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Physicochemical Properties of Cottage Cheese Produced Using Bromelain Enzyme Powder Derived from *Ananas comosus*

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ABSTRACT

Lampung Province is a producer of canned pineapple in Indonesia. Waste from the pineapple canning process, in the form of cobs and skins, is used as a powdered bromelain enzyme. Bromelain enzyme is thought to have the same function as rennet in the cheese-making process. This study aimed to determine the effect of using bromelain enzyme from *Ananas comosus* on the physical quality (curd yield, hardness, and adhesiveness) and chemical quality (protein, fat, and moisture content) of cottage cheese. The study was conducted using a Completely Randomized Design (CRD) with four treatment groups (0.5%, 0.6%, 0.7%, and 0.8% bromelain enzyme concentrations), each replicated five times. Results indicated that a bromelain concentration of 0.6% yielded the highest curd and cheese hardness, while a concentration of 0.7% produced the highest fat content and strongest adhesiveness. Additionally, a concentration of 0.8% resulted in the highest protein content, reducing the water content in the cheese. These findings suggest that bromelain enzyme concentrations between 0.6% and 0.8% are suitable for cheese production.

Keywords: Bromelain enzyme, Cottage cheese, Fresh cheese, Physicochemical properties, *Ananas comosus*

INTRODUCTION

Cow's milk is one of the main ingredients in cheese production. Cheese is a lump formed due to the coagulation of milk proteins, a process catalyzed by enzymes such as rennin or other proteolytic enzymes, and inoculated with a starter (Hutagalung et al., 2017). The starter typically consists of a group of lactic acid bacteria (LAB) that are capable of producing acid (Coelho et al., 2022). LAB begins the fermentation process by converting lactose to lactic acid, which lowers the pH and curdles the milk. Meanwhile, rennet is added to help form curds and separate proteins from whey.

Most Indonesians consider cheese an expensive food ingredient due to its difficult production process. The high price of cheese is partly attributed to the high cost of rennet enzymes, which are difficult to obtain. This is triggered by the enzyme production process taken from the stomachs of young ruminants and the rennet enzyme production process, which is not generally carried out in Indonesia. Currently, enzymes derived from plants are used as alternatives to replace the use of conventional enzymes. One such enzyme is bromelain, a proteinase-type enzyme extracted from the pineapple fruit *Ananas comosus* (Masri, 2013). Bromelain is found in all parts of the pineapple, including the fruit, stem, tubers, crown, and skin (Manzoor et al., 2016; Gul et al., 2021). The advantages of using this enzyme in Indonesia include its local availability, lower cost, and the ability to coagulate milk proteins, making it suitable for cheese production. Moreover, bromelain taken from *Ananas comosus* can affect the casein coagulation process, thereby influencing the physical properties of the resulting cheese (Komansilan et al., 2020).

Previous studies have explored the use of bromelain extract from *Ananas comosus* in cottage cheese production (Komansilan et al. 2024). The manufacture of *Ananas comosus* extract in the study by Komansilan et al. (2021) was based on Manoi (2017). *Ananas comosus* extract with concentrations of 1.5%, 3% and 4.5% has been applied in producing cottage cheese, leading to a yield of 8.46-10.62%, a texture of 3.67-3.81 N, a protein content of 11.50-12.64% a fat content of 2.13-5.21% and a water content of 56.01-57.65% (Komansilan et al., 2021). In contrast, this study employs powdered bromelain enzyme as a rennet substitute. This choice is motivated by the geographical context of Lampung Province, Indonesia—a major producer of canned *Ananas comosus* for export. The discarded pineapple parts (e.g., cores and peels) are processed into bromelain enzyme by PT Great Giant Pineapple subsidiary, namely PT Bromelin Indonesia. The objective of this study is to evaluate the effect of *Ananas comosus*-derived bromelain enzyme

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concentration on the physicochemical properties of cow's milk cheese, including chemical parameters (protein, fat, and moisture content) and physical characteristics (curd yield, hardness, and adhesiveness).

MATERIALS AND METHODS

Ethical approval

The current study was conducted according to the guidelines of the Department of Animal Husbandry, Faculty of Agriculture, University of Lampung, Bandar Lampung, Indonesia

Study materials and location

The research was carried out at the Animal Production Laboratory, Agricultural Product Analysis Laboratory, Faculty of Agriculture, University of Lampung, and the Agricultural Product Technology Laboratory (Lampung State Polytechnic, Indonesia). The materials used for the research were 20 liters of Holstein Friesian cow's milk, bromelain enzyme powder (PT Bromelain Indonesia, a public limited company), skim milk (PT Greendfields Indonesia tbk), mesophilic bacteria 8 log CFU/mL (*Lactococcus lactis* subsp lactis, *Lactococcus lactis* subsp cremoris, *Lactococcus lactis* subsp diacetylactis, *Leuconostoc mesenteroides* subsp mesenteroides) powder, $CaCl_2$ (PT. Subur Kimia Jaya), distilled water, and salt (merk Refina Salt, PT Unichem Candi Indonesia).

Study design

The experimental design used in this study was a Completely Randomized Design (CRD) with four treatments and five replications (1 L of cow's milk per replicate). The treatments given included 0.5% bromelain enzyme solution (T1), 0.6% bromelain enzyme solution (T2), 0.7% bromelain enzyme solution (T3), and 0.8% bromelain enzyme solution (T4).

Study procedure

The experiment was conducted in three stages, namely the stage of preparing starter media, the stage of preparing bromelain enzyme solution, and the stage of cheese production.

Starter media

One liter of skim milk was pasteurized at 90°C and then cooled to 40°C. Afterwards, 5 g of powdered mesophilic bacteria was added and stirred homogeneously. Finally, it was incubated for 24 hours before using the media.

Bromelain enzyme solution

The bromelain enzyme solution was prepared by dissolving the bromelain enzyme in distilled water at a ratio of 1g: 100 mL.

Cheese production

The stages of making cheese begin by preparing 1 L of fresh cow's milk and pasteurizing the milk at 72°C for 15 seconds. The milk reached a temperature of 32°C, and 7 ml/L of milk mesophilic starter and 8 ml/L of milk $CaCl_2$ solution were added to the solution. The milk was incubated for 30 minutes. The bromelain enzyme was added according to the treatment (0.5%, 0.6%, 0.7%, and 0.8%), and the solution was incubated for 40 minutes, cut the curd, and waited for the whey to separate from the curd. The next stage was to remove all the whey by filtering and put the curd into a container lined with filter cloth. The last step was pressing for 12 hours.

Curd yield

Yield testing was carried out by calculating the efficiency of the cheese produced. The cheese yield was obtained using Formula 1 (Nugroho et al., 2018).

Curd yield = $\frac{B}{A} \times 100\%$ (Formula 1)

A represents the weight of milk product used (g), and B denotes the weight of cheese formed (g).

Texture analysis (hardness and adhesiveness)

Cheese texture, including hardness and stickiness, were measured by cutting a rectangular cheese sample, then measuring it instrumentally with a texture analyzer (type Brookfield CT3, AMETEK Brookfield, USA) with a 4.5kg load cell, using a TA4R type probe with deformation, speed, and trigger conditions of 10mm, 2.5mm/sec, and 15g, respectively.

Protein content

The protein content of the resulting cottage cheese was measured using the Kjeldahl method (Komansilan et al., 2021).

Fat content

Fat content was measured via the Soxhlet method (AOAC, 2005). A boiling flask was first heated in the oven at 105°C and then weighed after being cooled down in the desiccator. As much as 2 grams of dry sample was placed into the filter (the Soxhlet extractor). A condenser was then placed above the boiling flask. Sixteen hours of reflux were then performed until the sample solution turned clear. The extracted sample was then dried in the oven at 105°C to allow for the evaporation of any solvent residue and reach a constant weight. The sample was then cooled in the desiccator and weighed. The fat content was measured by Formula 2:

$$\text{Fat (\%)} = \frac{C-B}{A} \times 100\% \quad \text{(Formula 2)}$$

A is the sample weight (g), B is the initial boiling flask weight, and C is the final boiling flask weight.

Water content

The water content of the resulting cottage cheese was measured using an oven as described by AOAC (AOAC, 2005). The sample (2g of cheese) was dried in the oven (105°C) for 5 hours. It was then cooled down on the desiccator for 30 minutes. The initial weight and final weight of the sample were then recorded using Formula 3.

$$\text{Water content (\%)} = \frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight}} \times 100\% \quad \text{(Formula 3)}$$

Data analysis

The data obtained in the study were analyzed using the analysis of variance, followed by the Least Significant Difference (LSD) test using Excel software.

RESULTS AND DISCUSSION

The results of curd yield, hardness, and adhesiveness of cheese using the enzyme bromelain are presented in Table 1. The results of cheese chemistry using the enzyme bromelain are presented in Table 2.

Table 1. Curd yield, hardness, and adhesiveness of cottage cheese

Parameter \ Bromelain enzyme concentration	T1	T2	T3	T4
Curd yield (%)	9.04 ± 0.93[b]	11.36 ± 0.81[a]	11.12 ± 0.99[a]	8.88 ± 1.86[b]
Hardness (N)	2.05 ± 0.44[b]	2.70 ± 0.66[a]	1.93 ± 0.48[b]	1.19 ± 0.27[c]
Adhesiveness (mj)	2.79 ± 0.81[a]	1.09 ± 0.15[b]	2.78 ± 1.34[a]	1.53 ± 0.69[b]

T1 represents 0.5% bromelain enzyme solution, T2 is 0.6% bromelain enzyme solution, T3 is 0.7% bromelain enzyme solution, and T4 is 0.8% bromelain enzyme solution. [abc] Different superscripts on the same row indicate significant differences ($p < 0.05$).

Table 2. Chemical content of cottage cheese

Parameter \ Bromelain enzyme concentration	T1	T2	T3	T4
Protein (%)	10.08 ± 0.86[b]	9.51 ± 0.72[b]	9.96 ± 0.41[b]	11.17 ± 1.02[a]
Fat (%)	0.62 ± 0.07[b]	1.30 ± 0.37[b]	2.20 ± 0.48[a]	0.94 ± 0.27[b]
Water (%)	46.38 ± 3.07[a]	48.60 ± 0.60[a]	47.12 ± 2.64[a]	41.76 ± 2.43[b]

T1 is 0.5% bromelain enzyme solution; T2 is 0.6% bromelain enzyme solution; T3 is 0.7% bromelain enzyme solution; and T4 is 0.8% bromelain enzyme solution. [abc] Different superscripts on the same row indicate significant differences ($p < 0.05$).

Curd yield, hardness, and adhesiveness of cottage cheese

The results of this study indicate that there are differences in cheese yield across different treatments. The highest yield was obtained using 0.7% bromelain enzyme, which is 11.12%. The use of lower (0.5% and 0.6%) and higher (0.8%) bromelain enzyme solutions did not increase the cheese yield. This has already been supported in a study conducted by Komansilan (2021), where the use of bromelain enzyme from 3% pineapple extract could produce the highest cheese yield compared to 1.5% and 4%, which was 12.64%. Cheese yield is very important in the cheese-making process (Khan and Masud, 2013) because it affects the costs and profits. Cheese yield is influenced by pH and enzyme concentrations that can initiate optimal coagulation of milk proteins. However, the use of vegetable enzymes cannot yet be used in cheese processing on an industrial scale since proteolytic activity can remove proteins from cheese.

Increasing the amount of bromelain enzyme solution resulted in reduced cheese yield. This is related to a decrease in pH value. Fox et al. (2017) note that cheese is coagulated by acidification to about pH 4.5-5.3, which leads to coagulation of casein at its isoelectric point (about pH 4.6). If the pH does not reach the isoelectric point, casein can still coagulate into curds, but the resulting cheese yield is decreased. The higher the acidity level, the more casein dissolves in the whey. The results of Wiedyantara et al.'s (2017) research show that the higher the concentration of fruit extract is, the higher the acidity level will be. This results in more casein dissolving in whey as well as reducing the yield percentage. Additionally, pineapple extract could influence the strength of the curd, thereby affecting the cheese yield. This is further supported by the study of Sumarmono and Suhartati (2012), who stated that the more fruit extract is added, the more proteolysis will occur, leading to an increased amount of casein dissolving in the whey. According to Yulianingsih et al. (2018), the higher enzyme content leads to excessive protein breakdown, resulting in smaller solid particles that pass through the filtration process.

Bromelain significantly affected cheese hardness ($p < 0.05$), with values ranging from 1.19-2.70 N, as compared to research conducted by Gutiérrez-Méndez et al. (2013), which showed that the hardness of fresh cheese was 7.59 ± 0.59 N. Apart from that, the cheese hardness value in this study was also lower compared to the study of Komansilan et al. (2021), which stated that the hardness of cheese using crude bromelain enzyme extract was between 3.38-3.81 N. This is due to increased proteolytic activity resulting from the acidic conditions of the bromelain enzyme, which triggers the breakdown of peptide bonds in the cheese. Poor cheese texture is due to imperfect curd separation. Aside from curd formation, water content in cheese could also affect its texture. Sulistyo et al. (2018) explained that water content correlates with cheese texture; the higher water content results in lower cheese texture.

The highest hardness value was obtained when administering 0.6% bromelain enzyme, while the lowest hardness value was achieved when administering 0.8% bromelain enzyme. Increasing the concentration of bromelain caused the acidity level to increase. This is in line with research by Komansilan et al. (2021), which illustrated that different concentrations of bromelain resulted in different curd formations, thereby affecting cheese texture. The higher the acid concentration in the coagulation product, the softer the texture of the cheese, since the higher acid concentration in the coagulation process will lead to higher proteolytic activity. The increase in proteolytic activity would cause protein instability during the proteolysis process. Therefore, increasing the acid concentration causes the protein to be more soluble in the whey, resulting in lower hardness (Nugroho et al., 2018).

The results of the study showed differences in adhesiveness across the treatments ($p < 0.05$). The adhesiveness of cheese in this study ranged from 1.09 to 2.79 mJ, higher than the study by Ali et al. (2021) conducted on fresh Baramily cheese, which was 1.17 ± 0.10 mJ. Treatments T1 and T3 showed the same completeness. According to Rahayu et al. (2010), the adhesiveness of cheese is formed by the coagulation matrix, namely casein, which can trap fat globules. O'Brien and O'Connor (2004) added that the smaller the size of the fat globules, the larger the surface area of the fat will be, thus affecting its hardness and adhesiveness.

Cheese in treatments T1 and T3 showed similar adhesiveness ($p < 0.05$). The adhesiveness of cheese is influenced by fat content (Bryant et al, 1995). The highest fat content was found in T3, being 2.20 ± 0.48%. This is in accordance with research by Setiawardani et al. (2016), which states that the factors influencing the adhesiveness of cheese are casein and fat. Milk fat is one of the components that play a role in forming the taste, aroma, and texture of the cheese produced (Estikomah, 2017). According to Bryant et al. (1995), the adhesiveness of cheese is not only influenced by the fat content but also by the protein content of the cheese. The types of cheese with higher adhesiveness are those that contain an open, loose protein matrix, such as found in high-fat cheese. As the protein matrix becomes denser, the cheese loses its adhesiveness. In addition to the fat content, the high acid concentration is thought to cause the cheese to become softer and stickier. Removing the fat will increase the protein content, changing the protein matrix, making it denser and, therefore, less sticky.

Protein, fat, and water content

The results of the study showed that there were differences in protein levels across the treatments ($p < 0.05$). The difference in protein content is due to the proteolytic properties of the bromelain enzyme. The protein content in this study increased proportionally with the increase in the concentration of the bromelain enzyme solution. This is due to the fact that bromelain is a protein. The highest protein content was found in T4, namely 11.17 ± 1.02. The observed protein content is low compared to the research by Komansilan et al. (2021), which used *Ananas comosus* extract (1.5%, 3%, and 4.5%), namely 11.5-12.64%.

The use of the bromelain enzyme at a concentration of 0.5-0.7% was thought to have the same acid content, so that the ability of the milk protein hydrolysis process to break down casein and free water was the same. Bromelain is a proteolytic enzyme that hydrolyzes casein into small peptides and free amino acids. Bromelain can attack peptide bonds in this fraction, which causes changes in the texture and consistency of the curd (Fox et al., 2017). If acid is added to milk, the milk will release hydrogen ions and attack other water molecules. The release of hydrogen ions causes the pH to decrease, resulting in changes in the casein micelle environment. The colloidal calcium hydroxy phosphate contained in the casein micelles dissolves and forms calcium ions (Ca^+), which penetrate the structure of other casein micelles and form strong internal calcium chains. This will change the casein micelles, starting with the joining of casein micelles through aggregation and ending with coagulation (Salvador et al., 2022). In addition to the concentration of bromelain enzyme solution, high water content can increase the mobility of proteolytic enzymes, thereby accelerating protein hydrolysis. According to Pramono (2019), the high-water content in milk can cause the resulting protein to become unstable. The high-water content in curd will cause its protein content to decrease, because the protein component is more soluble in water. The more proteins are dissolved in water, the lower the protein content in the cheese will be.

The use of 0.8% bromelain enzyme caused optimum acid conditions. This condition resulted in a protein hydrolysis process, where the casein micelles in milk released hydrogen ions so that the casein micelles were dissolved (whey). Dissolving casein micelles leads to the formation of calcium ions (Ca^+). These calcium ions trigger casein molecules to form denser casein clumps (curds; Arlene et al., 2015). Apart from the influence of Bromelain, treatment T4 had the highest protein content (11.17 ± 1.02) due to the low water content in this treatment. According to Pramono (2019), low water content in curd can cause the protein content in the curd to become stable, causing the protein content to become high.

The addition of bromelain taken from pineapple (*Ananas comosus*) to the treatment had a significant effect on the fat content of cow's milk cheese ($p < 0.05$). The average fat content in this study was below the fat content in the study by Komansilan et al. (2021), which was 2.13-5.21%. The use of bromelain did not significantly affect the results across T1, T2, and T4 treatments. Pramono (2019) stated that the amount of fat content in curd is not affected by the addition of bromelain. Bromelain can hydrolyze protein, but not to hydrolyze fat. According to Arlene et al. (2015), cheddar cheese made using bromelain enzyme has lower fat content compared to that made using rennet enzyme. This is because the rennet enzyme contains acid protease enzymes, *Rhizomucor miehei* mushroom, and lipase enzymes produced from the outer membrane into the culture medium. Meanwhile, according to Noviar et al. (2016), bromelain is only able to hydrolyze protein into amino acids but not to hydrolyze fat. However, fat content can be influenced by lipase derived from the raw materials used in making cheese. Lipase enzymes break down fats and convert them into simpler components, such as fatty acids and glycerol. Fatty acids are converted into various esters that produce taste, flavor, and texture (Gao et al., 2022).

The use of bromelain, accompanied by the use of starter, is thought to influence fat content in treatments T1, T2, and T4. The combination of protease enzymes from bromelain and lipase activity from starter bacteria can produce interactions that affect the fat content in cheese (Fox et al., 2017; Kariyawasam et al., 2019). Bromelain can break down proteins in the cheese matrix, which can change the distribution and stability of fat in the cheese. When proteins are hydrolyzed, fat can be released more easily during the cheese-making process. Meanwhile, starter bacteria play an important role in milk fermentation, especially in regulating coagulation and cheese ripening. Starter bacteria can also affect the fat content and composition during the cheese ripening process (McSweeney and Sousa, 2000).

T3 was significantly different with regard to fat content as compared to other treatments. This was thought to be due to the use of bromelain enzymes, accompanied by the use of a starter in T3, which produced more optimal acid conditions compared to other treatments. During the cheese-making process, other ingredients such as mesophilic bacteria were used as a starter culture. The acid that can be added in the acidification process is lactic acid, while microbial inoculation can be done by adding a starter culture (Estikomah, 2017). The use of starter can lower the pH, causing optimal acidic conditions so that more curd is produced and stabilizing the fat in the curd. In addition, bacterial starters produce lipase enzymes that can hydrolyze fat into free fatty acids and influence fat retention in cheese by controlling the curd structure.

The use of bromelain enzyme derived from *Ananas comosus* in treatments T1, T2, T3, and T4 had a significant effect on the water content of cow's milk cheese ($p < 0.05$). The use of 0.5-0.7% bromelain enzyme has the same effect on the water content of cheese. T4, in which 0.8% bromelain enzyme was used, could produce the lowest water content, namely $41.76 \pm 2.43\%$. This is supported by Yahya et al. (2024), who found that the addition of bromelain enzyme causes the water content in cream cheese to decrease along with increasing enzyme concentration. This difference is caused by increasing bromelain enzyme concentrations, which causes a different coagulation process (Komansilan et al., 2024). During cheese processing, high water content can facilitate protein hydrolysis by enzymes, producing amino acids that affect the taste and aroma of cheese. Conversely, low water content can limit this hydrolysis process, affecting the development of taste and texture (Syamsu and Elsahida, 2018).

CONCLUSION

The use of a bromelain enzyme solution concentration of 0.6% can produce the highest curd and cheese hardness. The use of a bromelain enzyme solution concentration of 0.7% can produce the highest fat content and the strongest adhesive power, whereas a bromelain concentration of 0.8% produces the highest protein content and reduces the water content in cheese. Moreover, bromelain enzyme solution concentrations of 0.6-0.8% can be applied in cheese making.

DECLARATIONS

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Authors' contributions

Nayla Salsabila designed and supervised the laboratory work, data analysis and interpretation, and manuscript editing. Nurul Afra Suryani made major contributions to the research design, realization, and supervision of laboratory work, data analysis and interpretation, and manuscript editing. Veronica Wanniatie made contributions to the research design, analysis, data interpretation, manuscript editing, revised the final version of the manuscript, and coordinated the authors. Arif Qisthon contributed to the research design and revised the final version of the manuscript. Dian Septinova participated in the research design, analysis, and data interpretation. Engki Zelfina participated in revising the final version of the manuscript. All authors have read and approved the final edition of the article.

Availability of data and materials

The findings of this study are available upon reasonable request from the corresponding author.

Competing interests

The authors have not declared any conflicts of interest.

Ethical considerations

This article is not submitted anywhere else, and the findings are analyzed and written under the supervision of all authors. All authors wrote the article and checked the last draft of the manuscript for the similarity index.

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