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Amino Acid, Fatty Acid and Curd Profile of Cottage Cheese using Crude Extract of Bromelain Enzyme (*Ananas comosus*) as Coagulant Source

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ABSTRACT

Background: The aim of this study was to analyze the profile of amino acids and fatty acids in cottage cheese using pineapple bromelain enzyme as a coagulant. Bromelain enzyme obtained from pineapple is used as a coagulant to replace rennet in the cheese-making process. Cottage cheese was made using fresh milk combined with bromelain enzyme at different concentrations.

Methods: The research method was a descriptive quantitative research method for the amino acid profile, saturated fatty acids and unsaturated fatty acids presented in table form and cheese curd was completely randomized design, [CRD] with treatment of 0%, 3%, 4% and 5% bromelain enzyme concentration. Variables observed include amino acid test, HPLC method, saturated fatty acids, unsaturated fatty acids with the gas chromatography mass spectrometry [GC-MS] method and cheese curd.

Result: The results showed that the profile of amino acids, saturated fatty acids and unsaturated fatty acids contained in cottage cheese was influenced by the concentration of bromelain enzyme, the higher the concentration of bromelain enzyme the higher the content of amino acids and unsaturated fatty acids while saturated fatty acids were lower in cottage cheese. The use of bromelain enzyme in making cottage cheese can produce cheese products with good quality in terms of amino acid and fatty acid profiles contained in the resulting cheese curd.

Key words: Amino acids, Bromelain enzyme, Cottage cheese, Curd, Fatty acids.

INTRODUCTION

The nutritional value, functional characteristics and bioactive potential of dairy products are significantly influenced by their amino acid and fatty acid profiles. Amino acids serve as the fundamental building blocks of proteins, whereas fatty acids, the primary constituents of lipids, play various physiological roles, including serving as energy sources, hormone precursors and structural components of cell membranes. Fatty acids are generally categorized into two groups: saturated fatty acids (SFAs) and unsaturated fatty acids (UFAs). While SFAs are associated with elevated blood cholesterol levels, UFAs are known to confer health benefits, particularly in supporting cardiovascular health (Fox *et al*., 2017; Sartika, 2008).

In cheese production, the type of coagulant enzyme used can influence the final nutritional composition, particularly the amino acid and fatty acid profiles. Traditionally, rennet-an enzyme complex derived from the stomachs of ruminant animals-has been widely used in cheese making. However, the use of animal-derived rennet raises concerns regarding halal compliance and sustainability. Additionally, the high cost and limited local availability of rennet in countries such as Indonesia contribute to elevated cheese production costs (Mijan *et al*., 2010; Kayagil, 2006).

Previous studies have demonstrated the coagulating potential of plant/fruit extracts, such as artichoke (Fguiri *et al*., 2024), papain enzyme (Malaka *et al*., 2024) and kiwi extract (Fguiri *et al*., 2023) as a rennet substitute in cheese making. Bromelain, a proteolytic enzyme extracted from pineapple (Ananas comosus), presents a promising natural alternative to animal-derived rennet. Bromelain has been widely studied for its protein-degrading capabilities and is commonly applied in the food, pharmaceutical and biotechnology industries (Pavan *et al*., 2012; Arshad *et al*., 2014). In the context of cheese making, bromelain may facilitate milk coagulation and influence the release of peptides and amino acids from milk proteins. Moreover, its proteolytic activity may also assist in the separation and profiling of lipids, thus affecting the fatty acid composition of cheese.

Cottage cheese is a fresh, unripened soft cheese known for its mild flavor, soft texture and favorable nutritional profile. In Indonesia, commercially available cheeses are

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predominantly hard cheeses that require long ripening times and higher production costs. Developing cottage cheese using bromelain as a milk coagulant offers a cost-effective and halal-compliant alternative while potentially enhancing nutritional quality.

There is limited information on how the use of bromelain as a coagulant affects the amino acid and fatty acid composition of cheese. Therefore, this study aims to evaluate the effects of bromelain enzyme from pineapple on the amino acid, saturated fatty acid and unsaturated fatty acid profiles in cottage cheese. The findings are expected to support the development of high-quality, nutritionally balanced and economically viable cheese products that meet consumer preferences and dietary requirements.

MATERIALS AND METHODS

Sample preparation and cheese making process

The preparation of cottage cheese and curd sample and also the analysis, were conducted in May to June 2024 at the Animal Products Laboratory of the Faculty of Animal Husbandry, Sam Ratulangi University, Manado. In this study, four treatments of bromelain enzyme (as the milk coagulant) concentration levels were used, which are K1 = 0%, K2 = 3%, K3 = 4% and K4 = 5%. At first, the bromelain enzyme was extracted by blending and pressing the juice from the pineapple fruit. The fresh milk (16 L) was pasteurized using the high temperature short time (HTST) method for 15 minutes at 70°C before being used to make the cottage cheese. After pasteurization, the bromelain enzyme was added with different levels of treatment to be tested and cooled to 400°C. When it is cooled down, the mixture is then incubated for 35 minutes, filtered through a gauze filter and then 1% salt is added. After being kept at -17°C for 24 hours, the curd was then examined.

Analysis procedure

Total curd yield

Curd is a parameter to determine the amount of curd formed after the milk casein is coagulated and has been separated from the whey. Total curd can be calculated using the following formula:

$$\text{Total curd [\%]} = \frac{\text{Curd weight}}{\text{Initial milk volume}} \times 100$$

Amino acid content

The identification of amino acids was carried out by analyzing their chemical reactions with functional groups common to all amino acids, particularly the amino (-NH,) and carboxyl (-COOH) groups, which produce distinctive reactions (Lehninger *et al*., 2008). A 5-gram sample was weighed and placed into a beaker, then mixed with 100 mL of acid orange reagent while stirring. After the solution became homogeneous, 10 mL of phosphate buffer was added. A chromatography paper measuring 3 cm in width and 10 cm in length was prepared and a sample spot was marked 3 cm from the top. The chromatography tube was filled with approximately 2 cm of carrier solution and the filter paper was inserted such that the spot remained 1 cm above the liquid level, allowing capillary action to separate the sample components based on molecular size and interaction with the carrier. This process was left overnight. The following day, the paper was sprayed with a dye reagent to reveal colored spots corresponding to different amino acids. Standard solutions of essential amino acids were prepared and spotted similarly for comparison. Each visible spot was then cut out and chemically processed to convert the amino acids into ammonium compounds. The absorbance of these compounds was measured and compared with the standards to determine the concentration of each amino acid in the sample.

Determination of fatty acid content

The fatty acid content was measured using the gas chromatography (GC) method. To begin the extraction process, 10 grams of the sample were placed into a 100 mL test tube, followed by the addition of 1.25 mL of concentrated ammonium hydroxide (NH_4OH). The mixture was homogenized, then 10 mL of ethanol (C_2H_5OH) was added and mixed thoroughly. The sample was subsequently extracted with 25 mL of diethyl ether and then with 25 mL of petroleum benzine (boiling point range 40-60°C). The mixture was shaken for one minute and allowed to stand until phase separation occurred, forming two distinct upper layers. These upper layers were carefully pipetted into a new tube and evaporated using a water bath at 45°C with a flow of nitrogen gas until dry. The remaining residue represented the fat or oil content of the sample. To prepare fatty acid methyl esters (FAMEs), the fat residue was reacted with 1.5 mL of a 20% boron trifluoride (BF_3) methanol solution in a sealed tube. The mixture was heated in a water bath at 45°C for 30 minutes, then cooled. The resulting methyl esters were extracted with 0.5-1 mL of n-hexane, forming two layers. The upper layer, containing the methyl ester-n-hexane mixture, was transferred to a bottle containing anhydrous sodium sulfate (Na_2SO_4) and allowed to stand for 5-10 minutes to remove any residual moisture. Finally, the clear hexane layer was transferred into a GC vial, flushed with nitrogen gas, sealed tightly and either stored in a freezer or directly analyzed by gas chromatography.

RESULTS AND DISCUSSION

Total cheese curd

The results of statistical analysis showed that the difference in bromelain enzyme concentration had a very significant effect [P≤0.01] on cheese curd. One of the most important parameters in assessing enzyme alternatives for cheese making is based on the weight of the cheese produced. Optimal cheese yield is important for cheese production because it will determine the costs and benefits. The total cheese yield during the process is influenced by several factors, such as enzyme concentration, temperature during the process. In this study, the total cheese curd was very significantly different between each treatment, namely 9.07% [K1], 11.16% [K2], 16.18% [K3] and 10.12% [K4], (Fig 1) . The use of 5% bromelain enzyme produced low cheese curd; this was due to the high proteolytic activity of bromelain at that concentration, which could cause excessive breakdown of milk protein. As a result, the curd structure became less dense and its quality decreased. Optimal enzyme concentration can initiate efficient milk coagulation, resulting in compact curd and higher cheese yield (Myagkonosov *et al*., 2023; Cai *et al*., 2024). The use of bromelain enzyme at too high a concentration, such as 5%, can cause over-hydrolysis of milk proteins, resulting in a less dense curd and decreased cheese quality (Walstra *et al*., 2006).

Amino acid profile

Amino acids are basic components of proteins that play an important role in various biological functions, including the formation and maintenance of body tissues. Amino acid profile is the type and composition of amino acids that make up cottage cheese, which includes various types of essential and non-essential amino acids that contribute to the nutritional value of a product (Geantaresa *et al.*, 2010). Cottage cheese also contains non-essential amino acids, making it a good source of protein. For example, the total amino acid content in cottage cheese can vary depending on the manufacturing method and raw materials used (Arifiansyah, 2020).

Table 1 shows the amino acid composition of cottage cheese made with varying concentrations of bromelain enzyme (0%, 3%, 4% and 5%) used as a coagulant. Overall, the results demonstrate that the addition of bromelain significantly increases the concentration of most amino acids. For instance, L-Glutamic Acid rises from 0.70 at 0% bromelain to 2.08 at 5%, while L-Leucine and L-Lysine also show sharp increases. Even amino acids present in smaller amounts, such as L-Histidine and L-Arginine, exhibit consistent upward trends. Bromelain is a proteolytic enzyme, meaning it breaks down proteins into smaller peptides and free amino acids (Komansilan *et al*., 2021; 2024). As the concentration of bromelain increases, it facilitates more extensive hydrolysis of milk proteins like casein and whey, making amino acids more accessible and measurable.

The highest increase of amino acid content at 5% bromelain enzyme was the glutamic acid ($C_5H_9NO_4$) because glutamate is one of the amino acids that is most easily produced through the hydrolysis process by this enzyme. The bromelain enzyme has a high affinity for proteins containing glutamine, producing glutamate in greater amounts compared to other amino acids, such as L-glutamine ($C_5H_{10}N_2O_3$) (Anggraini *et al*., 2013). This enzymatic activity improves the nutritional profile of the cheese by increasing its amino acid content and potentially enhancing its digestibility and flavor. Glutamic acid is known for providing umami, one of the five basic tastes (along with sweet, sour, bitter and salty). Cottage cheese contains glutamic acid, which contributes to its savory taste, which is often one of the main attractions of cheese products. As well as improving the quality of the taste of cottage cheese (Abdelmontaleb *et al*., 2021). Research shows that the use of bromelain enzyme from pineapple fruit can affect the casein coagulation process and increase proteolytic activity, which in turn affects the physical and chemical quality of cheese (Walther *et al*., 2008).

Saturated fatty acid profile

The results presented in Table 2 indicate that the addition of bromelain enzyme from pineapple extract leads to a

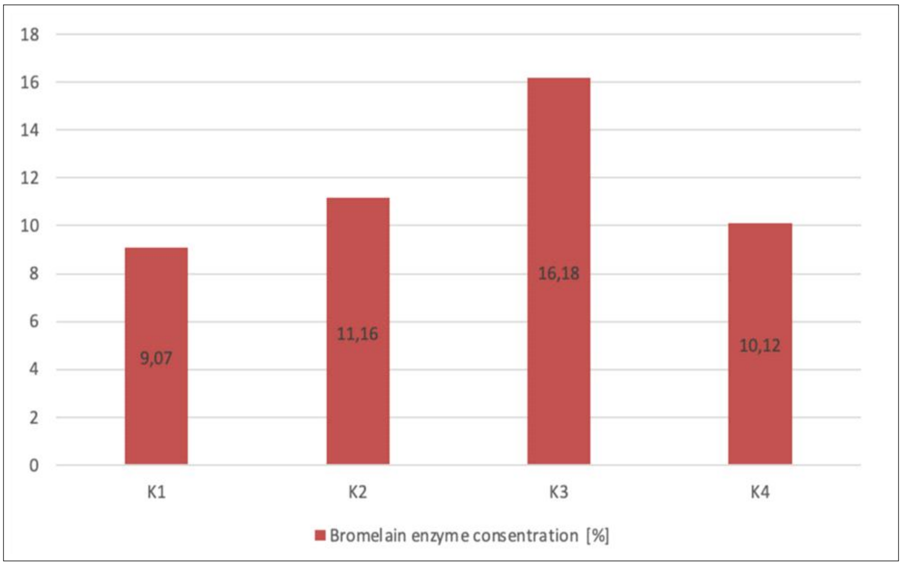

Fig 1: Total curd yield of cottage cheese added with bromelain enzyme as a coagulant.

progressive reduction in the content of saturated fatty acids in cottage cheese. As the enzyme concentration increases from 0% to 5%, the levels of nearly all saturated fatty acids, including butyrate, hexanoate, octanoate, decanoate and notably palmitic acid (C16:0), decrease consistently. Palmitic acid ($C_{16}H_{32}O_2$) remains the most abundant saturated fatty acid across all treatments, although its concentration drops from 35.93% at 0% bromelain to 35.20% at 5%. Meanwhile, three types of long-chain saturated fatty acids, namely docosanoate ($C_{23}H_{46}O_2$), tricosanoate ($C_{23}H_{46}O_2$) and lignocerate ($C_{24}H_{48}O_2$) acids, remain below detectable levels (<0.1%) in all samples.

Table 1: Amino acid composition of cottage cheese with added bromelain enzyme as a coagulant.

Variable	Bromelain enzyme concentration			
Amino acid	0%	3%	4%	5%
L- Aspartic acid	0.40	0.61	0.65	0.69
L- Glutamic acid	0.70	2.04	2.07	2.08
L- Histidine	0.07	0.20	0.22	0.24
L- Serine	0.25	0.34	0.36	0.38
L- Glutamin	0.01	0.02	0.03	0.05
L- Threonine- + L- Glycine	0.15	0.19	0.21	0.24
L- Arginine	0.10	0.20	0.22	0.25
L- Alanine	0.21	0.27	0.30	0.32
L- Tyrosine	0.11	0.15	0.18	0.24
L- Thrytophan + L- Methionine	0.05	0.09	0.11	0.12
L- Valine	0.05	0.22	0.24	0.26
L- Phenylalanine	0.30	0.31	0.32	0.33
L- Isoleucine	0.04	0.18	0.20	0.22
L- Leucine	0.06	0.62	0.64	0.66
L- Lycine	0.07	0.58	0.59	0.60

Table 2: Saturated fatty acid composition of cottage cheese added with bromelain enzyme as a coagulant.

Variable	Bromelain enzyme concentration			
Saturated fatty acid	0%	3%	4%	5%
Butyrate acid	0.98	0.97	0.87	0.85
Hexanoate acid	1.29	1.27	1.14	1.11
Octanoate acid	1.16	1.15	1.08	1.05
Decanoate acid	2.96	2.94	2.15	2.13
Undecanoate acid	0.29	0.27	0.18	0.16
Laurate acid	4.36	3.34	3.32	3.34
Tridecanoate acid	0.05	0.03	0.02	0.01
Myristate acid	11.62	11.59	11.09	11.08
Pentadecanoate acid	1.10	1.09	1.05	1.01
Palmitate acid	35.93	35.91	35.84	35.20
Heptadecanoate acid	0.36	0.34	0.32	0.30
Stearate acid	16.99	16.90	16.70	16.20
Arachidate acid	1.40	1.38	1.35	1.30
Heneicosanoate acid	0.78	0.76	0.45	0.40
Docosanoate acid	<0.1	<0.1	<0.1	<0.1
Tricosanoate acid	<0.1	<0.1	<0.1	<0.1
Lignocerate acid	<0.1	<0.1	<0.1	<0.1

The decrease in saturated fatty acid content with increasing bromelain concentration is likely due to enzymatic lipid degradation that occurs during the cheese-making process. Bromelain, being a proteolytic enzyme, may indirectly influence fat breakdown by disrupting protein-fat interactions and weakening fat globule membranes, facilitating the release and potential hydrolysis of fatty acids. This aligns with previous findings that fat degradation can occur with enzymatic assistance during cheese production (Kayagil, 2006). Additionally, the variation in fatty acid levels may be influenced by the structural composition of milk fat and its membrane, which affects fat retention or release during curd formation (Collins *et al*., 2003). Overall, the results suggest that the use of bromelain as a coagulant not only impacts protein hydrolysis but also contributes to the modification of fat composition in cottage cheese, potentially improving its nutritional profile by reducing saturated fat content.

Unsaturated fatty acid profile

As shown in Table 3, the addition of bromelain enzyme from pineapple extract led to a progressive increase in the content of unsaturated fatty acids in cottage cheese. The most substantial increase was observed in methyl trans-9-elaidate acid, which rose from 13.03% in the control to 13.15% at 5% bromelain concentration, making it the predominant unsaturated fatty acid across all treatments. Other unsaturated fatty acids, such as cis-9-oleate, palmitoleate and linoleate, also showed incremental increases with rising enzyme levels. In contrast, several long-chain unsaturated fatty acids (cis-11-eicosenoate, 11,14-eicosadienoate, cis-11,14,17-eicosatrienoate and docosahexaenoate) remained below detectable levels (<0.1%) across all samples.

The increased presence of unsaturated fatty acids, particularly methyl trans-9-elaidate, is attributed to the proteolytic activity of bromelain, which enhances the release of triglyceride-bound lipids during protein hydrolysis. As bromelain breaks down casein and other milk proteins, it disrupts fat-protein complexes, promoting the liberation and transformation of lipids into free fatty acids (Shah *et al*., 2014). The enzymatic hydrolysis reaches optimal efficiency at 5% concentration, facilitating the formation of more stable and readily formed unsaturated fatty acids. The chemical structure of methyl trans-9-elaidate may favor greater interaction with bromelain, allowing it to accumulate at higher levels than other fatty acids (Shingfield *et al*., 2013).

The use of bromelain as a milk coagulant significantly influences the physicochemical and nutritional properties of cheese compared to traditional rennet and other alternative enzymes. Bromelain, being a broad-spectrum proteolytic enzyme, tends to produce a softer curd structure and may accelerate protein hydrolysis, leading to higher levels of free amino acids in the final product. This can

Table 3: Unsaturated fatty acid composition of cottage cheese added with bromelain enzyme as a coagulant.

Variable	Bromelain enzyme concentration			
Unsaturated fatty acid profile	0%	3 %	4 %	5%
Myristoleic acid methyl ester	1.39	1.43	1.45	1.48
cis-10-pentadecenoate acid	0.20	0.22	0.25	0.27
Palmitoleate acid	1.29	1.34	1.36	1.38
cis-10-heptadecenoate	0.30	0.32	0.35	0.38
trans-9-elaidate acid	13.03	13.09	13.12	13.15
cis-9-oleate acid	2.38	2.44	2.46	2.48
Linolelaidate acid	0.25	0.33	0.35	0.37
Linoleate acid	0.32	0.35	0.38	0.40
gamma-linolenic acid methyl ester	0.15	0.17	0.18	0.20
cis-11-eicosenoate acid	<0.01	<0.01	<0.01	<0.01
Linolenate acid	0.10	0.47	0.50	0.54
11,14-eicosadienoate acid	<0.1	<0.1	<0.1	<0.1
cis -8,11, 14-eicosatrienoate acid	0.12	0.15	0.17	0.18
cis -11,14, 17-eicosatrienoate	<0.1	<0.1	<0.1	<0.1
Erucate acid	<0.1	<0.1	<0.1	<0.1
cis -5,8,11,14-eicosatetraenoate acid	0.10	0.13	0.16	0.18
cis-13, 16-docosapentaenoate acid	0.15	0.17	0.20	0.22
cis -5,8,11,14,17-eicosapentaenoate acid	0.1	0.1	0.1	0.1
Nervonate acid	0.10	0.12	0.18	0.18
cis-4,7,10,13,16, 19-docosahexaenoate acid	<0.1	<0.1	<0.1	<0.1

enhance the nutritional value and bioactivity of the cheese, but may also affect textural integrity and yield. In contrast, rennet-particularly chymosin-has a highly specific cleavage site in κ-casein, resulting in a firmer curd, better moisture retention and more consistent sensory attributes (Fox *et al*., 2017). However, rennet's animal origin poses limitations in terms of cost, availability and religious or ethical acceptability.

When compared to other plant-derived enzymes, such as papain (from papaya), ficin (from figs), or cardosins (from thistle), bromelain exhibits comparable or superior coagulation ability, but its higher proteolytic activity can lead to excessive proteolysis if not carefully controlled. Over-hydrolysis may result in bitterness or poor curd formation (Shah *et al*., 2014). Microbial coagulants, often produced from genetically modified strains of Rhizomucor miehei or Aspergillus niger, offer high yield and process stability, but may also vary in specificity and performance. Compared to these enzymes, bromelain stands out for its natural origin, environmental friendliness and potential cost-effectiveness, especially in tropical regions where pineapple is abundantly available. However, optimizing its concentration and activity remains critical to balance curd firmness, yield and nutritional quality, making it a viable but still underutilized coagulant in cheese technology.

Overall, these findings suggest that bromelain not only functions effectively as a milk coagulant but also contributes to a favorable modification of the lipid profile in cottage cheese. The increase in unsaturated fatty acids, particularly trans-9-elaidate, indicates an improvement in the nutritional quality of the product through enzymatic enhancement of lipid metabolism during cheese manufacture (Anggraini *et al*., 2013; Mahmood and Usman, 2010).

CONCLUSION

It can be concluded that the concentration of bromelain enzyme up to 5% produced a profile of amino acids, saturated fatty acids and unsaturated fatty acids contained in cottage cheese. The higher the concentration of bromelain enzyme, the higher the content of amino acids and unsaturated fatty acids, while saturated fatty acids were lower in cottage cheese. Meanwhile, the 4% bromelain enzyme produced the highest total cheese yield (curd). Bromelain enzyme from pineapple extract can be used as a cheap substitute for rennet in making cottage cheese.

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Disclaimers

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Conflict of interest

There was no conflict of interest associated with this research by any of the authors.

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