p-ISSN 1410-5659 e-ISSN 2621-5144 Jurnal Ilmu Ternak Universitas Padjadjaran, November 2025, 25(3):224-235
Published by: DOI: 10.24198/jit.v25i3.65814
Unpad Press Available online at http://jurnal.unpad.ac.id/jurnalilmuternak

APPLICATION OF PARTIALLY PURIFIED THIOL PROTEASE FROM FRANGIPANI STEM (*PLUMERIA OBTUSA*) AS A COAGULANT AGENT IN CREAM CHEESE PRODUCTION

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Abstract

Frangipani contains thiol protease enzymes, particularly in its stem. The stem's thiol protease qualifies as a vegetable rennet, which can be used as a milk coagulant to substitute animal-based rennet in cream cheese. This study aimed to determine the proteolytic activity of thiol protease, as well as the physicochemical (yield, spreadability, protein content, and moisture content) and organoleptic characteristics (taste, color, aroma, texture, and overall preference) of cream cheese made using thiol protease from frangipani stems as a substitute for animal rennet. A completely randomized design was used for physicochemical testing, and a randomized block design was used for organoleptic testing, followed by Duncan's Multiple Range Test. Treatments included: PK (0.2% animal rennet), P1 (0.2% frangipani stem thiol protease), P2 (0.4% frangipani stem thiol protease), and P3 (0.6% frangipani stem thiol protease). The results showed that the concentration of thiol protease was directly proportional to the proteolytic activity; different concentrations of frangipani stem thiol protease significantly affected ($P<0.05$) physicochemical parameters (yield, spreadability, protein, and moisture content). Organoleptic evaluation also revealed significant differences ($P<0.05$) in color, taste, aroma, texture, and overall preference. The best treatment was P3 (0.6%), with a yield of 20.68%, moisture content of 73.25%, protein content of 14.73%, and excellent spreadability.

Keywords: frangipani stem, cream cheese, thiol protease, physicochemical, organoleptic

APLIKASI THIOL PROTEASE HASIL PEMURNIAN PARSIAL DARI BATANG KAMBOJA (*PLUMERIA OBTUSA*) SEBAGAI KOAGULAN PRODUKSI KRIM KEJU

Abstrak

Kamboja mengandung enzim thiol protease, terutama pada batangnya. Thiol protease pada batang kamboja dapat digunakan sebagai rennet nabati, yang dapat digunakan sebagai koagulan susu untuk menggantikan rennet hewani dalam krim keju. Penelitian ini bertujuan untuk menentukan aktivitas proteolitik thiol protease, karakteristik fisikokimia (rendemen, daya oles, kadar protein, dan kadar air), serta organoleptik (rasa, warna, aroma, tekstur, dan tingkat kesukaan) krim keju yang dibuat menggunakan thiol protease dari batang kamboja sebagai pengganti rennet hewani. Rancangan acak lengkap digunakan untuk uji fisikokimia, dan rancangan acak kelompok digunakan untuk uji organoleptik, dilanjutkan dengan Uji Jarak Berganda Duncan. Perlakuan yang digunakan adalah: PK (0,2% rennet hewan), P1 (0,2% protease tiol batang kamboja), P2 (0,4% protease tiol batang kamboja), dan P3 (0,6% protease tiol batang kamboja). Hasil penelitian menunjukkan bahwa konsentrasi thiol protease berbanding lurus dengan aktivitas proteolitik. Perbedaan konsentrasi thiol protease batang kamboja berpengaruh nyata ($P<0,05$) terhadap parameter fisikokimia (rendemen, daya oles, protein, dan kadar air). Hasil uji organoleptik juga menunjukkan perbedaan nyata ($P<0,05$) pada warna, rasa, aroma, tekstur, dan tingkat kesukaan. Perlakuan terbaik adalah P3 (0,6%), dengan rendemen 20,68%, kadar air 73,25%, kadar protein 14,73%, dan daya oles yang sangat baik.

Kata kunci: batang kamboja, krim keju, thiol protease, fisikokimia, organoleptik

INTRODUCTION

Frangipani *(Plumeria obtusa)* is a plant from the Rosaceae family, commonly known as the frangipani flower. In Indonesia, frangipani is widely used as an ornamental plant and as a traditional wound treatment, due to its content of agoniadin, plumierid, plumeric acid, lipeol, and serotinic acid (Nurcahyo & Purgiyanti, 2017). Frangipani possesses numerous beneficial properties due to its antioxidant and phytochemical content (Fikayuniar et al., 2023). Its use in wound treatment is possibly linked to the presence of thiol protease, which serves as an effective agent in wound healing (Retnowati et al., 2020). However, the utilization of frangipani remains limited to ornamental and medicinal purposes. The frangipani stem is often discarded and considered to have no economic value, despite its promising potential (Sanjaya et al., 2024). Therefore, maximizing its use could enhance both its functional value and economic potential. Frangipani is known to contain several enzymes, one of which is thiol protease, a cysteine protease with a cysteine residue in its catalytic site, acting as an endopeptidase with an optimal pH of 7.5 and optimal temperature of 55 °C (Laksanawati et al., 2023). In the food industry, thiol protease helps reduce time and production costs by accelerating protein hydrolysis. Proteases account for 60% of the total enzyme trade worldwide, serving as biocatalysts in the food, detergent, and leather industries. Therefore, extracting thiol protease from frangipani stems can add economic and functional value, particularly as a potential milk coagulant in cheese production.

Typically, cheese is produced using a milk coagulant called rennet, which comes in extract or powdered form. It is usually derived from the abomasum (fourth stomach) of suckling calves or other young ruminants. Rennet (chymosin) used in cheese production is a protease enzyme that catalyzes the hydrolysis of peptide bonds in milk proteins, allowing the milk to coagulate and form curds (Stocco et al., 2025). The availability of animal rennet is limited due to its source, while demand for cheese production is high, resulting in high prices. This has driven research into alternative sources of protease enzymes that could act as milk coagulants, such as microbial and plant-based rennets (Nicosia et al., 2022). Microbial rennet is obtained from specific fungi or cells from young animal stomachs, while vegetable rennet can be found in local plants like frangipani, with its stem containing thiol protease.

Cheese is a dairy product made from fermented milk—commonly from cows, goats, or sheep—and is widely consumed around the world, including in Indonesia, due to its high nutritional value. Cheese is a high-protein dairy product typically produced by coagulating milk using rennet (Adrian et al., 2015). Based on texture, cheese is categorized into four types: soft, semi-soft, hard, and very hard (Moatsou, 2019). Cream cheese is a soft, unaged cheese, also known as fresh cheese. It is characterized by a soft, smooth texture, white color, and slightly savory flavor. Cream cheese is commonly used as a spread, sandwich filling, cooking ingredient, salad dressing, snack topping, or accompaniment to baked potatoes (Guna et al., 2020). Research on the extraction and purification of thiol protease from frangipani stem has previously been conducted by Laksanawati et al. (2022), showing that the extracted enzyme has an activity of 0.29 U/mL. Therefore, research on the use of plumeria stem protease as a coagulant in cream cheese production is a promising field of study. This study aims to : (1) determine the proteolytic activity of partially purified thiol protease from fragipani stem in cream cheese production, (2) determine the physicochemical and organoleptic qualities of cream cheese with plumeria stem as a coagulant.

MATERIALS AND METHODS

Materials and Experimental Design

The equipment used in this study included: glassware, water bath ekstraksi memmert WTB serie 12L, hot plate stirrer AREC 7, vortex mixer MX-S, centrifuge tube hettich EBA299, UV-Vis AMV11 spectrophotometer, knife, tray, basin, pasteurizer, and strainer. The main materials were frangipani stems sourced from plantations in the Banyuwangi region; fresh cow's milk obtained from PT Bumi Rojo Koyo dairy farm in Banyuwangi, acetid acid glacial 99.5%, distilled water (aquadest) (CV Friend Lab, Indonesia), Disodium phosphate anhydrous (Na_2HPO_4) (Food Grade, Sigma-Aldrich), monosodium phospate (NaH_2PO_4) high-purity grade (Merck, Germany), ammonium sulfate $(NH_4)_2SO_4$ white powder (Merck, Germany), 96% ethanol, animal rennet commercial, salt (NaCl) white powder 94.7%, dialysis tube (cutoff 12–14 kDa) protein biologi IGCSE Cambridge, potassium sulfate (K_2SO_4) pure analysis (Merck, Germany), mercury (II) oxide (HgO) crystalline powder (Merck, Germany), sulfuric acid (H_2SO_4) 96% purity, sodium hydroxide (NaOH) white cystalline, 98%, sodium thiosulfate ($Na_2S_2O_3$) crystalline substance

98%, Boric acid (H_3BO_3) 99% purity solube in water, sodium bicarbonate ($NaHCO_3$) white crystalline powder 99% purity.

Extraction and Purification of Thiol Protease

This study was divided into two stages: thiol protease extraction and cream cheese production. A total of 100 g of frangipani stem was chopped and soaked in 96% ethanol for 30 minutes (1:1 b/v). The material was then rinsed with running water and blended for 2 minutes, followed by the addition of 0.1 M sodium phosphate buffer at pH 7.0 (1:4 b/v). The mixture was filtered using filter paper to obtain the filtrate and then centrifuged at 3,000 rpm for 10 minutes at 4°C to obtain the supernatant. The extract was precipitated with 100 mL of 60b/v ammonium sulfate at 55°C under constant stirring and stored in a refrigerator for 24 hours. The semi-purified thiol protease was separated from the supernatant by adding 16 mL of 0.05 M phosphate buffer at pH 7.5. Next, dialysis was performed using a dialysis membrane (a cellophane tube with a 10 kDa cutoff). Dialysis was carried out with 0.05 M phosphate buffer at pH 7.5, with the buffer solution replaced periodically with lower concentrations. The dialyzed protease extract was washed with distilled water and stored (Laksanawati et al., 2022).

The second stage involved cream cheese production, based on the method described by Putra et al. (2022). Fresh cow's milk was pasteurized at 75°C for 15 seconds, then cooled to 55°C. Citric acid (0.75b/v) was added to the pasteurized milk, along with 0.2% commercial animal rennet (as a control), and thiol protease in concentrations: P1 (0.2v/v), P2 (0.4v/v), and P3 (0.6v/v). The resulting curd was strained and salted at a concentration of 1% of the curd weight. The resulting cream cheese was analyzed for yield, moisture content, protein content, spreadability, and organoleptic properties (aroma, taste, color, texture, and panelist preference). Proteolytic activity can be determined using milk as a substrate at 55°C and pH 7.5. Pasteurized milk is dissolved in phosphate buffer, then reacted with the thiol sulfhydryl extract of frangipani stems at various concentrations. 700 µl of phosphate buffer solution with an optimum pH of 0.01 M is added and incubated for 30 minutes. The incubation is stopped by adding 500 µl of 1.2 M TCA, followed by centrifugation at 10,000 rpm for 10 minutes. The supernatant is collected and measured with a spectrophotometer at a wavelength of 275 nm. One unit (1U) of enzyme activity is defined as the amount of enzyme that releases 1 1g of tyrosine per 1 mL in 1 minute.

Cream Cheese Production and Analytical Procedures

Yield testing was conducted by comparing the weight of the cream cheese produced to the initial weight of the fresh milk used (Adrianto et al., 2020). Spreadability was evaluated using a hedonic ranking test, assessing how easily panelists could spread the cream cheese on white bread (Yahya et al., 2024). The hedonic test was conducted by ranking the panelists' levels of preference for the spreadability of cream cheese on white bread. The order of the intensity of ease in spreading cream cheese on bread was (1) very difficult to spread, (2) not easy to spread, (3) somewhat easy to spread, (4) easy to spread, and (5) very easy to spread. Protein content was measured using the Kjeldahl method, which includes digestion and protein determination (Lanur et al., 2025). Moisture content was determined using the oven-drying method, which involves measuring the weight loss after drying (Yunita et al., 2025). Organoleptic testing assessed preferences based on differences in color, texture, aroma, taste, and overall likability (Negara et al., 2016). Organoleptic tests involved 30 untrained panelists as replicated. The organoleptic testing scale for cream cheese is as follows: (1) color by looking at the color of the cream cheese, (2) taste produced by the cream cheese, (3) aroma produced by the cream cheese, (4) touching or holding the cream cheese to observe its texture, (5) overall preference for the resulting cream cheese. Information on the organoleptic test quality scale is presented in Table 1.

This study employed a completely randomized design (CRD) for physicochemical testing and a randomized block design without factorial treatment for organoleptic testing. The treatments included PK (0.2% animal rennet), P1 (0.2% frangipani stem thiol protease), P2 (0.4% frangipani stem thiol protease), and P3 (0.6% frangipani stem thiol protease). Each treatment was repeated four times, resulting in a total of 16 samples. The data were analyzed using analysis of variance (ANOVA), and if a significant difference (P<0.05) was found, Duncan's Multiple Range Test was used for further analysis.

RESULTS AND DISCUSSION

Proteolytic Activity of Thiol Protease From Frangipani Stem

The effect of thiol protease concentration on the proteolytic activity is shown in Figure 1. During the process of making cream cheese using the thiol protease coagulant derived from frangipani stems, a cleavage of k-casein occurs on the surface of casein micelles. This cleavage triggers milk coagulation. Furthermore, thiol protease can also hydrolyze proteins other than casein in milk. The results showed that the concentration of the thiol protease enzyme is directly proportional to the value of proteolytic activity in cheese making. The higher the concentration of thiol protease added, the higher the proteolytic activity. In this study, the proteolytic activity value was high enough that the thiol protease could break down protein bonds other than k-casein in milk, causing the appearance of short peptide bonds that contribute to the cheese's slightly bitter aftertaste. High proteolytic activity characterizes most plant-based proteases, particularly during the early stages of cheese ripening, where they continuously hydrolyze casein, resulting in the formation of short peptides that can impart a bitter taste (Nicosia et al., 2022). Proteolysis is likely the most important biochemical event during the ripening of most cheese varieties, with a significant impact on flavor and texture (Laksanawati et al., 2023).

Physicochemical Characteristics of Cream Cheese

The observed physicochemical qualities in this study included yield, spreadability, moisture content, and protein content, as presented in Table 2.

- **Yield.**

  This study compared the use of commercial animal rennet and various concentrations of thiol protease in the production of cream cheese. The addition of frangipani stem thiol protease significantly affected (P<0.05) the yield obtained. Table 2 shows that PK had the lowest yield at 18.44% and was not significantly different from P1 (18.92%). The control (PK) and P1 treatments produced similar yields because they used the same enzyme concentration, albeit from different rennet types. Furthermore, the proteolytic activity was also similar. This suggests that the performance of the plumeria stem thiol protease and commercial rennet is similar in coagulating milk proteins. P3 had the highest yield at 20.68%, not significantly different from P2 (19.84%). The highest cream cheese yield was achieved with treatment P3 (20.68%), followed by P2 (19.84%), P1 (18.92%), and PK (18.44%). This suggests that the higher the concentration of thiol protease added, the greater the yield obtained, as more casein in the milk is coagulated by the thiol protease from the frangipani stem.

  Thiol protease from frangipani stem has a sulfhydryl (-SH) group at its active site and acts by cleaving κ-casein in milk proteins. The peptide bond in κ-casein is broken by thiol protease, resulting in structural changes to the protein and a loss of protein solubility, which leads to coagulation and curd formation. The more protease used, the more κ-casein is cleaved, and the more curd is formed (Ardat et al., 2022). Thiol protease from frangipani stem is categorized as an exopeptidase, which acts on the carboxyl group (hydrophobic) and amino acid group (hydrophilic) of peptides (Wicaksono & Winarti, 2021). The high yield is attributed to enzymatic activity, specifically the hydrolysis process in which milk casein is broken down and subsequently agglomerates. More thiol protease leads to more curd and increases the non-enzymatic agglomeration of casein micelles. The combination of casein hydrolysis and agglomeration causes a physical change in the milk, namely, coagulation. Higher concentrations of thiol protease optimize the milk coagulation process (Adrian et al., 2015). Similar results were reported by Lanur et al. (2025), who found that fresh cheese made with 0.06% noni fruit protease yielded

10.86%. The typical yield for cheese is 4.7–5.7%. The yield in this study was higher, at around 18–20%, likely due to the higher protease activity, which resulted in more effective coagulation and a greater yield.

- **Spreadability**

Spreadability refers to the ability of a spread, such as cream or jam, to be applied evenly over a medium like bread. A good spread should easily spread over the surface of bread and form an even layer. Spreadability in this study was assessed using a hedonic ranking test with 30 panelists, who ranked the samples based on ease of spreading cream cheese onto white bread (Guna et al., 2020). The difference in thiol protease concentration significantly affected (P<0.05) the spreadability of the cream cheese. The most easily spreadable samples were P2 and P3. The addition of P2 (0.4%) to P3 (0.6%) thiol protease produces the same spreadability because both treatments have the same superscripts. P2 and P3 had higher moisture content, resulting in a smoother and less clumpy texture. Panelists generally rated P1 and PK lower due to their less smooth and slightly clumpy textures. Increasing the concentration of frangipani stem thiol protease made the cream cheese easier to spread.

The results showed that moisture content also influences spreadability, making the cheese less clumpy and softer.` The results showed that moisture content also influences spreadability, making the cheese less clumpy and softer. Spreadability is considered good if the cream cheese, when spread onto bread or another medium, remains compact, not clumpy, and spreads evenly. A good cream cheese texture is soft, non-grainy, and exhibits good spreadability at room temperature (Nisak et al., 2022). Guna et al. (2020) reported that adding 0.5% porang flour produced cream cheese with the best spreadability. Spreadability is a specific quality indicator of cream cheese, used to evaluate consistency and texture when applied to food surfaces.

- **Protein Content**

Milk proteins coagulate under heat, acidic conditions, or in the presence of protease enzymes (Soeparno et al., 2001). Table 2 shows that the addition of thiol protease from frangipani stem significantly affected (P<0.05) the protein content of the cream cheese. P3 (0.6%) had the highest protein content at 14.73%, while P1 had the lowest at 11.12%. According to the Duncan test, PK and P1 were not significantly different, while P2 and P3 differed significantly. These results indicate that the higher the concentration of thiol protease used, the greater the breakdown of protein bonds, resulting in a higher protein content in the cream cheese. As the concentration of protease increases, it breaks more protein bonds into peptides, resulting in a greater amount of curd (Welin et al., 2023). This is also supported by Kurniawan et al. (2022), who noted that enzymatic reaction rates (v) are directly proportional to enzyme concentration (E).

The enzymatic reaction that occurs in cream cheese production involves the hydrolysis of casein proteins in milk, followed by an agglomeration process. The first stage involves the hydrolysis of casein, resulting in para-casein and macropeptides. The macropeptides, containing up to 30% κ-casein, diffuse into the liquid phase. The removal of macropeptides reduces surface tension and colloidal stability of micelles, allowing acid-induced coagulation. This is followed by agglomeration, which produces a uniform protein mass as micelles aggregate (Masyhur et al., 2016). The findings of this study show a consistent increase in both yield and protein content. According to Wulandari et al. (2021), the protein content of cheese ranges from 10% to 30%. Based on the different concentrations of frangipani stem thiol protease used, the protein content of the cream cheese increased with higher enzyme concentrations.

- **Moisture Content**

Moisture content in cheese indicates the amount of free and bound water present and is important for determining freshness, shelf life, and texture (Raisanti et al., 2022). Table 2 shows that the addition of frangipani stem thiol protease significantly affected (P<0.05) the moisture content of cream cheese. PK and P1 shared the same notation, meaning no significant difference between them, while P2 and P3 differed significantly. Higher thiol protease concentrations resulted in higher cream cheese moisture content. P3 (0.6%) had significantly higher moisture than P2 (0.4%). That cream cheese should have a moisture content of more than 67% to a maximum of 80%. The results confirm that adding frangipani stem thiol protease increases the moisture content of cream cheese. The more enzyme added, the more hydrolysis occurs, and the more water is bound. Cream cheese made with thiol protease concentrations of 0.4% and 0.6% experienced an increase in moisture content, as higher concentrations of thiol protease led to more extensive hydrolysis and greater water retention. This is because at higher concentrations, curd formation becomes less effective at separating from whey, resulting in more retained water (Andriani et al., 2019).

Differences in moisture content may also be due to the three forms of water in cheese: 1) bound within the curd matrix, 2) retained in hygroscopic curd particles, and 3) as free water. The amount of free water is influenced by pressing force during curd and whey separation (Hamzah et al., 2022). Guna et al. (2020) reported that cream cheese made with porang flour had a moisture content of 63–68%. Based on the results of this study, the product qualifies as soft cheese (cottage cheese) since its moisture content exceeds 40% (Hamzah et al., 2022). Soft cheese must have a moisture content below 80%, semi-soft cheese ranges from 45–55%, and hard cheese has a moisture content between 20–24% (Bulkaini et al., 2020).

Organoleptic Test

The organoleptic quality parameters observed in this study included the color, taste, aroma, texture, and overall preference for cream cheese made using frangipani stem thiol protease. The organoleptic test results are presented in Table 3.

- **Color**

Color is a key visual aspect of a product and typically the first characteristic assessed by consumers. The quality of a food product is often judged based on its color—products that match expected colors tend to be rated more favorably. The addition of frangipani stem thiol protease significantly affected (P<0.05) the color of the cream cheese. P2 was not significantly different from PK: 3.10 (white) and P1: 3.13 (white), and P2: 3.47 (white) was also not significantly different from P3: 3.87 (bright white). Overall, all cream cheese samples in this study were white in color. This is consistent with Yahya et al. (2024), who stated that cream cheese is generally white in color. The white color observed was likely due to the thiol protease from frangipani stem not containing any distinctive pigments, so the final cream cheese color was mostly influenced by the original color of the milk. Fresh milk is white due to its high content of carotene and riboflavin. The higher yield in this study also correlated with increased protein content, which in turn led to higher levels of carotene and riboflavin in the final product, resulting in a cream cheese that appeared whiter. This supports Arifiansyah et al. (2015), who explained that carotene and riboflavin are key contributors to the color of milk. The color of milk can range from a bluish-white to a golden yellow, depending on the type of animal, feed, fat content, and solids content. In a previous study by Yahdiyani et al. (2015), cream cheese made with microbial rennet and CMC (carboxymethyl cellulose) addition also produced a white color. Cream cheese products from all treatments are shown in Figure 2.

- **Taste**

Taste is one of the most important attributes evaluated in a product, as it directly influences consumer perception and acceptance (Poetri & Siahaan, 2024). Taste is assessed based on the sensory response from taste buds, which affects the consumer's judgment, particularly in food products. The addition of frangipani stem thiol protease significantly affected (P<0.05) the taste of the resulting cream cheese. PK: 3.23 (milky taste) has no significant difference with P1: 3.53 (milky taste). P2: 3.93 (salty, milky taste) has a significant difference from P3: 4.33 (very salty, milky taste). The results of the study showed that PK has no significant difference from P1. P2 has a significant difference from P3. The addition of thiol protease concentration from P2 (0.4%) to P3 (0.6%) causes the taste to become saltier because, in addition to the presence of salt, thiol protease also contributes to providing cheese flavor through the process of breaking down proteins into peptides. The results show that as the concentration of frangipani stem thiol protease increased, the cream cheese tasted saltier. This is likely due to the proteolytic nature of thiol protease, which breaks down proteins into amino acids, some of which contribute to a salty flavor. This supports the findings of Puspita et al. (2018), who explained that proteolytic enzymes break down proteins into amino acids that play a role in the formation of savory taste in food. The addition of salt in the cheese-making process also contributes to a distinct salty cheese flavor. According to Jessica & Kusumawati (2025), frangipani stem thiol protease exhibits proteolytic activity, which breaks down proteins into amino acids (particularly glutamic acid) that enhance savory or salty taste. Taste can also be influenced by chemical compounds, temperature, concentration, and interactions with other taste components. In this study, salt was added during the production of cream cheese (Safari et al., 2024).

- **Aroma**

Aroma is the smell perceived through chemical stimuli detected by olfactory nerves in the nasal cavity. The addition of frangipani stem thiol protease significantly affected ($P<0.05$) the aroma of the resulting cream cheese. The results of the study showed that PK: 2.60 (slightly milky aroma) had no significant difference compared to P1: 2.60 (slightly milky aroma), and P2: 3.07 (slightly milky aroma) had a significant difference compared to P3: 3.63 (distinct milky aroma). As the concentration of frangipani stem thiol protease increased, the resulting aroma became more distinctly milky. This is because higher enzyme concentrations led to increased protein hydrolysis, resulting in higher protein levels that are associated with more milk globules. These globules contain free fatty acids that contribute to the cheese's aroma. This aligns with the findings of Melia et al. (2010), who reported that the distinctive milky smell is caused by specific aromatic compounds and some volatile fatty acids present in milk. Milk typically carries the natural scent of the animal from which it comes, and its fat content easily absorbs surrounding odors. In general, cheese has a characteristic aroma produced by the activity of lactic acid bacteria, which play a role in developing both aroma and acidity (Winarsih & Rasyidah, 2022). According to Jessica & Kusumawati (2025), three main metabolic processes contribute to cheese aroma during processing them glycolysis, proteolysis, and lipolysis. Thus, the distinctive aroma of cheese arises from these combined processes.

- **Texture**

Texture refers to the characteristics of a material resulting from a combination of several physical properties, including size, shape, quantity, and structural elements, as perceived by touch, mouthfeel, and visual observation. Texture is also a key factor influencing consumer acceptance of food products (Rustagi, 2020). The addition of frangipani stem thiol protease significantly affected ($P<0.05$) the texture of the resulting cream cheese. PK: 3.67 (soft) shows no significant difference compared to P1: 3.83 (soft). P2: 4.10 (soft) has no significant difference with P3: 4.37 (soft). P3 has a significant difference from PK. The results show that as the concentration of frangipani stem thiol protease increased, the cream cheese texture became progressively softer. This is likely due to the increased moisture content at higher protease concentrations, resulting in a softer and more tender texture of the cream cheese. This finding aligns with Nisak et al. (2022), who stated that cream cheese should have a soft, tofu-like texture and a slightly salty taste. According to Arziyah et al. (2022), texture and consistency affect the flavor experience of food products. Additionally, fat content clearly affects texture and mouthfeel in relation to spreadability, although texture variation may not be systematically affected if fat content remains constant (Safari et al., 2024).

- **Overall Preference**

The results of the study showed that the addition of frangipani stem thiol protease had a significant effect (P<0.05) on panelists' overall preference for the cream cheese. The average overall preference scores were as follows: PK, 2.03 (slightly like); P1, 3.43 (somewhat like); P2, 3.83 (like); P3, 4.33 (like). These results indicate that most panelists preferred the cream cheese sample from treatment P3 and liked the sample from PK the least. Panelists favored P3 cream cheese because it had a soft texture, a brighter white color, and was easy to spread. Although PK also had a soft texture, it was more difficult to spread compared to P3, which affected its overall preference rating. There was no significant difference in preference between P1 and P2. These findings suggest that increasing the concentration of frangipani stem thiol protease in cream cheese production can improve the acceptability of the product among panelists.

Table 1. Organoleptic Quality Attributes of Cream Cheese

Scale	Quality Attributes				
	Color	Taste	Aroma	Texture	Overall Preference
1	Yellowish white	Tasteless	Not typical milk	Not very soft	Do not like
2	Slightly yellowish white	Slightly milky	Bit typical milk	Not soft	slightly like
3	White	Milky	slightly milky	Bit soft	somewhat like
4	Bright white	Salty milky	distinct milky	Soft	Like
5	Very bright white	Very salty milky	Very milky	Very soft	Very like

Table 2. Physicochemical Test Results of Cream Cheese

Treatments	Parameters			
	Yield (%)	Spreadability	Protein Content (%)	Moisture Content (%)
PK	18.92±0.40^{b}	3.40±0.81^{c}	11.69±0.37^{c}	69.60±0.09^{c}
P1	18.44±0.17^{b}	3.87±0.51^{b}	11.12±0.26^{c}	68.98±0.49^{c}
P2	19.84±0.36^{a}	4.10±0.48^{ab}	13.46±0.70^{b}	72.05±0.80^{b}
P3	20.68±1.01^{a}	4.43±0.77^{a}	14.73±0.36^{a}	73.25±0.53^{a}
Average	19.42±1.01	3.95±0.75	12.75±1.52	70.97±1.85

Note: PK (0.2% commercial animal rennet), P1 (0.2% thiol protease), P2 (0.4% thiol protease), P3 (0.6% thiol protease). Letters $^{a, b, c,}$ and ^{d} in the same column indicate significant differences (P<0.05).

Table 3. Organoleptic Test Results of Cream Cheese

Treatments	Parameters				
	Color	Taste	Aroma	Texture	Overall Preference
PK	3.10±0.92^{b}	3.23±1.10^{c}	2.60±1.16^{c}	3.67±0.92^{c}	2.03±0.56^{c}
P1	3.13±1.01^{b}	3.53±0.82^{c}	2.60±1.00^{c}	3.83±0.59^{bc}	3.43±0.93^{b}
P2	3.47±0.78^{ab}	3.93±0.45^{b}	3.07±0.83^{b}	4.10±0.30^{ab}	3.83±0.79^{b}
P3	3.87±1.01^{a}	4.33±0.55^{a}	3.63±1.16^{a}	4.37±0.76^{a}	4.33±0.99^{a}
Average	3.39±0.97	3.76±0.87	2.98±1.12	3.99±0.73	3.41±1.19

Note: PK (0.2% commercial animal rennet), P1 (0.2% thiol protease), P2 (0.4% thiol protease), P3 (0.6% thiol protease). Letters $^{a, b, c,}$ and ^{d} in the same column indicate significant differences (P<0.05).

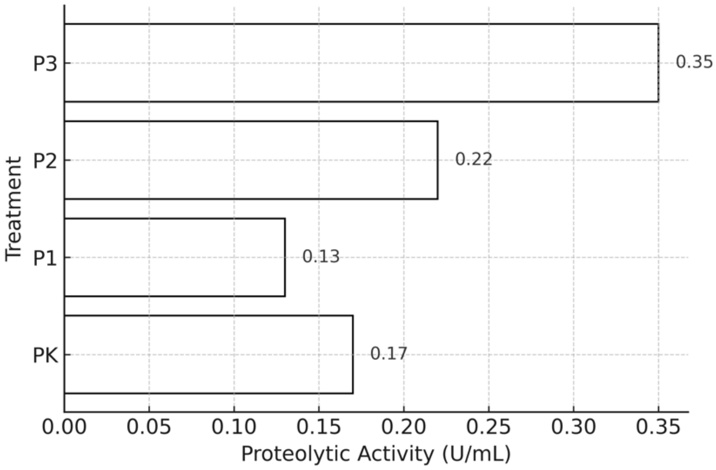

Figure 1. The effect of thiol protease concentration on the proteolytic activity

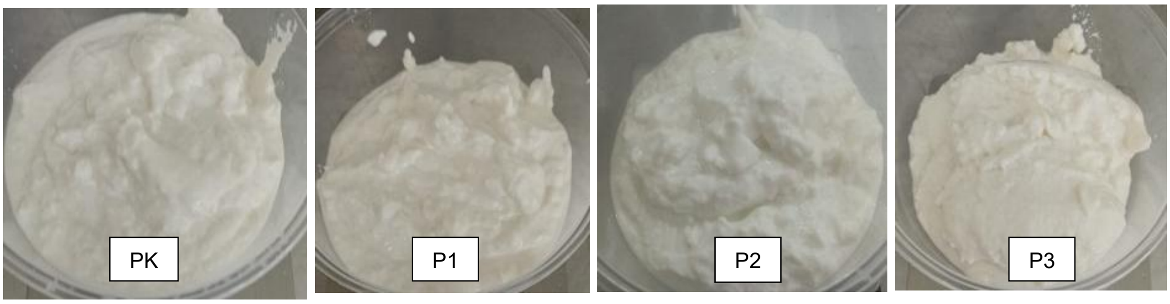

Figure 2. Cream cheese products from all treatments

CONCLUSION

Based on the research results, it can be concluded that the thiol protease from fragipani stems can be partially purified and applied to the production of cream cheese. The higher the concentration of thiol protease, the higher the proteolytic activity. The application of thiol protease from frangipani stems in cream cheese production significantly affected the physicochemical properties of the cream cheese, including yield, spreadability, protein content, and water content. Furthermore, the use of thiol protease from frangipani stems in cream cheese production also significantly affected the organoleptic properties of the cream cheese, including color, taste, aroma, texture, and preference. The best treatment for the physicochemical and organoleptic properties of the cream cheese produced was P3, which included the addition of 0.6% thiol protease from frangipani stems, yielding 20.68%, with a water content of 73.25%, a protein content of 14.73%, and a spreadability level of 14.73%. The researchers also appreciated the taste, color, aroma, and texture.

ACKNOWLEDGEMENT

We would like to convey our gratitude to Politeknik Negeri Banyuwangi for your financial support of this work under the grant PBRIP 2025. There is no conflict of interest for the present research.

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