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Factors affecting the coagulation of milk protein during quark cheese processing in Vietnam

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Abstract:

In recent years, cheese consumption in Vietnam is markedly rising, leading to the attention of local milk processors on cheese making. Quark-type cheese, a traditional food in Central Europe, is a type of well-known acid-coagulated cheese product. Milk coagulation properties (MCPs) play an important role to the success of the cheese-making process and are influenced by different parameters. In this study, the quality of raw milk for cheese making and the coagulation conditions in quark-type cheese processing were investigated. Raw milk collected from Phu Dong dairy farm met the requirement of Vietnamese standard for cheese making. The appropriate conditions for coagulation were identified: the heat treatment of milk at 60°C for 15 minutes, coagulation temperature at 40°C and pH 5.5, $CaCl_2$ concentration at 0.04g/l. Under these conditions, a short coagulation time (72.33 seconds) and high curd yields (50.70%) were obtained. These results suggested that quark-type cheese using raw milk in Vietnam could be successfully developed at larger scale.

Keywords: **coagulation conditions, curd yield, quark-type cheese, raw milk quality.**

Classification numbers: **3.4, 3.5**

1. Introduction

In Vietnam, cheese has been considered a western dish and not a major focus of dairy manufacturers because of deficiencies in domestic raw milk. Indeed, nearly 86% of cheese products are imported in Vietnam [1]. However, an increase in cheese consumption has been recently recognized. Cheese consumption has risen by 8.3%/yr from 2016 to 2020, expanding to 9.3%/yr in 2023. Due to the increasing cheese demand, processing cheese products from local raw milk has recently gained a lot of attention from dairy manufacturers in Vietnam.

Quark-type cheese, a traditional food in the cuisines of Central Europe, is one of the most well-known acid-coagulated cheese types with a smooth texture and a mild, slightly sour flavour. Quark cheese technologies and manufacture have been thoroughly reviewed [2], and was modified until today. In quark-type cheese production, milk is pasteurized before cooling to 28-30°C, followed by acidic and rennet coagulation [3]. Compared with other cheese products, quark-type cheese could be produced faster.

Milk coagulation properties (MCPs) play an important role in the success of the cheese-making process and are influenced by different parameters such as raw milk quality, heat treatment of raw milk, concentration of Ca^{2+}, rennet addition, and pH for coagulation by rennet. Previously, Z. Miloradovic, et al. (2018) studied the effect of heat treatment on the characteristics of Quark cheese made from cow and goat milk [4]. The results showed a significant influence of heat treatment on the texture and relative protein proportions in cow quark cheese, while there was no influence on the texture of goat quark cheese. Recently, C.D. Thybo, et al. (2020) [5] reported a balance between micellar and free calcium in curd and serum phases during direct acidification of bovine milk by changing the organic acid type. Citric acid was

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more efficient than lactic acid at lowering free calcium concentration, while the effect of temperature was larger for acidification with lactic acid. Moreover, acidification temperature had little effect on curd yield [5]. As those parameters influence cheese yield and quality at both laboratory and industry levels [6], it is important to investigate them for raw milk specific to each region.

Thus, the aim of this study is to evaluate Vietnamese raw milk quality for cheesemaking, and to discover the effect of factors in the coagulation of milk to apply to quark-type cheese processing.

2. Materials and methods

2.1. Materials

Raw milk was collected from Phu Dong Dairy Farm, Gia Lam, Hanoi, Vietnam in the morning from March to September 2022. Raw milk was kept at 4°C immediately after milking and transported to the laboratory for analysis of dry matter, protein and fat content, acidity, and freshness. Before the preparation of cheese samples, the raw milk was stored for no longer than 24 h at 4°C.

The enzyme Marzyme® (Danisco, USA) was produced by fermentation of pure broth of the fungus *Rhizomucor miehei*. The optimal pH 5.5-6.0; optimal temperature is 36-40°C, and its enzyme activity is 703-738 IMCU/ml. The recommended dosage from the product manufacturer is 24-48 IMCU/l milk.

$CaCl_2$ salt was obtained from Solvay, Italy. Lactic acid solution was obtained from PURAC® FCC, Thailand.

2.2. Methods

2.2.1. Raw milk analysis

Density, pH and total dry matter of raw milk were analysed according to TCVN 7405:2018 (ISO 6731:2010). Titratable acidity was measured according to AOAC 947.05. Protein and fat content were determined using the Kjeldahl method (ISO 8968-1:2014) and Gerber method (ISO 19662:2018), respectively.

2.2.2. Heat treatment for cheesemaking milk

500 ml of raw milk was prepared in a 1-l glass container and then heated indirectly in a water bath at 60°C for 15 min, 60°C for 30 min, and then 72°C for 15 min (Table 1).

Table 1. Experimental design of heating treatment.

Sample	Temperature (°C)	Time (min)
Raw milk	-	-
Heated milk 1	60	15
Heated milk 2	60	30
Heated milk 3	72	15

Milk after heat treatment was analysed for total micro-organisms, *E. coli*, Coliform, *S. aureus*, mould, and yeast [7].

2.2.3. Quark cheese processing

500 ml of raw milk was prepared in a 1-l glass container. The raw milk was pasteurized as in Section 2.2.2 followed by a cool down to 25°C. Heated milk was adjusted to pH 5.5 using lactic acid and then the enzyme was added. After coagulation, the curd was cut into 1-cm^3 cubes and gently stirred at 38°C before draining. The curd was transferred to moulds with pressure of 0.03 kg/cm^2 and kept at room temperature for 1 h. Cheese was withdrawn from moulds, wrapped in plastic bags, and stored at 4°C.

2.2.4. Coagulation time

Coagulation time was determined according to IDF Standard 157: 2007/ISO 11815 and Z. Miloradovic, et al. with some modifications [4]. A spatula was inserted into the gel several times. Coagulation time was defined as the interval between the addition of the enzyme to the milk and the appearance of visible clusters of casein on the surface of the spatula.

2.2.5. Coagulation conditions

There are two main factors affecting coagulation for quark cheese processing: temperature and pH. A total of 16 samples were prepared with different temperature and pH conditions as shown in Table 2.

Table 2. Experimental design of coagulation conditions.

Sample	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Temperature (°C)	30				35				40				45			
pH	5.0	5.5	6.0	6.6	5.0	5.5	6.0	6.6	5.0	5.5	6.0	6.6	5.0	5.5	6.0	6.6

2.2.6. Curd yield of Quark cheese

Curd yield was calculated as follows:

$$\text{Curd yield} = \frac{\textit{Mass of cheese x Dry matter of cheese}}{\textit{Mass of milk x Dry matter of milk}}$$

2.2.7. Textural property analysis of Quark cheese

Textural properties were analysed by the texture analyser TA.XT plus (Stable Micro System, Godalming, UK) according to K. Szkolnicka, et al. (2021) [8] with some modification. Texture profile analysis (TPA) includes the assessment of the following characteristics: hardness ($g.cm/s^{-2}$) (the peak force during the penetration of the sample), springiness (the distance of the detected height during the second compression divided by the original compression distance), and cohesiveness (the area of work during the second compression divided by the area of work during the first compression). After 24 h of refrigerated storage (4°C), the samples underwent textural analysis. The cheese sample was taken out from 4°C and cut into 40x40 mm squares with 25 mm height. The samples were penetrated with a cylindrical 25-mm diameter aluminium probe. The test speed was at 2 mm/s and trigger force was at 5 g.

2.2.8. Statistical analyses

Physicochemical and textural analyses were performed in triplicate. The obtained results were statistically analysed at the significance level p=0.05 using Microsoft Excel. Mean values and standard deviations were calculated and the values were compared by ANOVA.

3. Results and discussion

3.1. Raw milk quality

Raw milk was immediately kept at 4°C after collection. Physical and chemical properties of the raw milk are presented in Table 3.

Table 3. Physicochemical parameters of raw milk.

Analytical parameters	Raw milk	Vietnamese standard TCVN 7405:2018
Dry matter (%)	12.9±0.5	≥11.5
Fat (%)	4.0±0.4	≥3.2
Protein (%)	3.1 ± 0.2	≥2.8
Density (g/ml)	1.027	≥1.026
pH	6.6	-
Acidity (°T)	15-17	-

The results show that raw milk collected from Phu Dong Dairy Farm met the requirements of Vietnamese standard TCVN 7405:2018. However, fat and protein content were significantly higher than that specified in TCVN 7405:2018, which were suitable for coagulation process during Quark cheese making.

3.2. Effect of heat treatment on the coagulation of milk

Effect of heating treatment on the microbial quality of milk for cheese processing: Table 4 shows that raw milk only met the requirement of total number of aerobic microorganisms ($3x10^6$ CFU/ml), while other requirements were not met. "Heated milk 1" almost reached the microbiological criteria for pasteurized milk TCVN 5860:2019 (10^4 CFU/ml). However, the ability of coagulation of milk by rennin is known to decrease with heat treatment >70°C. Heat-treated milk has longer coagulation times and a gel structure softer than unheated milk [9]. The accessibility of k-casein bonds (Phe105-Met106) could be reduced by forming complexes with whey protein or precipitation of calcium phosphate by heating [10]. Although cheese made from unpasteurized milk is considered to have a better flavour, most producers pasteurize the milk to eliminate risks from microorganisms.

Table 4. Effect of heating treatment on the microbial quality of milk for cheese processing.

CFU/ml	Raw milk	Heated milk 1	Heated milk 2	Heated milk 3
Total micro-organisms	$2.6x10^6$	$6x10^4$	$7.5x10^3$	<10
E. coli	$3.2x10^3$	N.D	N.D	N.D
Coliform	N.D	N.D	N.D	N.D
S. aureus	$8x10^3$	N.D	N.D	N.D
Yeast & Mould	$1.2x10^3$	N.D	N.D	N.D

To study the effect of heat treatment on coagulation time and curd yield of fresh milk, the heat treatment was caried out as in Table 1 and the results are shown in Fig. 1. The results indicate that the curd yield of "Heated milk 2" was the highest and the coagulation time of "Heated milk 1" was the shortest. However, there are no significant differences compared to the raw milk (p>0.05). Heat-treated milk made protein recovery content in cheese increase due to denaturation of whey protein.

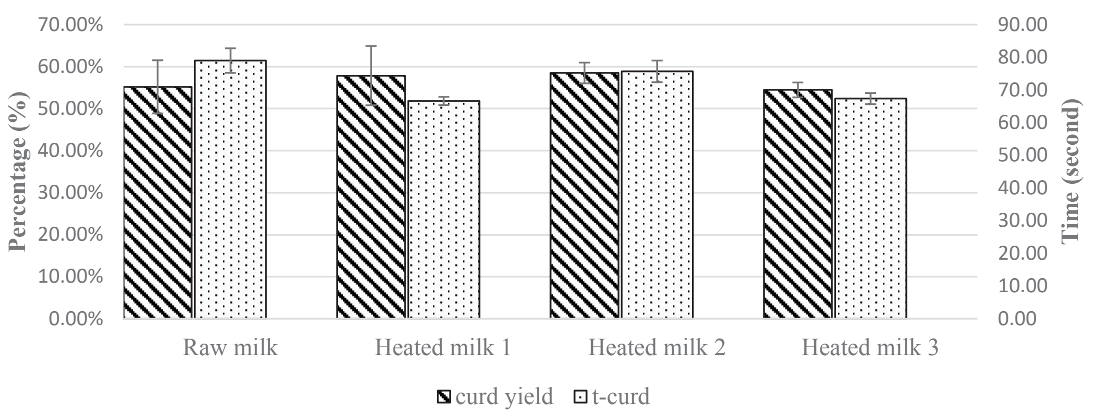

Fig. 1. Effect of heat treatment on the coagulation time and curd yield in coagulation.

The formation of whey protein/k-casein complexes at the surfaces of casein micelles or in the whey phase interferes with the contraction of curds and increase the moisture-holding capacity. However, excessive moisture is a common drawback of cheeses made from heated milk [9]. The recovery efficiency of the unpasteurized sample is like the others, but the quality of cheese drops significantly, especially in the texture aspect.

The effect of heat treatment on the texture of quark cheese is presented in Table 5. The longer the heating time and higher the temperature, the lower the quality of cheese. Especially with harsh pasteurizing temperatures, the hardness and cohesion of cheese made from "Heated milk 3" decreased from time to time. In general, the quality of cheese was negatively affected by heating. Cheeses produced from milk heated more than 70°C had lower hardness, fracture stress, fracture strain, surface hardness, and fracture hardness than milk cheeses with milder heating [2]. These trends are reflected in the interactive effects of altered composition (such as higher moisture content) and the effect of denatured whey/ protein/para-k-casein complexes on para-micelles. Casein forms the load-bearing protein network of cheese texture. Higher humidity also made the cheese softer and crumblier than cheese from milk with a mild heating setting [6].

For the "Heated milk 2" sample, hardness increased with storage time due to the loss of whey. The quality of cheese is not stable because the cohesion and flexibility decreased during storage. Overall, cheese from "Heated milk 1" had a similar structure to the unpasteurized sample.

3.3. Effect of coagulation conditions for quark cheese processing

At pH 5.0, coagulation time was not determined for the four temperature ranges because milk at pH 5.0 reaches the isoelectric point of casein in milk medium (pH 4.6-4.7) wherein casein was coagulated by the H^+ ions of lactic acid that binds to the negatively charged casein micelle and reduces the charge, causing the casein micelles to clump together, causing agglomeration.

In contrast, coagulation did not occur at pH 6.6. After adding enzymes and holding the investigated temperature for 1 h, the milk remained liquid and the fat scum easily emerged. This was because pH 6.6 is outside the optimum

Table 5. Effect of heat treatment on the structure of curd after storage.

Sample/storage time	Hardness ($g.cm/s^{-2}$)		Cohesiveness		Springiness	
	24 h	*72 h*	*24 h*	*72 h*	*24 h*	*72 h*
Raw milk	3.60±0.09	3.94±0.52	0.57±0.05	0.50±0.04	2.32±0.09	2.40±0.26
Heated milk 1	4.78±0.77	4.48±0.79	0.50±0.05	0.53±0.04	2.41±0.53	2.40±0.62
Heated milk 2	3.85±0.43	4.10±0.93	0.48±0.05	0.41±0.07	1.97±0.39	1.62±0.14
Heated milk 3	2.70±0.36	2.61±0.25	0.47±0.09	0.43±0.08	1.02±0.07	1.00±0.13

Values are expressed as mean ±SD (n=3). Values within a column not sharing a common superscript differ (p<0.05).

pH range of MARZYME®, which caused the enzyme to hydrolyse κ-casein very slowly making it difficult for the milk to coagulate to form a milky mass.

At 30 and 35°C, the coagulation time was higher than those at 40 and 45°C. At pH 6.0, the time until coagulation appeared was higher than at pH 5.5. In general, the lower the pH and the higher the temperature, the faster the coagulation time. When coagulating at pH 5.5 and 6.0, it gave a smooth and firm milky mass, and the coagulation time was short, especially at 40 and 45°C (Fig. 2).

The curd yield in samples with pH 5.0 was the lowest because the casein had coagulated immediately after lowering the pH. At a coagulation pH of 5.0, cheese products had the hardest texture, while the lowest hardness was achieved at a coagulation pH of 6.0. In fact, when storing cheese, for samples with pH 6.0, the whey in the cheese mass escaped the most, whereas samples at pH 5.0 and 5.5 were almost absent. This also affects product quality after storage (Fig. 3).

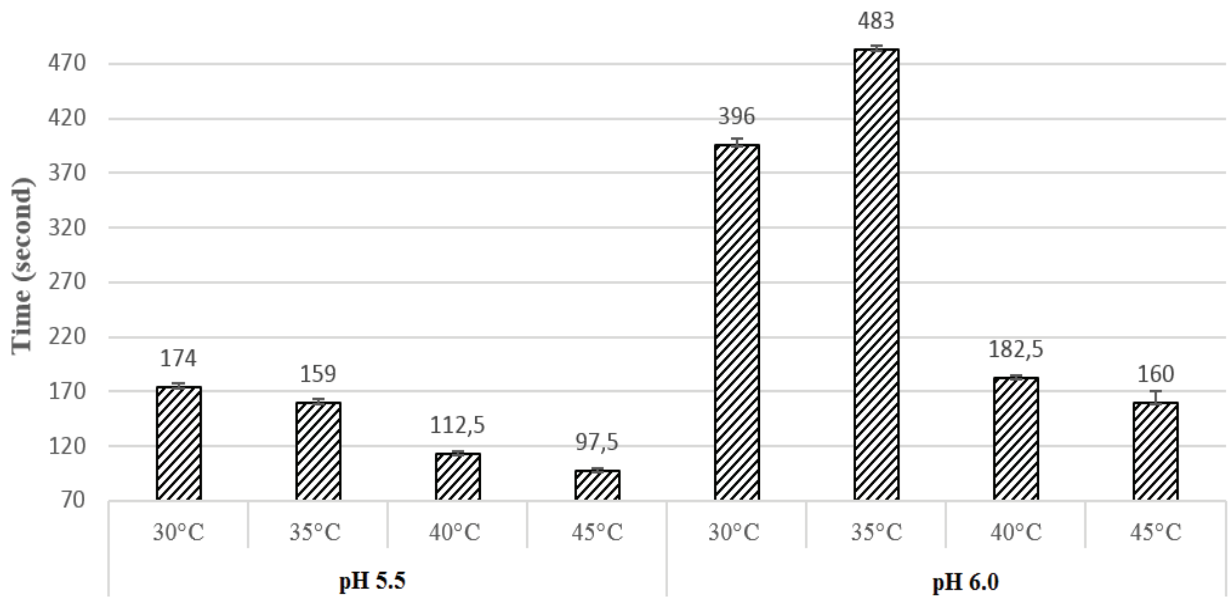

Fig. 2. Time of coagulation appearance with different conditions of temperature and pH.

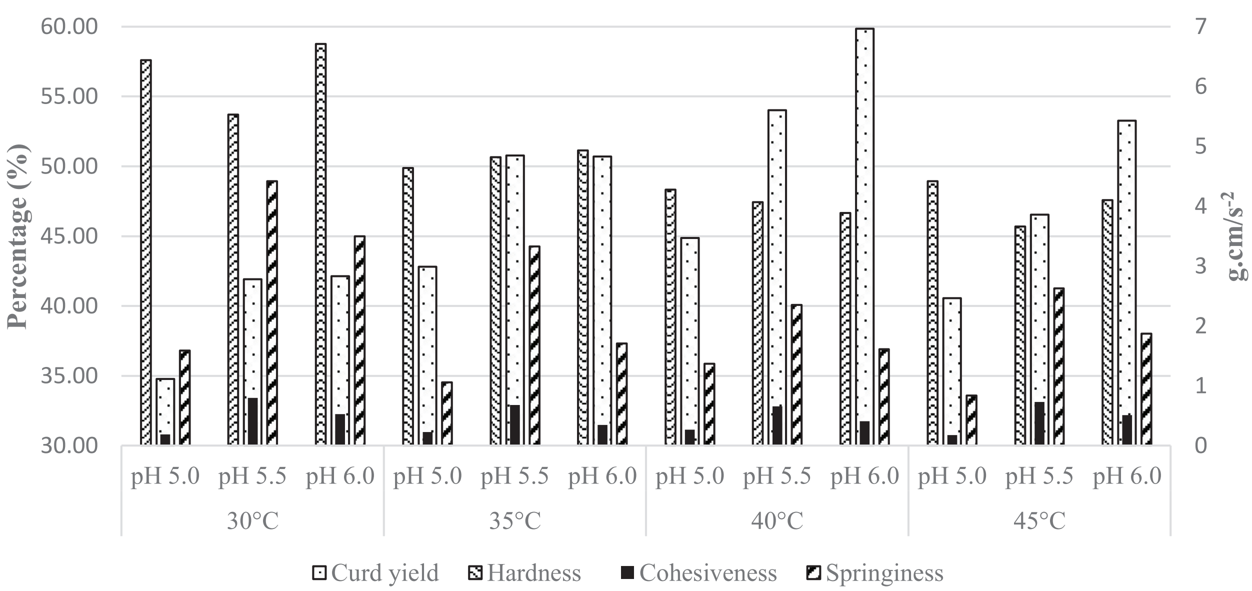

Fig. 3. Curd yield and curd structure with different pH and temperature conditions.

3.4. Effect of Ca^{2+} on the coagulation of milk in cheese processing

3.4.1. Effect of Ca^{2+} on the coagulation time

Calcium addition contributes to the increase of bridging between casein micelles, further facilitating coagulant formation [11]. Through its effect on the milk-salt system, the addition of Ca^{2+} also slightly lowers the pH of milk, promotes rennin activity, and reduces whey protein denaturation. The effect of calcium ions on the coagulation time of fresh milk is illustrated in Fig. 4.

From Fig. 4, it was clearly seen that there was a significant difference in the time of coagulation appearance between samples with different concentrations of $CaCl_2$ ($p<0.05$). The time until the appearance of coagulation decreased gradually when the concentration of $CaCl_2$ increased in the range from 0.4-0.8 g/l milk. At a concentration of $CaCl_2$ 0.8 g/l, the shortest coagulation time was found, which was not too different from the time at a concentration of 0.6 g/l milk ($p<0.05$).

3.4.2. Effect of Ca^{2+} on the curd yield and curd structure

The concentration of $CaCl_2$ also affects the yield of cheese, but this difference was not too significant at the measured concentrations ($p>0.05$). The curd yield was described as the nutrients in the recovered cheese contained in the milk, so yield would be related to the ability to retain moisture and especially protein, fat, and calcium in the milk mass. The moisture content of the cheese was not significantly affected by the addition of calcium at different concentrations at any pH during milk coagulation [11].

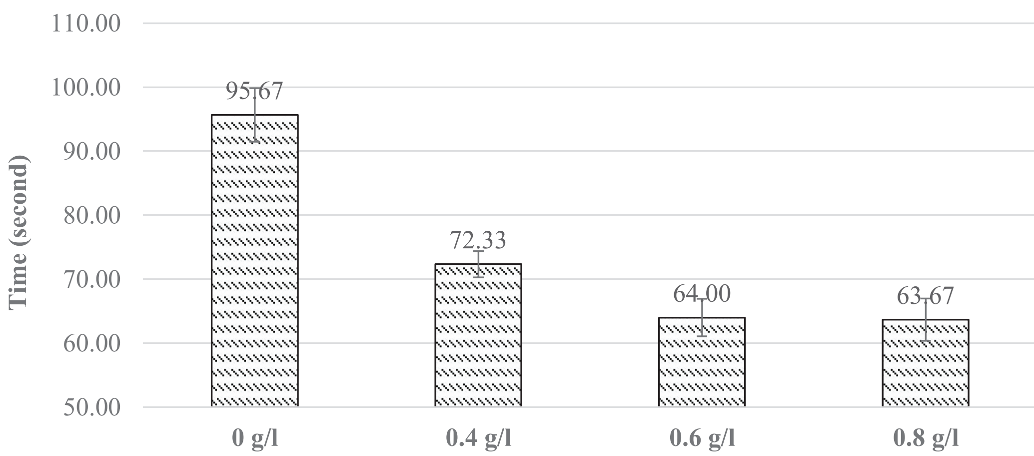

Fig. 4. Time of coagulation appearance with different concentrations of $CaCl_2$.

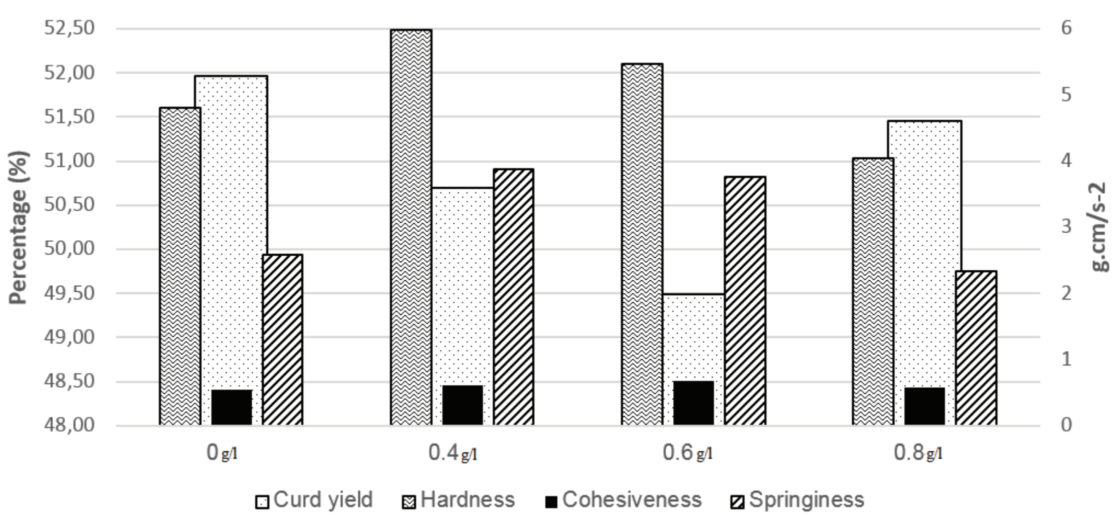

Fig. 5. Effect of $CaCl_2$ concentration on curd yield and curd structure.

At a concentration of 0.8 g/l, the best recovery efficiency was obtained, however, the difference was not large when compared with concentrations of 0.4 and 0.6 g/l ($p>0.05$). Regarding the dry matter content, although there was no significant difference, the results obtained at a concentration of 0.8 g/l were lower than that of the sample at 0.4 g/l. According to L. Ong, et al. (2013) [11], there was no significant difference ($p>0.05$) in the amount of fat retained in the final cheese. On the other hand, when tasting the cheese at concentrations of 0.6 and 0.8 g/l, we could feel the brackish taste of $CaCl_2$ salt in cheese the cheese [11].

The texture of raw curd increased gradually and was better than the sample without $CaCl_2$ at concentrations from 0 to 0.6 g/l, but the difference between concentrations was not significant ($p<0.05$). However, at a $CaCl_2$ concentration of 0.8 g/l, the structure was not only bad at concentrations of 0.4 and 0.6 g/l, but worse than the sample without $CaCl_2$. In addition, the product quality at a concentration of 0.8 g/l was also unstable when measuring the structure.

$CaCl_2$ with a concentration of 0.4 g/l in milk gave the best results in terms of coagulation time, product recovery efficiency, and product concentration as well as when the samples were tasted.

4. Conclusions

This study shows that raw milk from Phu Dong Dairy Farm, Gia Lam, Hanoi meets the standards for quark cheese processing. The optimal conditions for quark processing were determined: heat treatment of milk at 60°C for 15 min, coagulation temperature at 40°C, coagulation pH at 5.5, and concentration of $CaCl_2$ at 0.04 g/l milk. These results suggest that quark cheese processing could be further carried out on a pilot scale with high commercial potential to apply on larger scales.

CRediT author statement

Chinh Nghia Nguyen: Writing - Reviewing and Editing; Hang Ngan Dinh: Methodology, Writing, Data analysis; Thu Trang Nguyen: Writing, Data analysis; Ha Trang Nguyen, Thi Trang Nguyen, Giang Hoang: Data analysis; Ky Son Chu: Reviewing, Editing; Thu Trang Vu: Methodology, Reviewing, Editing.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

[1] Invest & Export Brazil (2020), *Vietnam Market Study on Cheese and Dairy Products,* 201pp.

[2] T.P. Guinee (2021), "Effect of high-temperature treatment of milk and whey protein denaturation on the properties of rennet-curd cheese: A review", *International Dairy Journal*, **121**, DOI: 10.1016/j.idairyj.2021.105095.

[3] A.M. Cadavid, L. Bohigas, M. Toldra, et al. (2020), "Improving quark-type cheese yield and quality by treating semi-skimmed cow milk with microbial transglutaminase", *LWT*, **131**, DOI: 10.1016/j.lwt.2020.109756.

[4] Z. Miloradovic, J. Miocinovic, N. Kljajevic, et al. (2018), "The influence of milk heat treatment on composition, texture, colour and sensory characteristics of cows' and goats' Quark-type cheeses", *Small Ruminant Research*, **169**, pp.154-159, DOI: 10.1016/j.smallrumres.2018.09.012.

[5] C.D. Thybo, S.K. Lillevang, L.H. Skibsted, et al. (2020), "Calcium balance during direct acidification of milk for Mozzarella cheese production", *LWT*, **131**, DOI: 10.1016/j.lwt.2020.109677.

[6] T. Troch, E. Lefebure, V. Baeten, et al. (2017), "Cow milk coagulation: Process description, variation factors and evaluation methodologies: A review", *Base*, **21(4)**, pp.276-287, DOI: 10.25518/1780-4507.13692.

[7] L.T. Mai, N.T. Hien, P.T. Thuy (2005), *Analytical Methods in Fermentation Technology Field*, Science and Technics Publishing House, 336pp (in Vietnamese).

[8] K. Szkolnicka, I. Dmytrow, A.M. Malek (2021), "The characteristics of quark cheese made from buttermilk during refrigerated storage", *Foods*, **10(8)**, DOI: 10.3390/foods10081783.

[9] A.B. Hougaard, Y. Ardo, R.H. Ipsen (2010), "Cheese made from instant infusion pasteurized milk: Rennet coagulation, cheese composition, texture and ripening", *Int. Dairy J.*, **20(7)**, pp.449-458, DOI: 10.1016/j.idairyj.2010.01.005.

[10] M. Britten, H.J. Giroux (2022), "Rennet coagulation of heated milk: A review", *Int. Dairy J.*, **124**, DOI: 10.1016/j.idairyj.2021.105179.

[11] L. Ong, R.R. Dagastine, S.E. Kentish, et al. (2013), "The effect of calcium chloride addition on the microstructure and composition of Cheddar cheese", *Int. Dairy J.*, **33(2)**, pp.135-141, DOI: 10.1016/j.idairyj.2013.03.002.