EFFECT OF TRANSGLUTAMINASE ON FRESH CHEESE QUALITY PROPERTIES USING CITRIC ACID AS A COAGULANT

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ABSTRACT

The purpose of this study was to evaluate the effect of microbial transglutaminase (MTGase) on cheese quality properties using citric acid as a coagulant agent. The experimental results showed that citric acid has a temperature-dependent ability to agglomerate milk proteins. The process of protein coagulation at 50°C by citric acid (2.0%) gave fresh cheese with yield production (% H), dry matter content (% TS) and cheese solids yield (CSY) of 12.81%, 41.17%, 38.73%, respectively. The reconstituted milk was incubated with MTGase under different conditions of enzyme concentration (0-6.0 IU / g protein), processing temperature (30-50 °C) and time (60-180 min.). Enzyme treatment at 37 °C with enzyme concentration of 2 IU/g protein for 2 hrs showed the highest values of yield (15.03%), dry matter content (46.96%) and cheese solids yield (46.83%). Analytical results of the sensory score, acidity, whey separation and color difference of fresh cheese samples during storage (28 days) showed that using MTGase better maintained the quality of the product. Furthermore, MTGase also improved water holding capacity (reduced whey separation) during storage and did not affect the color of fresh cheese products. Moreover, the physicochemical and microbiological parameters of the product were also determined. The results showed that the quality of fresh cheese coagulated by citric acid met CODEX STAN 221-2001 for unripened cheese including fresh cheese.

***Keywords**: Transglutaminase; MTGase; fresh cheese; cross–linking; citric acid.*

1. INTRODUCTION

Fresh cheeses are ready-to-eat unripened cheeses that are produced by coagulating milk, cream or whey through acidification, or acidification with a small amount of rennet or a combination of acid and heat in cottage cheese [1]. Fresh cheeses have the highest moisture content of all cheeses. This makes them a great ingredient for spreading and filling. In most cheeses, this type has a mild and delicious flavor [2]. Fresh cheeses have a limited shelf life, about 2-4 weeks refrigerated [3].

Based on the casein coagulation method, cheese can be divided into many different categories such as acid coagulation, enzyme coagulation and temperature coagulation (Figure 1) [1]. Quark and paneer cheeses are two famous acid-coagulated fresh cheeses that outperform others on the market [2]. Quark (in German-speaking countries) or Tvorog (in Eastern European countries) is essentially a viscous form of milk protein. It has pale yellow milky white color, smooth, uniform, soft, tough structure, mild acidic, high moisture content (82% w / w) [4]. Paneer is a type of fresh cheese obtained by coagulating milk with heat and acids, wrapping almost all fat, casein, and whey protein. Paneer has a white appearance, has a firm texture and a nutty flavor. The preparation of paneer using different milks and different techniques leads to large differences in the physicochemical, microbiological and sensory qualities of products [5].

Citric acid, a tricarboxylic acid ($C_6H_8O_7.H_2O$) is a common metabolite of plants and animals and is found in the juices

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of fruits and pineapples. In addition, citric acid can be produced from fermentation of carbohydrates containing media using the bacteria *Candida spp.* or non-toxic strains of *Aspergillus niger*. Citric acid is a versatile and non-toxic additive. It is recognized as safe worldwide by GRAS, approved by FAO/WHO.

Transglutaminase (EC 2.3.2.13, protein-glutamine γ-glutamyl transferase) catalyzes in vitro cross-linking reaction in whey proteins, soy proteins, wheat proteins, beef myosin, casein and crude actomyosin refined from mechanically deboned poultry meat. In recent years, this enzyme was also used to gelatinize various food proteins through the formation of cross-links resulting in the improvement of functional properties of food. Basically, the targets of transglutaminase reaction may be (a) modification of texture, (b) protection of lysine in food proteins from various chemical reactions, (c) encapsulation of lipids and/or lipid-soluble materials, (d) formation of heat- and water-resistant films, (e) prevention of gelation under heat processing, (f) improvement of elasticity and water-holding capacity, (g) modification of solubility and functional properties, and (h) production of highly nutritional protein-based products [6].

The current Vietnamese domestic raw milk has only been able to fulfil 30-40% of consumer demand and has met only production of drinking milk [7]. Most of the cheese in the Vietnamese market today is imported products from other nations. Besides, there are not many studies focusing on the effect of MTGase addition on physicochemical and sensory properties of fresh cheese made from milk powder. Based on practical needs and current trends in domestic production, in this research, we built a process of producing fresh cheese using whole milk powder as a material and citric acid as a coagulant agent. Using citric acid for cheese production has great potential for industrial scale application as it can help simplify the acidification process, reduce production costs and time leading to significant economic benefits.

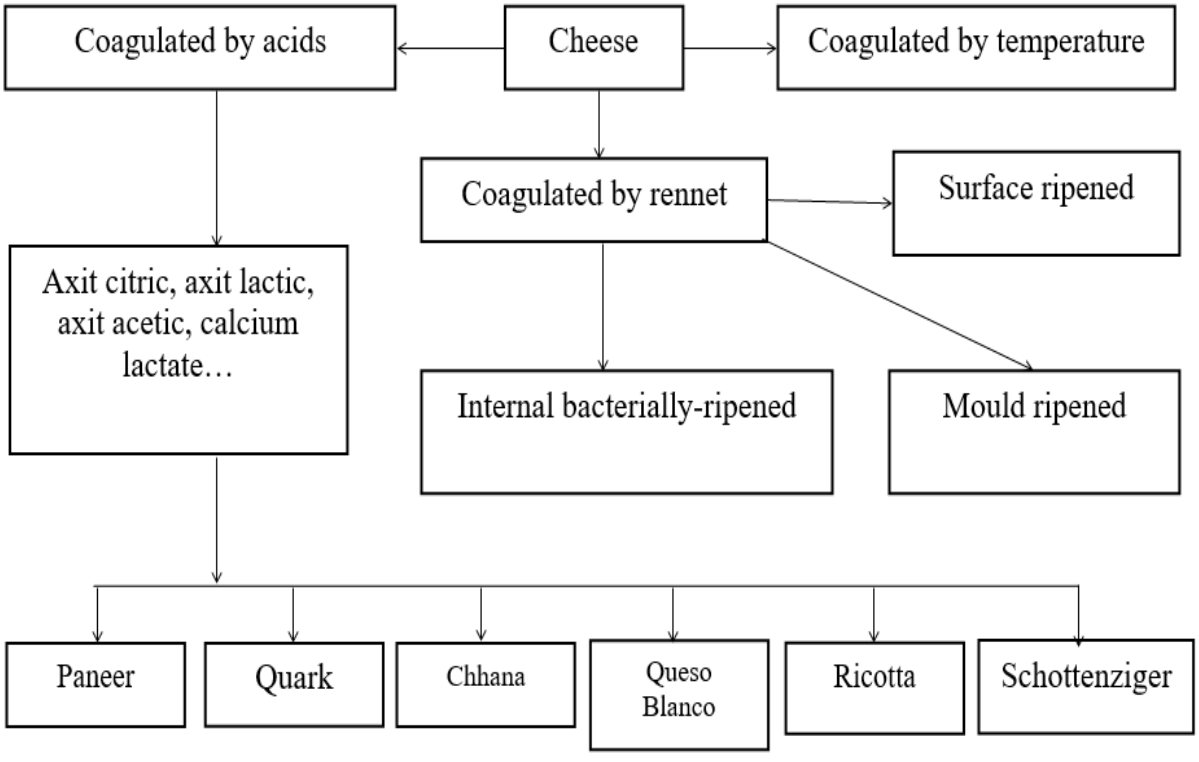

Figure 1. *Schematic classification of cheese based on coagulation method*

2. MATERIALS AND METHODS

2.1 Materials

Whole milk powder was supplied by Fonterra Ltd, New Zealand with moisture content of 2,79%, protein content of 23.78%, lipid content of 28.28%, lactose content of 39.00% and ash content of 5.80% according to the product's certificate of analysis.

Citric acid with CAS number is 77-92-9. Transglutaminase (Saprona TG 1E) supplied from Germany with an activity of 106 U/g.

2.2 Methods

Fresh cheese preparation. Whole milk powder (46.3 g) was reconstituted with water (253.7 g) at 50 °C for 30 minutes [8] to obtain a milk solution with the desired dry matter content of 15%. The chemical composition of the reconstituted milk solution was determined as: protein content 4.36%, lipid content 3.44% and moisture content 84.93% (TS = 15.07%).

Enzymatic treatment. a) MTGase concentration: Reconstituted milk (300 g) after heat treatment was lowered to the temperature of the enzyme treatment (37 °C). Then an amount of enzyme (0; 0.5; 1.0; 1.5; 2.0; 2.5; 3.0; 4.0; 6.0 IU/g protein) was dissolved in a milk solution. The mixture was then incubated for 2hrs in a water bath. The mixture was then pasteurized at 85°C for 30 minutes to eliminate bacteria and inactivate enzymes. *b) Temperature:* While the enzyme concentration (2.0 IU/g protein) was kept constant, the temperature of the mixture of reconstituted milk-enzyme was varied in a range from 30 to 50 °C (30; 37; 45; 50°C) for 2 hrs. *c) Treated time:* The enzyme concentration (2.0 IU/g protein) and temperature (37 °C) were fixed while the treated time was changed in a range from 0 to 3 hrs (0; 1.0; 1.5; 2.0; 2.5; 3.0 hrs). For all experiments, the reference sample (R) was done without MTGase treatment.

Acid coagulation. MTGase-treated reconstituted milk (300 g) after pasteurization was lowered to the coagulation temperature (37°C; 50°C; 85°C). Then, citric acid at investigated concentrations (1.0, 1.5, 2.0, 3.0%) was added to the mixture until the protein precipitated completely (pH = 4.6).

The curd is then transferred into a cylinder (Ø = 5.6 cm) for whey extraction. A 1 kg dumbbell is used to press until the cheese block height reaches 2.0 cm, then stop. The cheese was then refrigerated at 4 ± 2°C.

The quality properties (The yield of fresh cheese production, cheese solids yield, whey separation, texture profile analysis and sensory evaluation) of the samples were determined.

The yield of fresh cheese production. The yield (H, %) was determined by the formula:

$$\boldsymbol{H = \frac{m_1}{m_0} \times 100} H = \frac{m_1}{m_0} \times 100 H = \frac{m_1}{m_0} \times 100 \quad (1)$$

Where, m_1 was the weight of fresh cheese (g); m_0 was the weight of reconstituted milk solution (g).

Cheese solids yield (CSY). The CSY value is understood as the ratio of the amount of solids recovered in the cheese product to the initial solids weight [9]. CSY is calculated by the formula:

$$\text{CSY} = \frac{H * TS_{cheese} H * TS_{cheese}}{TS_{milk} \quad TS_{milk}} \quad (2)$$

Where, H was The yield of fresh cheese production, TS_{cheese} was the total solid content of cheese (%), TS_{milk} was the total solid content of reconstituted milk (%).

Whey separation. Whey separation (syneresis) was determined according to a method of Dmytrów et al. (2010) [10]. Cheese samples (25 g) were weighed and put into zip-lock packages. The whey leached out from samples at 25ºC was weighed after 20 hrs. Percentage of whey separation (*Wh*, %) was calculated by the formula:

$$Wh = \frac{m_1 m_1}{m_0 m_0} \times 100, \quad (3)$$

Where, m_1 was the weight of separated whey from sample (g); m_o was the initial weight of sample (g).

Texture profile analysis (TPA). The texture characteristics of the cheeses were analyzed by a Broofield (USA) CT3 texture analyzer. Fresh cheese samples were kept refrigerated (4 ± 2 °C) in separate plastic containers until analyzed. The samples were taken out of the refrigerator about 1 hour before being analyzed and kept in the box, then cut into cylindrical blocks (diameter 2.5 cm, height 2 cm). The temperature of samples was 25°C. Values of hardness, elasticity and adhesion were measured. Parameters for measurement were: (a) a cylinder force (TA-AACC36) with diameter of 3.6 cm; TA-BT-KIT fixture; (b) test speed of 1.0 mm / s; (c) pretest speed of 2.0 mm / s; recovery time of 5.0 s; Trigger load of 5.0 g and target distance of 3.0 mm [11, 12].

Microstructure observation. Scanning electron micrograph (SEM) was taken according to a method of Lobato-Calleros et al. (2001) [13]. Cylindrical cheese samples 0.5 cm in diameter by 0.5 cm in height were fixed in 2% buffered glutaraldehyde (0.1M phosphate buffer, pH 7.2, 6 h), and then subsequently dehydrated in increasing concentrations of aqueous ethanol solutions (50, 60, 70, 80, 90 and 100%, 30 min per each one) and placed in pure acetone in 1 hour. After that, samples were dried in a vacuum dryer (50°C, 6h). The cheese samples were mounted on a stub and coated with a thin layer of gold in a Fine Coat Ion Sputter JFC 1100 (Jeol Ltd., Akishima, Japan) before taking a photograph.

Sensory evaluation. Sensory properties of cheese samples were evaluated by a panel of 7 deeply trained assessors consisting of 6 females and 1 male with the age of 20-21. The sensory test was taken according to the ISO 22935-3: 2009 [14] with the scale of 0 to 5 points using a commercial product as reference samples. Evaluated attributes were appearance, texture and flavour. The appearance was judged by the colour and smoothness of the cheeses. The texture was evaluated through hardness (defined as the force required to bite entirely through a sample placed between molar teeth) and firmness (defined as the amount of resistance to compression offered by a 1 cm thick slice of cheese when pushed between the thumb and the index finger until fingers touch each other) [15]. The term ‘‘flavour’’ has many definitions but within this study, this term will be defined as the ‘‘impressions perceived via the chemical senses from a product in the mouth’’ [15]. Sensory evaluation sessions were conducted in the individual booth under fluorescent light. The samples were coded with three random digit numbers and presented monadically. The testing room was cleaned without a strange odour.

Colour space measurements. The colour space for the fresh cheese samples was determined by a CR-400 chroma meter (Minolta, Japan) according to Mokrzycki and Tatol (2011) [16]. The colour parameters of MTGase-treated samples were compared with those of the non-enzyme treated cheese. The L, a, and b values represent white/black, red/green and yellow/blue, respectively. The difference in colour ($\triangle E$) was calculated using the formula (4):

$$\Delta E = \sqrt{((L_i - L_0)^2 + (a_i - a_0)^2 + (b_i - b_0)^2)}$$
$$\Delta E = \sqrt{((L_i - L_0)^2 + (a_i - a_0)^2 + (b_i - b_0)^2)} \quad (4)$$

Where, (L_o, a_o, b_o) and (L_i, a_i, b_i) were colour parameters of the non-enzyme treated cheese, and the enzyme-treated samples, respectively. Based on the $\triangle E$ value, the difference in colour between the samples was expressed as: $0 < \triangle E < 1$ (the observer did not notice the difference in colour); $1 < \triangle E < 2$ (only experienced observers were able to notice the difference in colour); $2 < \triangle E < 3.5$ (inexperienced observers might notice colour differences); and $\triangle E > 3.5$ (there was a clear colour difference between the two samples).

Chemical analyses. Total solid content (TS), fat content (F) and protein content (P) were estimated by ISO 13580:2005, ISO 1735:2004 and Kjeldahl

method (ISO 89681:2014), respectively. Titratable acidity (TA) of products were determined following ISO/TS 11869:2012.

Each experiment was done three times. Experimental data was statistically analyzed by one-way ANOVA ($p < 0.05$) with the Minitab (version 16) software program.

3 RESULTS AND DISCUSSION

3.1 Results in acid coagulation process

A) Effect of temperature of citric acid coagulation process on fresh cheese quality

The temperature of citric acid treatment had a negligible effect on fresh cheese yield (Table 1). With a total dry matter content of the reconstituted milk of 15.07%, cheese yield on average was 12.4%. However, during the making process, we found that, at the temperature of 37°C, the protein precipitate was quite sticky, leading to difficulties in manipulation during whey separation, loss of dry matter on the surface of the tools… Otherwise, at 50 °C and 85 °C the whey separation process was easier to perform. Furthermore, experimental results showed that the dry matter loss in whey at treated temperature of 85 °C was higher than that at treated temperature of 50 °C. Therefore, 50 °C was selected for the citric acid coagulation in the next following experiments.

Table 1. *Effect of temperature in citric acid coagulation process on some fresh cheese qualities*

Temperature, oC	H, %	TS_{whey}, %	TS_{cheese}, %	CSY, %	Hardness, g
37	12,20±0,51^a	8,07±0,15^b	41,05±0,58^a	33,23±0,23^b	275,00± 0,71^c
50	12,81±0,44^a	8,13±0,19^b	41,17±0,21^a	34,99±0,11^a	320,25±14,50^b
85	12,16±0,88^a	9,51±0,86^a	40,80±0,58^a	32,92±0,50^b	446,75±12,37^a

The same letters (a-c) showed insignificant difference ($p < 0.05$)

Table 2. *Effect of citric acid concentration on cheese yield and texture properties*

Citric acid concentration,%	H, %	TS_{cheese}, %	CSY, %	Hardness, g	Springiness, mm	Adhesiveness, mJ
1,0	11,49 ±0,32^b	47,27±0,40^b	38,87± 0,90^b	320,75±3.18^a	2,97±0,8^a	0,29±0,014^a
1,5	12,30±0,11^a	47,64±0,26^b	39,56±0,62^b	323,00±8,83^a	2,8±0.03^a	0,22±0,021^a
2,0	12,63 ±0,20^a	48,71±0,07^a	43,94±0,60^a	320,25±0,35^a	2,79±0,23^a	0,33±0,106^a
3,0	11,68±0,10^b	44,38±0,22^c	40,05±0,11^b	460,25±35^b	3,17±0,21^a	0,49±0,078^a

The same letters (a-c) showed insignificant difference ($p < 0.05$)

B) Effects of citric acid concentration on cheese quality

The analytical results (Table 2) showed that, when the citric acid concentration increased from 1.0% to 2.0%, the recovery efficiency (H, %) increased from 11.49% to 12.63%. However, when the concentration of citric acid increased to 3.0%, the recovery efficiency and the dry matter content in cheese decreased (p <0.05). The dry matter content in cheese (TS_{cheese}) increased gradually with increasing concentration of processed acid. At that time, the highest cheese solids yield (CSY) was reached at the coagulating acid concentration of 2%. However, this factor did not significantly affect the texture properties of the product (hardness, springiness and adhesiveness). There was only an increase in hardness at 3% concentration.

Our results are similar to those of Khan (2014) [17]; Bankar (2016) [18] when authors did the research to determine the effect of different types of acids, including citric acid, tartaric acid and malic acid each at 2, 3 and 5% concentrations on the quality of paneer made using reconstituted milk. The results also showed that using 2% citric acid

to coagulate milk proteins gave statistically comparable quality parameters so that the concentration of citric acid of 2.0% was selected as the most suitable level of coagulant for fresh cheese production.

3.2 Results in Transglutaminase treatment

A) Effects of MTGase concentration on cheese quality

The results (Table 3) showed that when the concentration of the enzyme MTGase increased from 0.5 to 2.0 (IU/g protein), the quality parameters including solid content (TS_{cheese}), cheese solids yield (CSY) increased gradually. However, when the enzyme level continued to increase from 2.0 (IU / g protein) to 6.0 (IU / g protein), these quality properties of cheese gradually decreased. This could be explained that at the MTGase concentration of 2 IU/g protein all the proteins in milk might completely be linked, the cross-linking reaction did not take place anymore. The increase in the MTGase levels could lead to an abundance of linkages in the gel network which might compete and break each other [19].

The results of texture analysis (Table 3) showed that beyond springiness, hardness and adhesiveness of fresh cheese products supplemented with MTGase significantly changed among samples. Specifically, when increasing the concentration of MTGase from 0.5 to 2.0 IU/g protein, hardness and adhesiveness tend to increase. However, when the concentration of enzyme continued to increase from 2.0 to 6.0 IU/g protein, these texture parameters decreased. This can be explained by the effect of enzyme addition which might increase the water-holding capacity and strength of the gel through protein cross-linking [20], and emulsification [21], thereby helping to improve the texture of the fresh cheese product. However, continuing to increase the enzyme concentration the gel network became tighter, the competition between the linkages increased and could break the cross-links created by MTGase [19]. Consequently, weakening the gel network and reducing the quality of the cheese texture.

From the analytical results we found that using enzyme MTGase concentration of 2.0 IU/g protein gave the better quality properties for the fresh cheese product. The hardness of 380 g wasn't higher than the value in the products of the same type (480 g for Quark [22].

B) Effect of temperature in enzymatic treatment on cheese quality

The experimental results (Table 4) showed that when the temperature of the enzyme treatment increased, the yield of the product (H, %) and CSY increased, especially the higher values (14.95% and 46.37%, respectively) were found when temperature reached 37°C. Treatment temperature seemed to not affect the texture of the cheese product. Only the adhesiveness was reduced when the treatment temperature reached 50°C. Therefore, 37 °C was selected as the temperature for reconstituted milk treatment with MTGase during the production of fresh cheeses.

Table 3. *Effect of MTGase concentration on the quality of fresh cheese*

MTGase conc., IU/g protein	H, %	TS_{cheese}, %	CSY, %	Hardness, g	Springiness, mm	Adhesiveness, mJ
0 (R)	13.96 ± 0.13^{b}	42.19 ± 0.12^{f}	39.08 ± 0.11^{f}	317.00 ± 19.8^{d}	2.46 ± 0.03^{b}	0.15 ± 0.02^{d}
0.5	15.28 ± 0.55^{a}	43.89 ± 0.20^{d}	44.51 ± 0.21^{d}	321.25 ± 6.01^{d}	2.55 ± 0.0^{ab}	0.17 ± 0.04^{d}
1.0	15.06 ± 0.59^{a}	44.95 ± 0.17^{c}	44.95 ± 0.17^{c}	351.75 ± 22.98^{bc}	2.59 ± 0.06^{ab}	0.24 ± 0.02^{b}
1.5	15.21 ± 0.35^{a}	44.09 ± 0.26^{d}	44.51 ± 0.26^{d}	364.00 ± 12.73^{abc}	2.63 ± 0.01^{a}	0.25 ± 0.03^{ab}
2.0	15.03 ± 0.06^{a}	46.96 ± 0.35^{a}	46.83 ± 0.35^{a}	380.75 ± 11.67^{a}	2.61 ± 0.16^{a}	0.29 ± 0.01^{a}
2.5	15.07 ± 0.81^{a}	46.05 ± 0.09^{b}	46.04 ± 0.09^{b}	369.75 ± 8.84^{ab}	2.65 ± 0.04^{a}	0.25 ± 0.02^{ab}

3.0	15.19±0.21^{a}	44.26±0.05^{d}	44.62±0.06^{cd}	350.75 ± 0.35^{bc}	2.62 ± 0.0 ^{a}	0.26 ± 0.02^{ab}
4.0	14.65±0.54^{ab}	42.83± 0.18^{e}	41.64± 0.18^{e}	351.50 ± 8.84^{bc}	2.61 ± 0.00^{ab}	0.23 ± 0.02^{bc}
6.0	15.07±0.07^{a}	41.15±0.07^{g}	41.22± 0.16^{e}	340.50± 3.54^{cd}	2.53± 0.06^{ab}	0.19± 0.04^{cd}

The same letters (a-g) showed insignificant difference ($p < 0.05$)

Table 4. *The effect graph of enzyme temperature on milk solution treatment temperature on cheese quality*

Temperature, ^{o}C	H, %	TS_{cheese}, %	CSY, %	Hardness, g	Springiness, mm	Adhesiveness, mJ
37 (R)	13.96±0.13^{cd}	42.19±0.12^{d}	39.08±0.11^{d}	317.00 ± 19.8^{a}	2.46 ± 0.03^{b}	0.15± 0.02^{ab}
30	14.20±0.14^{c}	47.28±0.24^{a}	44.56±0.22^{b}	321.25±6.72^{a}	2.52±0.04^{a}	0.19±0.02^{a}
37	14.95±0.15^{a}	47.32±0.22^{a}	46.37±0.03^{a}	384.00±20.51^{a}	2.51±0.05^{a}	0.16±0.04^{ab}
45	14.57±0.18^{b}	45.46±0.12^{c}	43.94±0.11^{c}	362.00±38.89^{a}	2.51±0.04^{a}	0.12±0.02^{ab}
50	14.41±0.07^{bc}	45.89±0.28^{b}	43.88±0.26^{c}	363.50±48.79^{a}	2.53±0.08^{a}	0.09±0.03^{b}

The same letters (a-d) showed insignificant difference ($p < 0.05$)

Table 5. *Graphs of effects of enzyme-based lactation processing time on cheese quality*

Time, hrs	H, %	TS_{cheese}, %	CSY, %	Hardness, g	Springiness, mm	Adhesiveness, mJ
37 (R)	13.96±0.13^{b}	42.19±0.12^{d}	39.08±0.11^{d}	317.00± 19.08^{b}	2.46 ± 0.03^{b}	0.15± 0.02^{ab}
1	13.94±0.22^{b}	43.40±0.17^{c}	40.48±0.22^{c}	294.50± 7.77^{c}	2.51±0.01^{b}	0.11± 0.01^{ab}
1.5	14.10±0.20^{b}	44.32±0.34^{b}	41.14±0.32^{b}	296.25± 8.13^{bc}	2.61±0.01^{a}	0.18± 0.11^{a}
2	15.15±0.22^{a}	47.10±0.27^{a}	47.31±0.29^{a}	386.25±11.76^{a}	2.54±0.04^{ab}	0.20± 0.05^{a}
2.5	14.80±0.32^{a}	47.06±0.05^{a}	46.83±0.40^{a}	393.25±32.88^{a}	2.58±0.08^{ab}	0.13± 0.08^{ab}
3	14.98±0.17^{a}	47.05±0.07^{a}	46.91±0.36^{a}	324.00±17.68^{b}	2.57±0.03^{ab}	0.07± 0.01^{b}

The same letters (a-c) showed insignificant difference ($p < 0.05$)

C) Effect of enzyme-treated time on cheese quality

The analytical results (Table 5) showed that, when increasing the enzyme-treated time from 1 to 2 hrs, the product yield increased from 13.94% to 15.15%. Continuing to increase the processing time from 2 to 3 hrs, the yield did not change. Similarly, the dry matter content in cheese (TS_{cheese}) and the cheese solid yield (CSY) also increased gradually and reached the maximum values (47.10% and 47.31% respectively) at the second hour of treatment.

The analytical results (Table 5) even showed that the hardness, springiness and adhesiveness increased with increasing processing time from 1 to 2 hrs. The higher values of texture qualities were reached at 2 hrs of treatment process. Furthermore, increasing enzyme-treated time caused the deduction of texture properties. This can be explained protein structure sensitivity increases the level of protein polymerization; however, the level protein polymerization results in a weak, brittle, and fracturability gel lattice structure. Therefore, enzyme processing time has a significant impact and is a key factor in cheese production to avoid the formation of excessive protein cross-linking that leads to deterioration of the structural properties of the final product [23].

From the results, enzyme-treated time of 2 hrs was chosen for fresh cheese production.

Based on the experimental results, the technological process of manufacture of fresh cheese was created and presented in Figure 2.

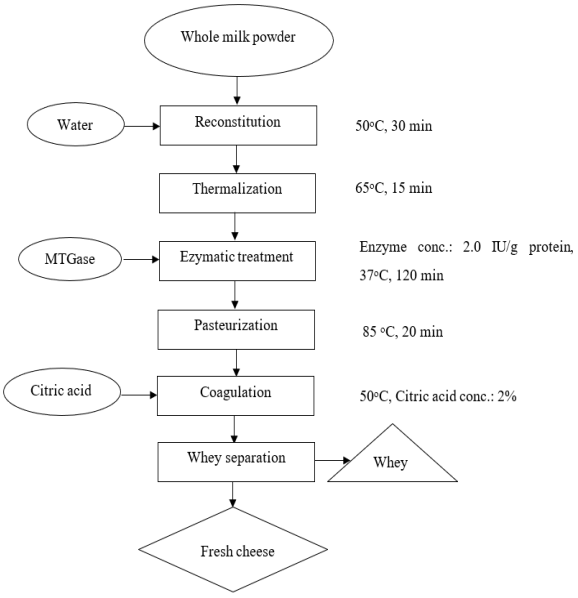

Figure 2. *Flowchart of technological process of manufacture of fresh cheese coagulated by citric acid*

3.3 Quality properties of fresh cheese

Fresh cheese sample was produced under the conditions of MTGase treatment above (Figure 2). Practically, the protein, lipid and total solid contents (%) of this cheese were 8.90, 30.45 and 46.96, respectively (Table 6). As a result, reconstituted milk from whole milk powder caused the high fat content of the cheese. At the same time, the protein content was also lower than some similar cheese products like Quark or paneer.

The scanning electron micrographs (SEM) of two fresh cheese samples with enzyme (M1) and without enzyme (M2) showed difference in microstructure of products (Figure 3). The microstructure of fresh cheese coagulated with enzyme (M2) forms micelle casein chains linked together in the interstitial space of various sizes and the liquid phase is fixed in the protein gel network. In addition, the protein gel network is roughly formed and the spacing between the polymers is large [24]. Meanwhile, the gel structure of the enzyme-treated fresh cheese sample (M1) was more homogeneous, fatty globules are evenly distributed in the protein gel network; a set of small congeners linked together resulting in a smoother gel network with smaller mesh sizes between the congeners. Therefore, cross-linking by MTGase prevents phase separation during protein coagulation.

3.4 Changes in fresh cheese quality during storage

Some of the quality parameters of citric acid-coagulated fresh cheese samples with MTGase treatment (M1) and without enzyme treatment (M2) are shown in Table 7. Whey separation did not occur during the first week (day 1-7) in both products. However, the analytical results showed that in the weeks which followed (day 7-28), there was more whey separated from the M2 samples than that from M1. The titratable acidity increased gradually during storage in both product groups. According to Lucey (2001) [27], the separation of whey during cheese preservation is the result of synergies. The reason is that all casein molecules have an extremely flexible structure, even the denatured casein molecules tend to form more compact micelle structures, leading to whey cleavage (i.e. is the release of water). This synergy is spontaneous and is understood to be a contraction of the gel without any external force acting, resulting in reordering of the gel network and causing the whey to separate.

Table 6. *Chemical composition of cheeses*

Cheese sample	Protein content, %	Lipid content, %	Total solid content, %	Listeria monocytogenes, CFU/g	Positive Staphylococci Coagulase, CFU/g
Fresh cheese	8.90	30.54	46.96	Not detected	Not detected
Quark[1]	≥ 12	<40	≥ 12	-	-
Paneer[2]	18.10	18.43	44.01	-	-

Note: "-": Unknown. [1] Data adapted from Fellows (2008)[25], [2]Data adapted from Kumar (2014) [26].

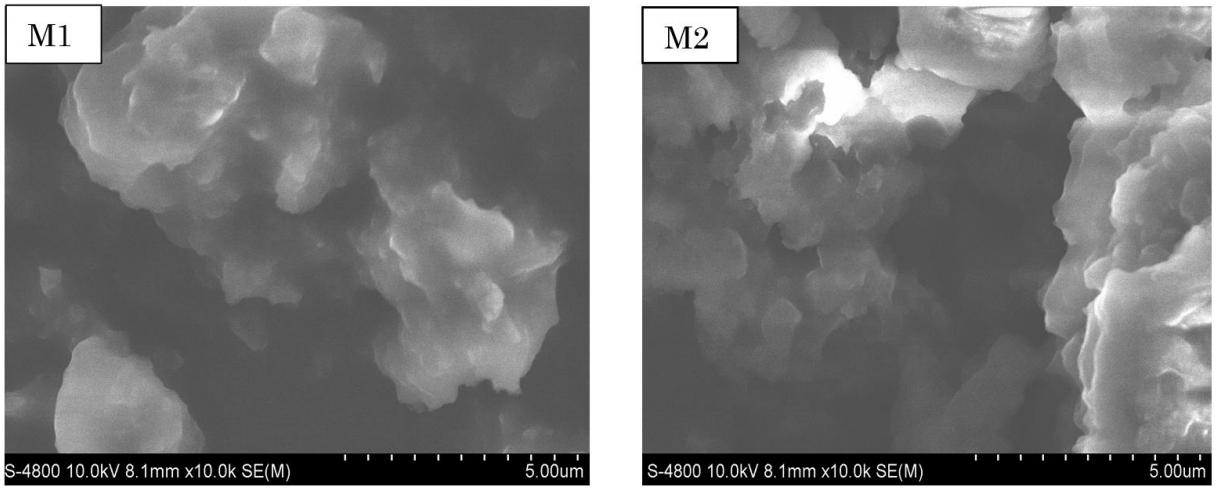

***Figure 3**. SEM images of fresh cheese samples. M1 - Fresh cheese with enzyme, M2 - Fresh cheese without enzyme*

***Table 7.** Quality properties of fresh cheese samples with/without MTGase*

Day	Sample	Whey separation, %	Titratable acidity, °T	Average sensory evaluation score	ΔE*
1	M1	0	11,78±0,04^{c}	4,52±0,26^{a}	1,41
	M2	0	12,25±0,07^{d}	4,43±0,16^{a}	
7	M1	0,05±0,02^{c}	11,03±0,11^{d}	4,50±0,39^{a}	1,02
	M2	0,64±0,47^{d}	12,60±0,14^{cd}	4,29±0,37^{ab}	
14	M1	0,24±0,12^{c}	12,05± 0,21^{cb}	4,43±0,25^{ab}	0,84
	M2	1,10±0,04^{c}	12,90± 0,14^{bc}	4,29±0,28^{ab}	
21	M1	0,73±0,17^{b}	12,28± 0,04^{b}	4,17±0,50^{ab}	1,32
	M2	1,63±0,17^{b}	13,15± 0,35^{ab}	4,10±0,25^{b}	
28	M1	1,76±0,30^{a}	13,00 ±0,28 ^{a}	4,07±0,33^{b}	1,10
	M2	3,33±0,19^{a}	13,60 ±0,14^{a}	4,02±0,30^{b}	

M1 - Fresh cheese with enzyme, M2 - Fresh cheese without enzyme

Analytical results (Table 7) showed that the average sensory evaluation scores of appearance, texture and flavour changed similarly between two fresh cheese samples (M1 and M2) during storage. Both products showed no significant change in appearance or flavour during 28 days of storage. However, sensory scores tended to decrease in the final weeks of storage (from day 21 to 28). There was no difference in colour between M1 and M2 samples during storage ($\triangle E < 2$).

4 CONCLUSION

On the strength of this study, the application of citric acid as a coagulant combined with MTGase helps the cheese production process shorten the production time compared to the coagulation process by normal fermentation. With this application, other natural acid sources such as lactic acid, malic acid ... can be used to create a variety of products with different flavors. However, the efficiency of fresh cheese produced by this method (12.81%) is still low, lower than that of fermentation methods [2]. The use of MTGase also contributes to improvement of product yield. The fresh cheeses that are coagulated by citric acid have the quality properties met CODEX STAN 221-2001 for unripened cheese.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

[1] Fox, P. F., Guinee, T. P., Cogan, T.M., McSweeney, P. L. (2017). Cheese: Historical Aspects, chapter 1. Fundamentals of Cheese Science, Second Edition. Springer, 1-8.
[2] Fox, P.F. and McSweeney, P. L. (2004). Cheese: An Overview. In: Fox, P.F. (ed.) Cheese: Chemistry, Physics and Microbiology. *Third Edition - Volume 1: General Aspects. Elsevier Applied Science, London, UK*, 1-16.
[3] Kanekanian, A. (2005). Dairy technology-principles of milk properties and processes. *International Journal of Dairy Technology*, *58*(4), 237.
[4] Schulz-Collins, D., & Senge, B. (2004). Acid-and acid/rennet-curd cheeses part A: Quark, cream cheese and related varieties. In *Cheese: Chemistry, physics and microbiology* (Vol. 2, pp. 301–328). Elsevier.
[5] Kumar, S., Rai, D. C., Niranjan, K., & Bhat, Z. F. (2014). Paneer-An Indian soft cheese variant: a review. *Journal of Food Science and Technology*, *51*(5), 821–831.
[6] Quaglia G. B. Gennaro L. (2003). Enzymes: Uses in Food Processing. Encyclopedia of Food Sciences and Nutrition (Second Edition). Pages 2125-2139.
[7] InvestVietnam (2020). Invest in Vietnam industry. Available online at: http://investvietnam.gov.vn/en/nghanh.nghd/27/dairy-industry.html
[8] Bylund, G. (1995). *Dairy processing handbook. Tetra Pak processing systems AB*.
[9] Salinas-Valdés, A., De la Rosa Millán, J., Serna-Saldívar, S. O., & Chuck-Hernández, C. (2015). Yield and textural characteristics of panela cheeses produced with dairy-vegetable protein (soybean or peanut) blends supplemented with transglutaminase. *Journal of Food Science*, *80*(12), S2950–S2956.
[10] Dmytrów. I. Jasinska. M.. Dmytrów. K. (2010). Effect of microbiological transglutaminase on selected physicochemical properties of tvarog. Italian Journal of Food Science: IJFS; Pinerolo. 22(4). pp 449-460.
[11] Baraka A. Abd El-Salam (2015). Effect of Milk Fat Replacement with Vegetable Oil and/or Whey Protein Concentrate on Microstructure, Texture and Sensory Characteristics of Fresh Soft Cheese. International Journal of Dairy Science 10 (3): 117-125.
[12] Benjamin O., Davidovich-Pinhas M., Shpigelman A., Rytwo G. Utilization of polysaccharides to modify salt release and texture of a fresh semi hard model cheese. Food Hydrocolloids (2017), doi: 10.1016/j.foodhyd.2017.08.033.
[13] Lobato-Calleros. C. Robles-Martinez J.C. Caballero-Perez J.F. and Aguirre-Mandujano E. (2001). Fat replacers in low-fat Mexican Manchego cheese. Journal of Texture Studies 32 (2001) 1-14.
[14] ISO 22935-3:2009 [IDF 99-3:2009]. Milk and milk products - Sensory analysis - Part 3: Guidance on a method for evaluation of compliance with product specifications for sensory properties by scoring. Available online at: https://www.iso.org/standard/41218.html
[15] Clark S., Costello M., Drake M.A., Bodyfelt F. (2009). The Sensory Evaluation of Dairy Products. 2nd edition. Springer Science & Business Media, LLC, 233 Spring Street, New York, Chap. 1, p. 17.
[16] Mokrzycki WS, Tatol M (2011). Colour difference ΔE - A survey. Machine Graphics and Vision 20(4):383-411.

[17] Khan, S. U., Pal, M. A., Wani, S. A., & Salahuddin, M. (2014). Effect of different coagulants at varying strengths on the quality of paneer made from reconstituted milk. *Journal of Food Science and Technology*, *51*(3), 565-570.
[18] Bankar, S. S., Raziuddin, M., & Zanjad, P. N. (2016). The influence of different coagulants on yield and sensory quality of cow milk chhana. *Indian Research Journal of Extension Education*, *14*(4), 61-64.
[19] Gauche, C., Vieira, J. T. C., Ogliari, P. J., & Bordignon-Luiz, M. T. (2008). Crosslinking of milk whey proteins by transglutaminase. *Process Biochemistry*, *43*(7), 788-794.
[20] Motoki, M. & Seguro, K. (1998). Transglutaminase and its use for food processing. *Trends in Food Science & Technology*, *9*(5), 204-210.
[21] DeJong, G. A. H., & Koppelman, S. J. (2002). Transglutaminase catalyzed reactions: impact on food applications. *Journal of Food Science*, *67*(8), 2798–2806.
[22] Pham TH, Pham KC, and Huynh AT et al. (2021). Effect of transglutaminase on quality properties of fresh cheese. International Journal of Advanced and Applied Sciences, 8(4): 44-53
[23] Mazuknaite, I., Guyot, C., Leskauskaite, D., & Kulozik, U. (2013). Influence of transglutaminase on the physical and chemical properties of acid milk gel and cottage type cheese. *Journal of Food, Agriculture and Environment*, *11*, 3-4.
[24] Ahmed, A., & Bajwa, U. (2019). Composition, texture and microstructure appraisal of paneer coagulated with sour fruit juices. *Journal of Food Science and Technology*, *56*(1), 253-261.
[25] Fellows, P. (2008). Cheese making, Practical Action, United Kingdom.
[26] Kumar, S., Rai, D. C., Niranjan, K., & Bhat, Z. F. (2014). Paneer-An Indian soft cheese variant: a review. *Journal of Food Science and Technology*, *51*(5), 821–831.
[27] Lucey, J. A. (2001). The relationship between rheological parameters and whey separation in milk gels. *Food Hydrocolloids*, *15*(4-6), 603-608.

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