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Optimization of Incubation Time and Coagulant Concentration of *Ficus carica L.* Latex for Fresh Goat Cheese Production

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ABSTRACT

Cheese coagulants from plant origin have emerged as promising alternatives to animal rennet in cheese production. Among them, latex from Ficus carica L. has shown potential as a natural milk-clotting agent for goat cheese production. This study aims to determine the optimal incubation time and fig tree latex extract concentration on the quality of goat cheese. The optimization of the production process of the goat cheese was carried out using Response Surface Methodology (RSM) with Central Composite Design (CCD). The independent variables were the concentration of the *Ficus carica L.* latex extract (0.2-0.5 mL/100 mL of milk) and the time of incubation (60-120 min). The goal was to achieve maximum protein content (%), ash content (%), and calcium content (%). The results of this study showed that the *Ficus carica L.* latex extract used in the study could coagulate milk with a strength of 354.69 MCU and a dominant protein molecular weight of about 20 kDa. The optimization results using RSM method with CCD design showed that the goat cheese was produced optimally in 0.5 mL/100 mL of milk for the concentration of *Ficus carica L.* latex extract, with 120 min incubation time. These optimal conditions resulted in a cheese with protein content of 18.56%, ash content of 2.32%, calcium content of 803.93 mg/100g, and desirability level of 0.93.

Keywords: *Central Composite Design, Cheese Coagulant, Ficus carica L., Goat cheese, Response Surface Methodology*

Introduction

Milk coagulation is the basis of the cheese-making process, and the selection of coagulants plays a crucial role in determining the quality of cheese. Animal rennet, predominantly obtained from the abomasum of calves or other mammalian origin, is the most prevalent milk coagulant. This proteolytic enzyme-rich enzyme, namely chymosin, facilitates the curdling of milk into whey (liquid) and curd (solid), subsequently utilized in cheese production (Uniacke-Lowe and Fox, 2017).

At the same time, plant coagulants are found to be an appealing and more feasible alternative. Certain countries have embraced traditional cheese-making methods incorporating plant-derived coagulants like papaya latex or *Ficus carica L.* latex. These alternatives house proteolytic enzymes, such as ficin in the *Ficus carica L.*, capable of coagulating milk and enabling cheese production. The adoption of plant-based coagulants is driven by sustainability considerations and adherence to religious practices, offering a viable alternative to animal rennet.

The selection of coagulants plays a crucial role in determining product quality, ethical considerations, and consumer preferences in cheese production. Animal coagulants like calf rennet are reliable and produce high-quality cheese (Liu *et al*., 2021), while plant coagulants offer ethical and dietary advantages, making them suitable for vegetarian and certain religious dietary requirements (Priyashantha *et al*., 2023a). However, plant-based options often face challenges related to consistency, sensory impact, and scalability (Mohsin *et al*., 2024). The choice between plant and animal coagulants ultimately depends on the specific needs and constraints of the cheese production process, including desired texture, flavor profile, production scale, and target consumer market.

The incorporation of coagulants from plant/vegetable sources has garnered significant interest, particularly in the realm of food applications. Consumer resistance to proteases from animal sources, owing to disease transmission concerns, has fueled this exploration. Meanwhile, recombinant proteases face restrictions in certain countries, limiting their use in human food (Morellon-Sterling *et al*., 2020).

Considerable research has been directed towards identifying milk-curdling enzymes as rennet substitutes. Genetically engineered bacteria have shown promise in this regard, offering suitable alternatives. Simultaneously, the focus has shifted to natural proteases from plants, considering factors such as religious and cultural preferences, benefits, and ease of accessibility (Mahajan and Badgujar, 2010).

Several studies have reported that proteases derived from various plants have been used in cheese production in various regions of the world, such as papain from papaya, bromelain from pineapple, ficin from fig plants, oryzacin, cucumisin from melon, sodom apple, and Jacaratia corumbensis (Mahajan and Badgujar, 2010; Mohsin *et al*., 2024). This plant protease has significant potential as a coagulant agent in cheese making. Typically, cheese produced with plant proteases tends to produce a higher cheese yield, but often produces a bitter taste. This bitter taste is mainly due to high enzymatic activity, which causes the formation of short-chain peptides during protein breakdown (Nicosia *et al*., 2022).

For centuries, the fig plant has been used as a human diet, and in some regions, *Ficus carica L.* latex contains ficin, which can coagulate milk in cheese production (Lazreg-Aref *et al*., 2018). Fig plant latex extract has become a significant ingredient in traditional cheese production, includes cow's milk and small ruminant dairy cattle, particularly goat and sheep milk (Nouani *et al*., 2009).

Fig latex exhibits strong proteolytic activity, which is important for the milk coagulation process in cheese-making. This activity is comparable to traditional rennet, although it may vary depending on many factors, such as the concentration and form of the extract used (Lomolino *et al*., 2015; Khan *et al*., 2023). Fig latex is a plant-based coagulant, making it suitable for vegetarians and those with religious dietary restrictions (Mozzon *et al*., 2020). It is an economical and sustainable alternative to animal rennet, reducing dependency on animal-derived products (Khan *et al*., 2024). This is particularly important as traditional rennet may not always meet halal standards due to its animal origin (Priyashantha *et al*., 2023b).

Derived from the fruit latex or leaf stems of the fig plant (*Ficus carica L.*), the proteolytic enzyme of ficin EC 3.4.22.3 is renowned for its sulfhydryl nature, containing crucial cysteine residues for activity (Baeyens-Volant *et al*., 2015). As a part of cysteinase isolated from *Ficus carica L.* latex, ficin possesses a distinct polypeptide chain, having a molecular weight of approximately 23.1 kDa, positioning it as a valuable milk coagulant in cheese-making and a key player in protein hydrolysis (Morellon-Sterling *et al*., 2020). *Ficus carica L.*, known for its superior milk coagulation activity, higher cheese yield, and reduced syneresis capacity compared to rennet, shows potential as a cheese coagulant from plant origin (Abraha *et al*., 2018). However, information about the optimal concentration and incubation time using fig tree latex in cheese-making remains elusive. This study aimed to determine the optimal incubation time and fig tree latex extract concentration on goat cheese.

Materials and Methods

Goat's Milk Quality Test

The source of goat milk for this research was Kacang goat breed, which was collected from a dairy goat farm in the Yogyakarta region, Indonesia. The milk underwent filtration to eliminate any dirt and foreign particles before analysis and cheese production. Tests conducted to assess the quality of fresh milk encompass a pH test, specific gravity test, water content test, ash content test, protein content test, fat content test, and calcium content test. The pH test utilizes a digital pH meter (Murti *et al*., 2021) and specific gravity is determined using a lactodensimeter. Water content, ash content, and fat content are evaluated through proximate testing methods, while the protein content is analyzed using the Kjeldahl method (AOAC, 2005), and calcium content is measured using an Atomic Absorption Spectrophotometer or AAS (Nielsen, 2010).

Fig Plant Latex Collection

Fig tree latex was collected in the morning by cutting the stems and leaves of healthy plants until the latex flowed out. Then, the latex was transferred into a clean glass bottle, taken to the laboratory, and cooled in the refrigerator until the experiment began (Mazri *et al*., 2018).

Crude Enzyme Preparation

Stock solution of the enzyme was obtained by homogenizing phosphate buffer solution with pH 7.0 at 1:9 (v/v) ratio and *Ficus carica L.* latex. It was 0.2 mL crude latex in each mL of solution, serving as the crude enzyme stock concentration. The latex filtrate sample was centrifuged at a speed of 3.200 rpm for 15 min at a temperature of 4°C. After centrifugation, the supernatant (crude enzyme) was employed for this research (Mazri *et al*., 2018). The SDS-PAGE test was carried out on the protein profile of fig tree latex (Nielsen, 2010).

Goat Cheese Production

In every experimental iteration, 100 mL of fresh goat's milk was heated until it reached a temperature of approximately ±50°C (Rana *et al*., 2017; Abraha *et al*., 2018). Subsequently, the fig plant latex extract was added according to the research treatment. The mixture was then incubated at a temperature of ±50°C, aligning with the designated durations prescribed by the treatment plan. Following the incubation period, the resultant mix underwent filtration using a cheese filter cloth, ultimately yielding the final of the goat cheese. The results of goat cheese in this research are shown in Figure 1.

Figure 1. Goat cheese production

Milk Clotting Activity (MCA)

The fig tree latex crude enzyme source, totaling 0.2 mL, was added to 2 mL of the substrate solution, containing 12% skimmed milk powder in 0.01M CaCl2 (12 g skimmed milk powder in 100 mL distilled water solution + 0.15 g $CaCl_2$). Then the time required for the formation of curd fragments was recorded. One (1) MCA unit indicates the volume of milk that can be coagulated by 1 mL of crude enzyme within 40 min (2.400 s). Milk clotting activity is measured by entering the formula below (Mazri *et al*., 2018)

$$\boldsymbol{MCA} = \frac{(\mathbf{2400\ x\ V})}{(\mathbf{t\ x\ v})}$$

MCA	=	Milk Clotting Activity
2400	=	Time conversion factor (s)
V	=	Volume of skim milk (mL)
t	=	coagulating time (s)
v	=	crude enzyme extract volume (mL)

Goat Cheese Analysis

The protein content in goat cheese was determined using the Kjeldahl method, while the ash content was analyzed through proximate analysis (AOAC, 2005). The calcium content was measured using Atomic Absorption Spectroscopy (AAS), which involved several steps, including sample preparation through the destruction method, preparation of a standard calcium solution, and analysis of the sample using AAS (Nielsen, 2010).

Experimental Design

The experimental design in this research is Response Surface Methodology (RSM) with Central Composite Design (CCD). RSM serves as a statistical technique used for experiment design, data analysis, and comprehending the correlation between independent variables and response variables. CCD is one of the experimental designs in RSM that is most commonly used to optimize the response of a system with two or more independent variables. This method begins by designing an experiment using a CCD design. Next, identify the factor variables (independent variables) that will be considered in the experiment, and the CCD model/design will be obtained (Njoku and Otisi, 2022; El-taweel *et al*., 2023).

The experiment was carried out using the CCD design, which was created to obtain a response from the system or process for each combination of factor values. Response analysis involves processing experimental data and calculating response values (response variables) for each combination of factor values. After the mathematical model is obtained, identify the most appropriate mathematical model based on the results of the response analysis. The final stage is the optimization stage, and the optimum formula will be obtained from the variables and responses from the treatment results (Montgomery, 2001).

The variables used refer to (Rana *et al*., 2017; Abraha *et al*., 2018) which were modified, namely the concentration of fig plant latex extract (2-5 mL of 1 mL of fig tree latex dissolved in 9 mL of phosphate buffer pH 7) and incubation time (60-120 min). The desired cheese yield response is maximum protein content (%), ash content (%), and calcium content (%). Based on the information described above, the coded levels of the experimental factors were established as presented in Table 1.

Table 1. The coded and uncoded values used in RSM method with CCD design

Point (code)	Independent variable	
	A	B
Minimum axial point (-α)	0.14	47.58
Minimal factorial point (-1)	0.2	60
Center point (0)	0.35	90
Maximum factorial point (1)	0.5	120
Maximum axial point (α)	0.56	132.42

A= Fig tree latex extract concentration (mL/100mL milk) and B= incubation time (min)

Determining the level of each independent variable in Table 1 is explained below.

1. **Determination of Factorial Points**

In this study, a factorial design was employed involving two independent variables, each tested at two levels. The levels of these variables were defined as follows:

a. **Variable A – Fig tree latex extract concentration (mL/100 mL milk)**
   Low level (-1) is 0.2, and High level (+1) is 0.5
b. **Variable B – Incubation time (min)**
   Low level (-1) is 60, and High level (+1) is 120

2. **Determination of Center Points**

For **Variable A (Concentration)**, the center point was calculated as the midpoint between the low and high levels (0.2 and 0.5 mL/100 mL), resulting in a center point of 0.35 mL/100 mL (coded as 0). The interval between levels is 0.15 mL/100 mL.
For **Variable B (Incubation Time)**, the center point was the midpoint between 60 and 120 min, which is 90 min (coded as 0), with a level interval of 30 min.

3. **Determination of Axial Points**

Rotatability criterion is used to determine axial points for each variable based on the Central Composite Design (CCD), using the formula $\alpha = (2^n)^{1/4}$, where *n* is the number of independent variables. In this experiment, there are two independent factors, α is calculated as $(2^2)^{1/4} = \sqrt{2} = \pm 1.4$. Rotatability in Response Surface Methodology (RSM) ensures that the experimental design provides equal precision of estimation in all directions from the center point. A rotatable design improves statistical analysis and helps in accurately mapping the response surface (Njoku and Otisi, 2022).

The axial point for each variable was calculated using the following general equation

$$\text{Axial Point} = (\text{Interval level} \times \alpha) + \text{Center Point}$$

Variable A (Concentration)

$$A = (0.15 \times \alpha) + 0.35$$

When α = -1.414:
A = (0.15 × -1.414) + 0.35 = 0.14 mL/100 mL milk

When α = +1.414:
A = (0.15 × 1.414) + 0.35 = 0.56 mL/100 mL milk

Variable B (Incubation Time)

$$B = (30 \times \alpha) + 90$$

When α = -1.414:
B = (30 × -1.414) + 90 = 47.58 min
When α = +1.414:
B = (30 × 1.414) + 90 = 132.42 min
Factor levels and treatment codes used in the study are presented in Table 2.

Table 2. Factor levels and treatment codes of RSM method with CCD design

Run	Type	Variable Code		Variable Value	
		A	B	A	B
1	Axial	0	1.41	0.35	132.42
2	Factorial	1	1	0.5	120
3	Center	0	0	0.35	90
4	Axial	1.41	0	0.56	90
5	Center	0	0	0.35	90
6	Axial	-1.41	0	0.14	90
7	Center	0	0	0.35	90
8	Factorial	1	-1	0.5	60
9	Center	0	0	0.35	90
10	Center	0	0	0.35	90
11	Axial	0	-1.41	0.35	47.58
12	Factorial	-1	-1	0.2	60
13	Factorial	-1	1	0.2	120

A= Fig tree latex extract concentration (mL/100mL milk) and B= incubation time (min)

The Design-Expert 13.0.5.0 application was used to obtain the CCD model and analyze the RSM data. The RSM method with CCD design is used for two (2) independent variables with 13 treatment runs consisting of five (5) center points, four (4) factorial points, and four (4) axial points. The independent variables of this research are the concentration of fig plant latex extract and the incubation time. The cheese response variables observed in this study included cheese protein content, ash content, and calcium content.

Results and Discussion

Goat's Milk Quality

The Kacang goat is one of the indigenous goat breeds in Indonesia. Kacang goats are known as a local breed that is spread across various regions in Indonesia. This goat has a small to medium body posture. Kacang goats have good adaptation to tropical environments and limited food conditions. These goats have various fur colors, such as black, brown, white, or a combination of these colors. Kacang goats are often used in people's livestock systems in Indonesia, especially for meat consumption purposes (Pamungkas *et al.*, 2008). However, there has not been much research on the potential of Kacang Goats as milk-producing livestock and the use of Kacang Goat milk as a dairy product. Kacang goat milk exhibits distinct physicochemical and nutritional characteristics when compared to milk from other goat breeds. In a comparative study involving five goat breeds—Saanen, Kacang, Jamnapari, Boer, and a Jamnapari-Saanen crossbreed—Kacang goat milk was found to have the lowest titratable acidity (0.06%), indicating a milder taste profile. While Jamnapari goat milk demonstrated the highest antioxidant capacity, Kacang goat milk still presented a significant antioxidant presence, surpassing that of cow milk. In addition, the protein content and ash content are similar to milk from other goats (Alyaqoubi *et al.*, 2015). Analysis of the Kacang Goat milk chemical composition used in this study is presented in Table 3.

Table 3. Chemical composition of Kacang goat milk

Parameter	Goat Milk
Total Solid (%)	15.6 ± 0.01
pH	6.6 ± 0.02
Density	1.03 ± 0.01
Protein (%)	4.3 ± 0.01
Fat (%)	6.5 ± 0.01
Solid non-Fat (%)	9.1 ± 0.01
Ash Content (%)	0.5 ± 0.01
Calcium (mg/100mL)	214.2 ± 0.1

Goat milk composition/quality is affected by numerous factors, including the overall diet, health, goat's breed, and stage of lactation. Hygiene practices during milking and storage also play a crucial role in maintaining milk composition (Annes Pereira *et al.*, 2025).

Typically, the general standards for good-quality fresh goat's milk encompass characteristics like its normal state, cleanliness, and a white or cream hue. Milk has a natural taste and is free from foreign ingredients and adulteration. When milk is checked with an alcohol test to observe the results of an alcohol test with ethyl alcohol, the precipitate should be fine or small. The pH value should be in the range of 6.5-6.8. Non-fat solids should not be less than 8.25%. Maximum freezing point –0.530°C. The minimum specific gravity is 1.028 at a temperature of 20°C (TAS, 2008).

Fresh goat milk quality test is based on the protein, fat, and non-fat solids content. Kacang Goat milk used in this study, when compared with the Thai Goat Milk Standard, is included in the premium milk category with standard requirements for protein content > 3.7%, fat content > 4%, and total solids > 13% (TAS, 2008). The calcium level contained in Kacang Goat Milk reaches 214.2 mg/100mL, indicating a higher calcium level compared to other goat milk, which has levels of around 134 mg/100gr (Park, 2010) and 183.57 mg/100gr (Pawlos *et al.*, 2020). The calcium content of goat's milk varies, influenced by many things such as genetic factors, type of animal, breed of animal, feed, age of the animal, stage of lactation, and environmental factors (Gaignon *et al.*, 2018; Nugraha, 2021). So the Kacang Goat milk used in the research is of good quality.

Optimization of Fresh Goat Cheese Production

Milk Clotting Activity (MCA) serves as a vital indicator in assessing the capability of fig plant latex extract to coagulate milk. In this research, MCA from fig plant latex extract was successfully measured, achieving a result of 354.69 Milk Clotting Units (MCU) and a clotting time of 68 s. Another study evaluated Dauphine and Marseillaise ficin forms from *Ficus carica L* which has milk clotting activity 266.7 MCU. Both types has identical milk clotting time, at 90 s (Daffri *et al.*, 2020). These results illustrate the extent to which fig tree latex extract can coagulate milk, which is an essential characteristic in the process of cheese making. One (1) MCA unit indicates the volume of milk that can be coagulated by one (1) mL of crude enzyme in 40 min/2400 s (Sukmana *et al.*, 2020). Milk clotting activity can be seen in Figure 2.

Figure 2. Milk Clotting Activity (MCA) of *Ficus carica L.* Latex

Milk Clotting Activity (MCA) plays a crucial role in optimizing both incubation time and coagulant concentration when using *Ficus carica L.* (fig) latex as a plant-based coagulant in fresh goat cheese production. As an indicator of the enzyme's ability to hydrolyze κ-casein and initiate curd formation, MCA directly influences overall quality of the cheese. High MCA enables shorter incubation periods and efficient curd development. Therefore, evaluating MCA is essential to determine the precise enzyme dosage and incubation conditions, especially when working with plant-derived enzymes known for their variable activity.

The results of the fig tree latex SDS-PAGE study indicate that the *Ficus carica L.* latex extract is dominated by proteins with a molecular weight of about 20 kDa (Figure 3). This protein profile provides a deeper understanding of the protein composition in fig tree latex extract, which may contribute to the previously observed milk-clotting activity. The protease enzyme in the latex of *Ficus carica L.* is known as ficin (Ramadan, 2023). Numerous investigations have been conducted to purify and biochemically analyze ficin. Ficin represents a distinct polypeptide chain with a molecular weight of 23.1 kDa (Morellon-Sterling *et al.*, 2020). It serves as a viable milk coagulant in cheese production and additionally contributes to protein hydrolysis processes (Siar *et al.*, 2020).

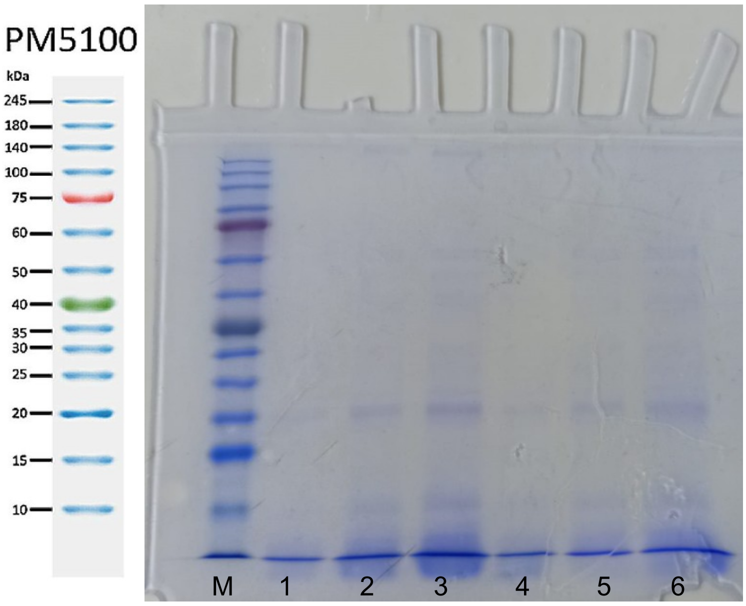

Figure 3. Fig plant latex protein profile
(M, 1. TC 5 µL, 2. TC 10 µL, 3. TC 20 µL, 4. C 5 µL, 5. C 10 µL, and 6. C 20 µL (M= Marker, TC = Without Centrifuge and C = with Centrifuge))

The results of the goat cheese composition using RSM method with CCD design are presented in Table 4. The results show that goat cheese has a protein content, ash content, and calcium content, respectively, ranging from 8.21-17.74%, 1.11-2.27%, and 665.73-818.37 mg/100g.

Table 4. Goat Cheese Response using RSM method with CCD design

Run	Type	Variable Code		Variable Value		Response		
		A	B	A	B	R-1	R-2	R-3
1	Axial	0	1.41	0.35	132.42	17.34	2.00	795.34
2	Factorial	1	1	0.5	120	17.33	2.00	788.19
3	Center	0	0	0.35	90	13.27	1.56	737.13
4	Axial	1.41	0	0.56	90	17.74	2.27	818.37
5	Center	0	0	0.35	90	12.64	1.48	714.59
6	Axial	-1.41	0	0.14	90	8.21	1.04	665.73
7	Center	0	0	0.35	90	14.10	1.24	745.37
8	Factorial	1	-1	0.5	60	14.03	1.62	719.88
9	Center	0	0	0.35	90	14.48	1.68	726.07
10	Center	0	0	0.35	90	15.14	1.74	736.30
11	Axial	0	-1.41	0.35	47.58	8.50	1.11	697.97
12	Factorial	-1	-1	0.2	60	9.72	1.19	685.18
13	Factorial	-1	1	0.2	120	11.84	1.39	711.64

A= Fig tree latex extract concentration (mL/100mL milk); B= incubation time (min); R-1 = Protein content response; R-2 = Ash content response; and R-3 = Calcium content response

The results of this cheese were almost the same as research on Cacioricotta cheese, which both used goat's milk and produced cheese with a protein content of 19.3% (Faccia ***et al***., 2019), in another study, Colaho cheese had a protein content of 22.48%, ash content 3.59% and calcium content 932 mg/100g (Messias ***et al***., 2022).

Many factors affected the cheese quality/characteristics. The production process involves several factors, including the raw milk material (source, composition, and quality), manufacturing process, coagulant type, the addition of calcium salts, whether or not bacterial starter is added, ripening, and other factors. This will not only affect the physical and chemical quality but will also affect the flavor of the cheese produced (Hargrove ***et al***., 1967; McSweeney ***et al***., 2004; McSweeney ***et al***., 2017; Uniacke-Lowe and Fox, 2017).

The lowest cheese protein content value was 8.206% which was obtained by a combination of treatment with fig tree latex extract concentration 0.14 mL/100mL milk and incubation time of 90 min. The highest protein content value of goat cheese was 17.739% which was obtained with a combination treatment of fig tree latex extract concentration 0.56 mL/100mL milk and incubation time 90 min. Figure 4 shows a 3D surface plot of goat cheese protein content.

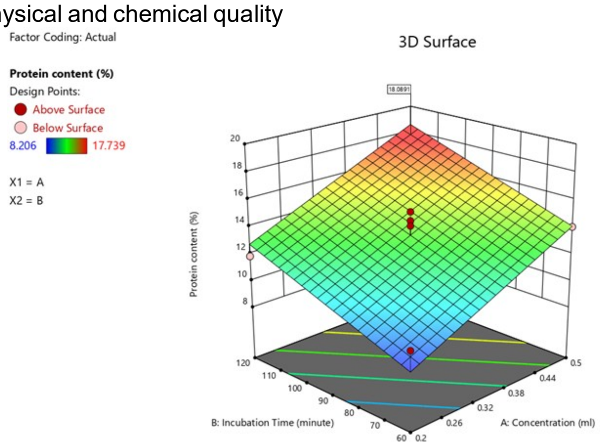

Figure 4. Goat cheese protein content 3D surface plot

Protein in cheese is a key component of cheese products. This protein plays a crucial role in shaping the texture, flavor, and consistency of cheese. The composition and concentration of proteins in cheese can differ based on the type of cheese produced, the manufacturing techniques employed, and the source of milk utilized. The protein in cheese is also an important source of nutrition because it contains essential amino acids and other important nutrients (Fox ***et al***., 2017).

The lowest goat cheese ash content value was 1.105% which was obtained by a combination treatment of fig tree latex extract concentration 0.35 mL/100mL milk and incubation time 47.6 min. The highest goat cheese ash content value was 2.265% which was obtained with a combination treatment of fig tree latex extract concentration 0.56 mL/100 mL milk and incubation time 90 min. Figure 5 shows a 3D surface plot of goat cheese ash content.

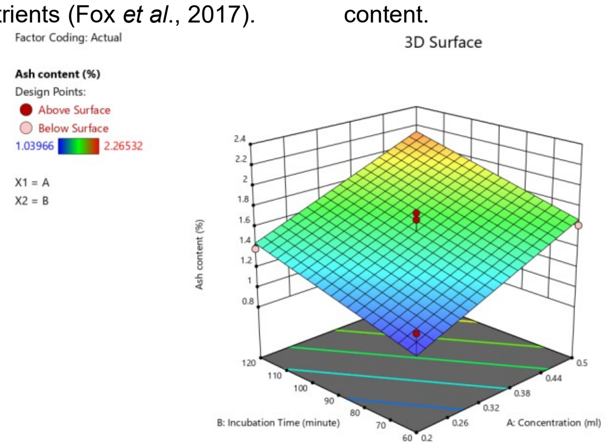

Figure 5. Goat cheese ash content 3D surface plot

Many factors influence ash content in cheese, such as the type of cheese, the manufacturing process/cheese production, and the composition of the milk used in cheese making. This content primarily reflects the mineral or salt content present in the milk. The minerals and salts in cheese play a significant role in determining its taste, texture, aroma, and appearance of the cheese. Ash content in cheese can vary greatly, depending on the type of cheese (Vacca *et al.*, 2020).

The lowest goat cheese calcium content value in cheese was 665.732 mg/100 g, which was obtained by a combination of treatment with fig tree latex extract concentration 0.14 mL/100 mL milk and an incubation time of 90 min. The highest value of calcium content in cheese was 818.371 mg/100 g, which was obtained with a combination of treatment of fig tree latex extract concentration 0.56 mL/100 mL milk and incubation time 90 min. Figure 6 shows a 3D surface plot of goat cheese calcium content.

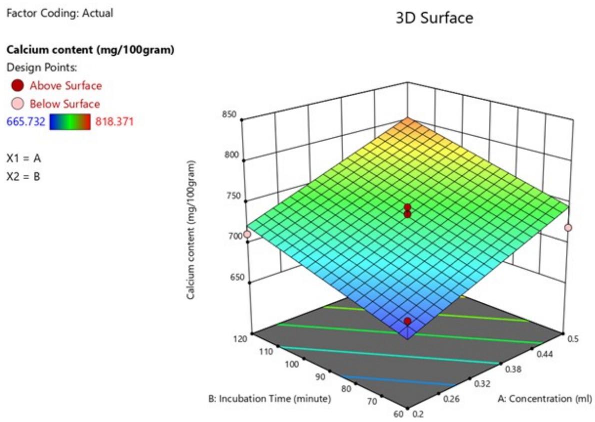

Figure 6. Goat cheese calcium content 3D surface plot

Calcium is an essential mineral contained in cheese products. Calcium is one of the main components obtained from milk, the main raw material in making cheese. Calcium plays an important role in maintaining healthy bones and teeth and is involved in various biochemical processes in the human body. Calcium levels in cheese can vary depending on the type of cheese made and the milk source used. Cheese is typically considered a rich source of calcium (McSweeney *et al.*, 2004; McSweeney *et al.*, 2017), and cheese consumption can make a significant contribution to meeting human daily calcium requirements (Pawlos *et al.*, 2021).

Ash content and calcium content exhibited a similar trend in this study. Ash content reflects the total mineral composition in cheese, and in this case, higher ash levels were consistently associated with increased calcium concentrations. This suggests that calcium is a major contributor to the overall mineral content in the cheese, highlighting its role in curd structure and firmness during the coagulation process.

Model fitting and analysis of variance

In this research response model, which uses the RSM method with CCD design, a fit summary of the research response is obtained as shown in Table 5. The suggested model for all responses in this research is a linear model, which is based on statistical testing. Other models that do not receive a recommendation (with the description "blank") can still be considered for use, while models that have the description "aliased" should be avoided.

Table 5. Research Response Fit Summary

Response	*Sequential p-value*	*Lack of Fit p-value*	*Adjusted R^2*	*Predicted R^2*	*Suggested Model*
Protein content	< 0.0001	0.2937	0.8577	0.7823	Linier
Ash content	0.0002	0.6973	0.7875	0.7034	Linier
Calcium content	< 0.0001	0.1361	0.8305	0.7139	Linier

The results of the Analysis of Variance and Fit Statistics concerning the response variables are presented in Tables 6 and 7. The results of this analysis show that the selected linear model meets the model value requirements. The model's performance is evaluated by checking its significance, lack of fit, and fit statistics. In this case, the condition is that the p-value of the model for both factors (A and B) is smaller than the value $\alpha = 0.05$, indicating that both are significant in the linear model. Meanwhile, the lack of fit value exceeds $\alpha = 0.05$. Apart from that, all responses have met the requirements for the Adjusted R^2 range value, with the Predicted R^2 difference being less than 0.2 and the Adeq Precision value being more than four (Variyana *et al.*, 2019). Based on the linear model and statistical calculations above, the actual equation value for each response is obtained in Table 8.

Table 6. Summary of ANOVA Research Response Models and Fit Statistics

Response	Source	Sum of Squares	df	Mean Square	F-value	p-value	Sig/not
Protein content	Model	107.88	2	53.94	37.15	< 0.0001	*sig.*
	Lack of Fit	10.62	6	1.77	1.81	0.2937	*not sig.*
Ash content	Model	1.39	2	0.6943	23.24	0.0002	*sig.*
	Lack of Fit	0.1474	6	0.0246	0.6494	0.6973	*not sig.*
Calcium content	Model	20,131.05	2	10,065.53	30.39	< 0.0001	*sig.*
	Lack of Fit	2,750.47	6	458.41	3.26	0.1361	*not sig.*

Table 7. Summary of Research Response Models Fit Statistics

Response	Adjusted R^2	Predicted R^2	Adeq Precision
Protein content	0.8577	0.7823	17.0469
Ash content	0.7875	0.7034	13.9128
Calcium content	0.8305	0.7139	16.0012

Table 8. Model Equation of Actual Response

Response		*Actual Equation*
Protein content	=	$-0.099870 + 19.39808*A + 0.074674*B$
Ash content	=	$0.059731 + 2.31253*A + 0.007692*B$
Calcium content	=	$551.3946 + 272.5959*A + 0.9686*B$

A= Concentration (mL/100mL milk) and B= Incubation time (min)

Model equations of actual response can be helpful in making predictions regarding the response at a particular level of each factor. In this context, it is important to note that factor levels must be measured in native units for each factor, so that the results can have an appropriate interpretation. However, it's important to acknowledge that this equation cannot be utilized to assess the relative influence of each factor. This is because the coefficients in the equation have been scaled to accommodate varying units for each factor, and the intercept point may not necessarily be positioned in the center of the design space.

It is essential to highlight that in this study, the utmost importance (scale 5) was attributed to the assessment of protein and calcium content in cheese. This prioritization stems from the study's preliminary nature, which is a precursor to the primary investigation concerning the calcium bioavailability of goat cheese. The optimization recommendations three (3) solutions of variable combinations of fig tree latex extract concentration and incubation time, aiming to enhance protein content, ash content, and calcium content, as detailed in Table 9. Among these outcomes, the optimal solution was determined to be a fig tree latex extract concentration of 0.5 mL/100 mL of milk paired with an incubation time of 120 min, attaining a high desirability value of 0.931. A desirability score nearing 1 signifies close alignment with the anticipated optimal conditions (Gutema *et al.*, 2022).

Table 9. Solutions for optimal response

Number	Concentration	Incubation Time	Protein content	Ash content	Calcium content	Desirability	
1	**0,500**	**120,000**	**18,560**	**2,139**	**803,930**	**0.931**	**Selected**
2	0,492	120,000	18,404	2,121	801,742	0.930	
3	0,500	116,812	18,322	2,115	800,842	0.930	

Therefore, this particular variable pairing is anticipated to yield cheese meeting the optimization objectives. Projections indicate that the resulting cheese would possess a protein content of around 18.560%, ash content of 2.139%, and calcium content of 803.930 mg/100 g.

Validation of variable optimization results on response

The optimization results of the variables of fig tree latex extract concentration and incubation time influence the responses being studied. Several parameters, such as protein content, ash content, and calcium content, showed a positive trend with an increase in these variables.

Table 10. Optimization Response Results Validation

Solution 1 of 3 Response	95% PI low	Data Mean	95% PI high
Protein content (%)	16.1187	17.3695	21.0013
Ash content (%)	1.78883	2.38	2.48931
Calcium content (mg/100g)	767.054	795.655	840.805

The validation results showed that after retesting with the recommended treatment combination, the mean data value for all responses was in the range of 95% PI Low and 95% PI High (Table 10). This means that the results of this combination can be used. In general, the test results illustrate that increasing the concentration of fig tree latex and incubation time will produce cheese that provides the same model. It should be noted that the use of coagulants of plant origin tends to bring out a bitter taste in the cheese. Therefore, this level of optimization must be in line with consumer preferences. A balance between the physical, chemical, textural, and organoleptic characteristics of cheese is very important to meet market needs and preferences (Nicosia *et al*., 2022).

Conclusion

In summary, the optimal formulation was achieved using 0.5 mL of fig tree (*Ficus carica L.*) latex extract per 100 mL of milk, with an incubation time of 120 min. Under these conditions, goat milk was effectively coagulated, producing cheese with a protein content of 18.560%, ash content of 2.319%, and calcium content of 803.930 mg per 100 g, yielding a desirability value of 0.931. These results demonstrate the potential of *Ficus carica L.* latex as an effective plant-based coagulant, offering a promising alternative to traditional animal-derived rennet in goat cheese production.

Conflict of interest

No potential conflict of interest relevant to this article was reported. All authors have agreed with the contents of the manuscript.

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Author’s contribution

The authors confirm their contribution to the paper as follows: study conception and design: WTN, TWM, YE, N; data collection: WTN; analysis and interpretation of results: WTN, TWM, YE, N, YYS, M, DLNF; draft manuscript preparation: WTN, TWM.

Ethics approval

Ethical approval for animal studies is not necessary in the present study because this article does not involve animal subjects.

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