Chemical composition of goat cheese with different types of coagulants, types and doses of calcium compounds

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Abstract. This study investigates the effects of different types of coagulants, types and doses of calcium compound on goat cheese production. The coagulants used in this study were fig latex extract (*Ficus carica L.*) and commercial rennet, while calcium chloride ($CaCl_2$) and calcium carbonate ($CaCO_3$) with doses of 0,10 and 20mg/100ml of milk. The research aims to determine how these variables influence the chemical composition of goat cheese. The results revealed that the type of coagulant affected the water content, ash content, protein content and calcium content of goat cheese produced. Fig plant latex extract produced cheese with higher water, ash, and calcium content, but with lower protein content than commercial rennet. The type of calcium compound did not affect the chemical composition of the cheese. While the concentration of calcium compound only affected the calcium content of the cheese. In summary, this study showed that the type of coagulant affected the chemical composition of the cheese, the addition of calcium compound $CaCl_2$ and $CaCO_3$ did not affect the chemical composition of the cheese, except for the protein content. The doses of calcium compound addition generally do not affect the chemical composition of cheese, except for the calcium content.

1. Introduction

Cheese is a nutrient-rich dairy product that serves as an important source of human protein and minerals. The popularity of cheese has spread globally, becoming an integral part of various dishes and beverages [1].

The fundamental process of cheesemaking relies on the coagulation of milk, a step primarily facilitated using coagulants [2]. Traditionally, animal rennet has been the predominant coagulant used in cheese production. However, commercial rennet produced through fermentation has become the most widely used type of rennet. Rennet contains proteolytic enzymes, primarily chymosin, which facilitate the separation of milk into curd (solid) and whey (liquid), an essential step in the cheesemaking process [3].

However, the growing interest in plant-based coagulants has led to exploring alternative sources, especially in regions that emphasize sustainability, cultural preferences, and halal requirements [4]. In addition, proteases from various plants have been utilized in cheese production across different regions of the world, including enzymes such as papain, bromelain, ficin, oryzasin, cucumisin, sodom apple, and *Jacaratia corumbensis* [5].

Plant based proteases have potential as coagulants in cheese-making. Generally, cheeses produced using these enzymes tend to have higher yields, but they also often develop a bitter taste due to the high enzymatic activity that leads to the production of shorter peptides [4].

This bitterness is caused by peptides formed during proteolysis. A peptide's ability to induce bitterness depends on its amino acid composition, particularly the presence of hydrophobic residues. Amino acids such as proline, valine, leucine, phenylalanine, and tryptophan are among the most influential in enhancing bitterness intensity [6].

Several countries have developed traditional cheeses using plant-based coagulants like fig latex. These coagulants, rich in proteolytic enzymes such as ficin, possess milk-clotting properties like those of rennet, making them viable substitutes [7].

The use of fig latex (*Ficus carica L.*) is particularly appealing due to its abundance, natural origins, and enzymatic properties. Ficin, the primary enzyme in fig latex, is a cysteine protease like papain but offers advantages like better stability in acidic conditions and greater shelf life [4–7]. This makes fig latex a promising alternative for cheesemaking, particularly in producing goat cheese.

In addition, fig latex also can coagulate milk. The active component in fig latex responsible for protein coagulation is the enzyme ficin (EC 3.4.22.3). Ficin, a cysteine protease similar to papain from papaya latex, exhibits a comparable catalytic mechanism [7]. However, ficin demonstrates better stability than papain in acidic conditions and in the presence of alcohol. Ficin

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also has a longer shelf life and requires a lower dose for its application, making it more suitable for biotechnological applications than papain [8].

Ficin exhibits proteolytic activity within a pH range of 5.0 to 8.0 and a temperature range of 45-55°C [9]. The optimal proteolytic activity of ficin occurs at a pH of 6.0-6.5 and a temperature of 50°C [10]. In comparison, chymosin, a protease found in rennet, has optimal proteolytic activity within a pH range of 5.5-6.5 and a temperature range of 37-43°C [11].

In addition to coagulants, calcium compounds play a critical role in cheesemaking by enhancing the structural integrity and texture of the final product, while also improving the yield and efficiency of coagulation [12].

Calcium chloride is frequently used in cheese-making to support coagulation, improve the efficiency of the process, and optimize cheese yield. The standard addition ranges from 0 to 0.5 g/L [13]. A 0-0.2 g/L range is typically applied in cheese making [14].

However, different types and doses of calcium compounds can significantly affect the characteristics of the cheese, including its texture, moisture content, and firmness[15–16]. The interaction between calcium compounds and coagulants, especially when comparing plant-based and commercial coagulants, may lead to diverse outcomes in cheese quality.

In this research, calcium chloride ($CaCl_2$) and calcium carbonate ($CaCO_3$) were selected as the calcium compounds due to their distinct effects on cheese production. Calcium chloride is widely used in cheese making because it provides rapid and strong coagulation, attributed to its high concentration of calcium ions, which are immediately available. The addition of $CaCl_2$ enhances the calcium bridging between casein micelles, promoting cross-linking and fat entrapment within the curd, ultimately improving cheese yield [12]. On the other hand, calcium carbonate is an inorganic salt primarily used to manage and treat low calcium conditions. Under acidic conditions, $CaCO_3$ dissociates into ionized calcium (Ca^{2+}) and carbonate anions ($CO3^{2-}$), which bind to free protons (H^+), thus raising the pH. Its solubility is pH-dependent, with increased precipitation at higher pH levels. In an alkaline environment, $CaCO_3$ precipitates spontaneously, whereas acidic conditions prevent precipitation and encourage dissolution [17]. The selection of both $CaCl_2$ and $CaCO_3$ allows for a comparative investigation into their varying impacts on the physicochemical properties and overall quality of the cheese produced.

Therefore, understanding the effect of different coagulant types (commercial rennet and fig latex) and the variations in calcium compound types and doses on the chemical composition of goat cheese is essential.

2. Materials and methods

2.1 Research design

The research variables include the type of coagulant, consisting of commercial rennet and fig latex extract. The calcium compounds used are calcium chloride ($CaCl_2$) and calcium carbonate ($CaCO_3$). The doses of calcium compounds are 0, 10, and 20 mg/100 ml of milk.

2.2 Goat's milk quality analysis

Goat's milk used for producing goat cheese was sourced from a dairy goat farm in the Yogyakarta region, Indonesia. Prior to analysis and cheese production, the milk was filtered to remove any impurities or foreign particles. The quality of the fresh milk was evaluated through several tests, including pH, specific gravity, water content, ash content, protein content, fat content, and calcium content. A digital pH meter was used to measure pH [18], while specific gravity was determined with a lactodensimeter. Water, ash, and fat contents were analysed using proximate testing methods, protein content was determined via the Kjeldahl method [19], and calcium content was measured using an Atomic Absorption Spectrophotometer (AAS) [20].

Table 1. Chemical composition of goat milk for cheese making

Parameter	Goat Milk
Fat (%)	6.49 ± 0.006
Solid non-Fat (%)	9.12 ± 0.012
Protein (%)	4.34 ± 0.006
Lactose (%)	4.1 ± 0.006
Ash Content (%)	0.53 ± 0.012
Total Solid (%)	15.61 ± 0.017
Density	1.031 ± 0.006
pH	6.58 ± 0.025
Calcium (mg/100ml)	214.2 ± 0.102

2.3 Collection of fig plant latex

Fig (*Ficus carica L.*) latex samples were collected in the morning by making incisions in the stems and leaves of healthy plants to allow the latex to flow. The latex was then transferred to a clean glass bottle, taken to the laboratory, and stored in a refrigerator until the experiment began [21].

2.4 Crude enzyme preparation

Fig latex was homogenized with a phosphate buffer solution pH 7 with a ratio of 1 ml of fig latex and 9 ml phosphate buffer solution pH 7. The latex filtrate sample was centrifuged at a speed of 3,200 rpm for 15 minutes at 4°C. The supernatant derived from latex enzymes, commonly referred to as "crude enzyme" was employed for research purposes [21].

2.5 Commercial rennet

The rennet used in this study is a commercial product, CHYMAX Powder Extra NB from CHR HANSEN, composed of sodium chloride, chymosin, and casein peptone.

2.6 Cheese production

2.6.1 Cheese production using rennet coagulant

One liter of fresh goat milk was pasteurized and then cooled to approximately 39°C. The milk's pH was adjusted to around 5.9 by adding vinegar. Rennet was then added and thoroughly stirred. The mixture was incubated at 39°C for 45 minutes. After incubation, the curd was cut into smaller pieces and strained using a cheesecloth, resulting in goat cheese.

2.6.2 Cheese production using fig latex extract coagulant

One liter of fresh goat milk was pasteurized and then heated to approximately 50°C, following the protocol described in previous studies [22–23]. Fig latex extract was then added, and the mixture was incubated at 50°C for 120 minutes, as per previous research. After incubation, the mixture was strained using a cheesecloth, producing the final goat cheese product.

2.7 Goat cheese analysis

Protein content was tested using the Kjeldahl method, and water and ash content were tested using proximate analysis [19]. Calcium content testing was carried out on goat cheese using Atomic Absorption Spectroscopy (AAS) with several stages, starting from sample preparation, making a standard calcium solution, and testing the sample on AAS [20].

2.8. Data analysis

The chemical composition data of goat cheese were analysed using SPSS software version 16. ANOVA (Analysis of Variance) was employed to compare the means of each treatment. If significant differences were found, the analysis was followed by a post hoc test using Duncan's test to identify treatments with significant differences.

3. Result and discussion

The chemical composition of cheese is shaped by numerous factors, including the type of milk used, processing techniques, choice of coagulant, addition of bacterial starter cultures, ripening conditions, and environmental influences. In this study, key chemical components that impact the characteristics and quality of the cheese, such as water content, ash content, protein content, and calcium content, were analysed.

3.1 Water content of goat cheese

The water content of cheese is a critical parameter that directly affects its texture, shelf life, and overall quality. In this study, the water content of goat cheese was evaluated under different types of coagulants, types and doses of calcium compound.

Table 2. Water content of goat cheese (%) with different types of coagulants, types and doses of calcium compound

Types of coagulants	Types of calcium compound	Doses of calcium compound (mg/100ml)	Water content (%)
Rennet	$CaCl_2$	0	45.16 ± 1.50 [a]
		10	44.22 ± 1.51 [a]
		20	44.72 ± 3.41 [a]
	$CaCO_3$	0	44.23 ± 2.30 [a]
		10	49.88 ± 3.80 [a]
		20	44.04 ± 3.13 [a]
Fig plant latex	$CaCl_2$	0	52.84 ± 0.13 [b]
		10	52.82 ± 0.16 [b]
		20	52.92 ± 0.59 [b]
	$CaCO_3$	0	52.83 ± 0.13 [b]
		10	52.31 ± 0.63 [b]
		20	52.56 ± 0.70 [b]

Mean ± standard deviation; [a-b] Means with different letters in the same row indicate statistically significant differences at $p < 0.05$.

Table 2 illustrates that different types of coagulants significantly affect the water content in goat cheese. Cheese made with fig latex extract had a higher water content than rennet. The choice of coagulant plays a crucial role in cheese production by coagulating the milk and forming curd, which is the initial stage of cheese-making. Whether using animal rennet, plant-based coagulants, or modified enzymes, the type of coagulant impacts the curd's composition and characteristics, subsequently influencing the water content of the final cheese product [24]. Therefore, selecting the appropriate coagulant is essential for achieving the desired quality and properties of the cheese.

Commercial rennet, typically derived from animal sources or other enzymes, is known for producing firmer and denser curds. This denser curd has a tighter structure that expels more whey, leading to lower water content in the cheese [25–26]. Reduced water content not only influences the cheese's texture but also enhances its strength and firmness, making it easier to cut and process [25]. The extent and rate of whey expulsion during curd stirring are affected by various factors such as curd shrinkage and pore structure, which ultimately determine the final water content of the cheese [27]. Additionally, the choice of specific enzymes can significantly impact curd properties including coagulation time, curd viscosity, and texture, underscoring the importance of enzyme selection in cheese production [26].

Conversely, natural coagulants like fig latex produce a softer and looser curd. This type of curd retains more water due to its less compact and more porous structure, resulting in cheese with a softer and wetter texture. While this texture may be preferable for certain cheese varieties, it can also decrease the cheese's durability and shelf life, as higher water content increases the risk of microbial growth [28–29].

Water content in cheese is a critical factor for producers. Lower water content generally enhances cheese texture, durability, and shelf life, and reduces the risk of microbiological spoilage [29–30]. Cheeses with reduced water content are more resistant to spoilage and have a longer storage life, which is beneficial in the food industry. Moreover, consumers often prefer denser and drier cheeses for a more satisfying eating experience [30].

3.2 Ash content of goat cheese

Ash content is a key indicator of cheese quality. It refers to the mineral residue remaining after all organic matter in the cheese has been completely burned away [31]. The table 3 presents the average ash content observed in goat cheese based on test results.

Table 3. Ash content of goat cheese (%) with different types of coagulants, types and doses of calcium compound

Types of coagulants	Types of calcium compound	Doses of calcium compound (mg/100ml)	Ash content (%)
Rennet	$CaCl_2$	0	2.23 ± 0.18 [a]
		10	2.25 ± 0.17 [a]
		20	2.25 ± 0.26 [a]
	$CaCO_3$	0	2.26 ± 0.14 [a]
		10	2.07 ± 0.23 [a]
		20	2.35 ± 0.04 [a]
Fig plant latex	$CaCl_2$	0	2.67 ± 0.00 [b]
		10	2.69 ± 0.06 [b]
		20	2.68 ± 0.04 [b]
	$CaCO_3$	0	2.66 ± 0.07 [b]
		10	2.77 ± 0.02 [b]
		20	2.75 ± 0.02 [b]

Mean ± standard deviation; [a-b] Means with different letters in the same row indicate statistically significant differences at $p < 0.05$.

Table 3 demonstrated that different types of coagulants significantly affect the ash content of goat cheese. Specifically, cheese made with fig latex extract had a higher ash content compared to cheese produced with rennet. Ash content represents the total mineral content, which can originate from various sources including the milk, the coagulant, and any added calcium compounds [32].

A higher ash content signifies a greater concentration of minerals, which can enhance the nutritional value of the cheese, as minerals are essential in the human diet [33]. It is important to note, however, that elevated mineral levels do not adversely affect the taste or texture of the cheese [34].

3.3 Protein content of goat cheese

The protein content in goat cheese is a crucial factor in assessing both its nutritional value and overall quality. Protein is a vital macronutrient necessary for human growth and development, contributing to muscle formation, metabolic regulation, and immune function [35]. Table 4 presents the results of protein content testing in goat cheese made with various coagulants, types, and doses of calcium compounds.

Table 4. Protein content of goat cheese (%) with different types of coagulants, types and doses of calcium compound

Types of coagulants	Types of calcium compound	Doses of calcium compound (mg/100ml)	Protein content (%)
Rennet	$CaCl_2$	0	20.21 ± 0.28 [bA]
		10	21.93 ± 0.51 [bB]
		20	21.59 ± 1.00 [bB]
		0	20.58 ± 0.56 [bA]

	$CaCO_3$	10	21.19 ± 1.53 [bB]
		20	20.84 ± 0.63 [bB]
Fig plant latex	$CaCl_2$	0	17.97 ± 0.05 [aA]
		10	18.16 ± 0.11 [aB]
		20	18.42 ± 0.22 [aB]
	$CaCO_3$	0	17.72 ± 0.10 [aA]
		10	18.24 ± 0.12 [aB]
		20	18.42 ± 0.28 [aB]

Mean ± standard deviation; [a-b] Means with different letters in the same row indicate statistically significant differences at $p < 0.05$ in types of coagulants and [A-B] Means with different letters in the same row indicate statistically significant differences at $p < 0.05$ in calcium compounds.

Table 4 indicates that different types of coagulants and types of calcium compounds significantly affect the protein content of goat cheese, with cheese made using rennet having higher protein levels compared to cheese made with fig latex extract. The type of coagulant plays a critical role in determining the final protein content of the cheese. Commercial rennet, commonly used in the cheese industry, is more effective at coagulating casein, the primary milk protein, compared to fig latex. This greater coagulation efficiency results in a denser curd with higher protein content [26].

Cheese with higher protein content offers greater nutritional value, as protein is essential for human health, including muscle development and metabolic function. Consequently, cheeses with higher protein levels are often preferred by health-conscious consumers [36].

When selecting a coagulant, producers should consider its coagulation efficiency and the desired characteristics of the final cheese product. For example, if a cheese with a dense texture and high protein content is desired, commercial rennet may be the better choice. Conversely, if a cheese with a softer texture and lower protein content is preferred, fig latex might be more suitable [37–38].

3.4 Calcium content of goat cheese

The calcium content in goat cheese is one of the parameters that affects the quality and texture of cheese. As one of the important sources of calcium in the human diet, the presence of calcium in goat cheese not only plays a role in maintaining healthy bones and teeth, but also in cardiovascular and nerve function [15].

Based on the results of the study, the calcium content of cheese with different types of coagulants, types and doses of calcium compound can be seen in the table 5.

Table 5. Calcium content of goat cheese (mg/100g) with different types of coagulants, types and doses of calcium compound

Types of coagulants	Types of calcium compound	Doses of calcium compound (mg/100ml)	Calcium content (mg/100g)
Rennet	$CaCl_2$	0	797.32 ± 1.69 [b]
		10	772.12 ± 5.57 [a]
		20	821.12 ± 6.56 [de]
	$CaCO_3$	0	830.11 ± 8.91 [ef]
		10	813.48 ± 11.68 [bcde]
		20	802.08 ± 4.96 [bc]
Fig plant latex	$CaCl_2$	0	817.35 ± 6.84 [cde]
		10	838.40 ± 2.96 [f]
		20	903.10 ± 4.11 [g]
	$CaCO_3$	0	819.78 ± 6.11 [de]
		10	806.45 ± 6.15 [bcd]
		20	902.33 ± 4.49 [g]

Mean ± standard deviation; [a-f] Means with different letters in the same row indicate statistically significant differences at $p < 0.05$.

Table 5 revealed that difference in coagulant types and calcium compound doses significantly influenced the calcium content in goat cheese. Cheese made with fig latex extract resulted in the highest calcium content.

These results suggest that the interaction between the type of coagulant, the type of calcium compound, and the doses added significantly influences the calcium content in goat cheese. The significant differences observed among the differences coagulant types indicate that the coagulation process is notably affected by the selection of coagulant as well as the type and dosage of the calcium compound employed. This finding aligns with previous research indicating that the type of coagulant can affect the physical and chemical properties of cheese [37–38]. The use of calcium carbonate and calcium chloride in the production of fresh acid rennet cheese from goat's milk, at a dosage of 20 mg/100 ml of milk, significantly increased the calcium content of the cheese [12].

Based on the discussion above, the chemical composition of goat cheese particularly in terms of water, ash, and protein content does not significantly affect by the addition of calcium compounds ($CaCl_2$ and $CaCO_3$) and varying doses of these compounds (0, 10, and 20 mg/100 ml). Instead, it is influenced by the type of coagulant used. Furthermore, the calcium content of the goat cheese is affected by the interaction between the type of coagulant, the type and doses of calcium compound applied, with the highest calcium content observed in goat cheese made using fig plant latex in combination with either $CaCl_2$ or $CaCO_3$ at a dosage of 20 mg/100 ml. This finding aligns with a study by [12], which demonstrated that using calcium carbonate and chloride in the production of fresh acid rennet cheese from goat's milk, with varying doses of calcium compounds, did not affect the moisture and protein retention. However, both $CaCl_2$ and $CaCO_3$ with dose 20mg/100ml of milk significantly increased the calcium content of the cheese.

In cheese production using rennet, chymosin cleaves the peptide bond of kappa-casein at the 105 (phenylalanine)-106 (methionine) amino acid sequence, forming para-kappa-casein and macro-glycopeptides [3]. Meanwhile, ficin from fig latex belongs to the cysteine protease group and consists of 210 amino acid (AA) residues. The active site of this enzyme comprises two AAs: Cys (Cys-25) and His (His-159). Protease from *Ficus carica L.* exhibits broad specificity toward basic and neutral amino acids such as Gly, Val, Leu, Ala, Ser, Asn, Arg, and His. Ficin cleaves proteins at the Tyr, Phe, and Val bonds [7]. Therefore, the difference in amino acid cleavage processes on kappa-casein by different coagulants results in varying chemical compositions. Although the use of different calcium compounds in this study generally does not affect the chemical composition of the cheese, due to their distinct properties, differences might occur in terms of texture and possibly organoleptic properties. As explained by [12], $CaCl_2$ and $CaCO_3$ can have different effects on the physicochemical properties and quality of the cheese, such as coagulation, fat entrapment, and pH adjustment.

In other research, the type and dosage of calcium compounds, particularly calcium chloride and calcium carbonate, are essential in influencing curd formation and improving the texture of cheese. Adding 20 mg/100 g of calcium carbonate effectively reduced syneresis and significantly increased the hardness of goat milk acid rennet gels, a key intermediate product in cheese-making. Calcium chloride also enhanced curd firmness and elasticity. Moreover, calcium compounds, especially calcium gluconate, helped to moderate the strong goat flavor, making the cheese more palatable. Thus, selecting the right calcium compound and dosage is critical for optimizing curd formation, texture, and overall cheese quality [15].

4. Conclusion

In conclusion, the type of coagulant affects the chemical composition of goat cheese, while the type of calcium compound ($CaCl_2$ and $CaCO_3$) and the doses of calcium compounds added (0, 10, and 20 mg/100 ml) generally do not affect the chemical composition of goat cheese, especially regarding water, ash, and protein content, except calcium compound affect for the protein content. Furthermore, the calcium content of the goat cheese is influenced by the interaction between the type of coagulant, the type and doses of calcium compound, with the highest calcium content observed in goat cheese made using fig plant latex in combination with either $CaCl_2$ or $CaCO_3$ at a dosage of 20 mg/100 ml.

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