fresh cheese production: fat loss in the whey or fat filling the curd cavities (Karwacka et al. 2024). Fat and moisture contents are known to act together in determining curd structure and cheese softness, as both components share space within the protein matrix and influence the openness of the curd and serum retention. Fat content is related to water content; decreasing fat content increases the water content of fresh cheese. This is because fat coats the broken-down proteins, making it difficult for the proteins to bind water in the curd, and the fat degrades, resulting in a decrease in fat content. The higher the concentration of protease enzymes added to fresh cheese production, the lower the fat content of the resulting cheese (Lanur et al. 2025). This statement supports the results of the lowest fat content in P3, which was obtained with the addition of high sulfhydryl protease enzymes from frangipani stems at a concentration of 0.06% compared to P1 and P2. Fat content contributes significantly to cheese flavor, texture, and overall acceptability. The fat levels obtained in this study were comparable to those generally reported for fresh cheese products and complied with the physicochemical characteristics described in NTC 5894.

Color

The results of the analysis of variance (ANOVA) proved that the difference in the concentration of thiol protease from frangipani stems had a significant effect (P<0.05) on the color of fresh cheese. The color of fresh cheese with the addition of thiol protease is shown in Figure 3. The higher the score given by the panelist, the more acceptable the color of the fresh cheese was considered to be. The PK treatment was not significantly different from the P1 treatment but was significantly different from the P1 and P2 treatments. The test results showed that the PK treatment (0.02% commercial enzyme) and P1 treatment (thiol protease 0.02%) had the lowest average value of 3.87 (white), while the highest average value was found in the P3 treatment (0.06% thiol protease) with a value of 4.33 (bright white). The color of fresh cheese in each treatment tended to differ due to the addition of thiol protease and commercial rennet; however, the differences were not pronounced, with all treatments ranging from white to bright white. The addition of thiol protease did not produce a color completely identical to that of the control fresh cheese, but the overall appearance remained within the normal range for fresh cheese. In general, the color of fresh cheese is more strongly influenced by the natural pigments and composition of cow's milk, particularly fat content, than by the type of coagulant used. Cottage cheese with a coagulant of the moringa leaf protease enzyme of 0.06% produces a bright white to white color. Cheeses with a high fat content, such as cottage cheese a white color, while cheeses with low fat content have a yellower color (Larasati et al. 2024). Feta cheese produced from milk with high fat has a bright white color with an L*(*lightness*) value of 85.8 (Khiabanian et al. 2022). Based on the panelists' assessment, the color of fresh cheese in the P1 treatment with a concentration of 0.02% thiol protease produces a white color and has a high fat content of 16.7% (Hammam et al. 2021). Organoleptic test of fresh cheese with the addition of thiol protease is showed in Table 4.

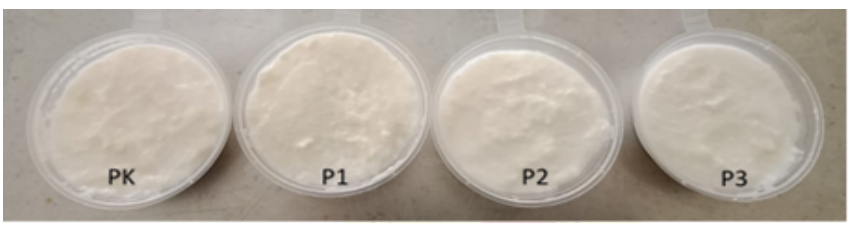


Figure 3. The color of fresh cheese with the addition of thiol protease.

Table 4. Organoleptic test of fresh cheese with the addition of thiol protease.

Parameters	Treatment			
	PK	P1	P2	P3
Color	3.87±0.43^{c}	3.93±0.52^{bc}	4.20±0.55^{ab}	4.33±0.71^{a}
Taste	3.70±0.79^{ab}	3.40±0.56^{b}	3.70±0.75^{ab}	3.97±0.85^{a}
Aroma	4.03±0.76^{a}	3.93±0.78^{a}	3.87±0.73^{a}	4.10±0.99^{a}
Texture	3.20±0.80^{c}	2.60±0.96^{d}	3.83±0.37^{b}	4.67±0.47^{a}
Favorite	3.27±0.82^{b}	4.07±0.74^{a}	3.83±1.02^{a}	2.80±0.99^{c}

Note: PK (control, commercial enzyme 0.02%), P1 (thiol protease 0.02%), P2 (thiol protease 0.04%), P3 (thiol protease 0.06%). Different ^{abcd} notations on the same line indicate a result of a real effect (P<0.05).

Taste

The results of the analysis of variance (ANOVA) showed that differences in thiol protease concentrations had a significant effect (P<0.05) on the taste of fresh cheese. The average test results showed that treatment P1 (thiol protease 0.02%) had the lowest average value of 3.40 (slightly salty and slightly bitter), and the highest average value was found in treatment P3 (thiol protease 0.06%) with a value of 3.97 (salty and slightly bitter). The bitter taste perceived in the fresh cheese in thought to be related to the proteolytic activity of thiol protease, which hydrolyzes casein into peptides and free amino acids. Plant-derived coagulants from flowers, leaves, or stems have often been reported to generate a slight bitterness in cheese, as extensive proteolysis can produce short hydrophobic peptides that contributed to bitter sensation when consumed (Nicosia et al. 2022). In this study, all treatments were still within the "slightly salty-slightly bitter" range, suggesting that the bitterness remained at low to moderate level. Fresh cheese produced from thiol protease coagulants also has a salty taste. The protease enzyme in the plumeria stem contains proteolytic activity, which breaks down proteins into amino acids, particularly glutamic acid, which contributes to the savory or salty flavor of foods. A key factor to consider when using enzymes as a rennet substitute is excessive proteolytic activity, which can lead to a bitter taste. Excessive concentrations of proteolytic enzymes can cause a bitter taste due to the formation of inherently bitter peptide bonds. High levels of fresh milk protein can impart a bitter taste to the resulting fresh cheese.

Aroma

The results of data analysis showed that differences in enzyme concentrations had no effect (P>0.05) on the aroma of fresh cheese. Different concentration levels did not affect the aroma of the fresh cheese produced. Treatment PK up to treatment P3 did not affect the aroma of the fresh cheese produced. The average aroma value ranged from 3.87 to 4.10 (distinct milky). This result can be explained by the fact that all treatments used the same type and amount of cow's milk and were processed under similar conditions, and thiol protease from frangipani stems does not have a strong characteristic odor, so it did not markedly influence the aroma of the fresh cheese. The aroma of fresh cheese is largely determined by the volatile fatty acid and other aroma-active compounds presents in milk fat; short-and medium-chain fatty acids such as butyric, caproic, caprylic, capric, and lauric acids are volatile and contribute to the characteristic aroma of fresh cow's milk (Kustyawati et al. 2012). This result indicates that the different treatments applied in this study did not substantially influence the volatile compounds responsible for cheese aroma formation. The relatively similar milk-based composition and processing conditions among samples may have contributed to the comparable aromatic profiles perceived by the panelists.

Texture

The results of data analysis showed that differences in thiol protease concentrations had a significant effect (P<0.05) on the texture of fresh cheese. The higher the panelists' assessment, the better the texture of the fresh cheese produced. Treatment P1 (thiol protease 0.02%) had the lowest average value of 2.60 (bit soft), while the highest average value was found in treatment P3 (thiol protease 0.06%) with a value of 4.67 (very soft). The increase in thiol protease concentration enhanced proteolytic activity,

leading to greater protein breakdown and producing a softer cheese texture. Consequently, treatment P3 showed the softest texture, whereas treatment P1 exhibited only a moderately soft texture due to the lower enzyme concentration used. Based on the data obtained, it can be seen that increasing the concentration of thiol protease was associated with a softer texture, higher moisture content, and an increase in yield. Milk naturally contains calcium bound to casein micelles. Coagulation of milk by protease enzymes breaks the bonds between calcium and casein micelles, allowing the calcium to dissolve in the whey. This reaction not only reduces the calcium content of the cheese but also results in a softer texture (Kommineni et al. 2022). Cheese texture can be influenced by the milk coagulation process used because the protein content in the milk affects the density of the resulting cheese. Increasing the concentration of coagulating agents can increase proteolytic activity and affect the moisture content of fresh cheese, which in turn contributes to the softer texture observed at higher thiol protease levels.

Favorite levels

The results of the analysis of variance (ANOVA) showed that the difference in the concentration of thiol protease from frangipani stem had a significant effect (P<0.05) on the preference for fresh cheese. The higher the panelist's assessment, the better the preference value for fresh cheese. Treatment P1 was not significantly different from treatment P2 but was significantly different in treatments PK and P3. The average test results showed that treatment P3 (thiol protease 0.06%) had the lowest average value of 2.80 (slightly liked), and the highest average value was in treatment P1 (thiol protease 0.02%) with a value of 4.07 (liked). This was because the fresh cheese produced with a concentration of 0.02% thiol protease had a slightly soft texture, a milky aroma, a white color, and a slightly bitter, salty taste, so that the panelists preferred it compared to treatments with concentrations of 0.04 and 0.06%. Fresh cheese with a 5% papain enzyme combination showed the most preferred results by panelists (Junior et al. 2018). All of these results were supported by the results of the overall organoleptic analysis, including texture, color, and flavor. Cottage cheese with a protease enzyme coagulant from noni fruit at a concentration of 0.02% was the cheese most preferred by panelists. This is due to the cheese's delicious taste, soft texture, distinctive milk color and aroma, and no fishy aftertaste. Overall, the physicochemical and sensory characteristics of the cheese produced in this study were generally comparable with commercial fresh cheese products and consistent with the quality characteristics described in the Colombian Technical Standard NTC 5894 for fresh cheese.

CONCLUSION

Thiol protease extracted from (*Plumeria obtusa*) stems successfully coagulated cow's milk for fresh cheese production. Overall, with 0.06% concentration (P3) produced cheese quality comparable to commercial rennet (PK), indicating that this enzymatic treatment can yield fresh cheese with acceptable consumer characteristics. P3 treatment resulted in higher protein content, acceptable moisture content, and improved texture, which contributed to greater panelist acceptance. The plant protease therefore shows promise as a sustainable alternative coagulant, helping to address rennet cost and availability challenges in small-scale dairy production. These findings advance vegetable rennet research by validating *Plumeria obtusa* stem protease performance through comprehensive physicochemical and sensory evaluation. Future studies should optimize extraction efficiency, test applicability across cheese varieties, investigate shelf-life stability, and validate industrial-scale performance.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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