

OPTIMIZATION OF DAIRY TREATMENT PROCESS WITH TRANSGLUTAMINASE IN THE MANUFACTURE OF FRESH CHEESE

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ABSTRACT

Microbial transglutaminase (MTGase) is an enzyme widely used in food industry. In this study, MTGases had been applied to produce fresh cheese made from whole milk powder. A set of 18 experiments was carried out to evaluate the influence of factors in reconstituted milk treatment with MTGase. A response surface methodology was applied to evaluate quality properties of fresh cheese via objective functions of hardness, yield, protein content, total solid content and sensory evaluation score. In this model, three factors were enzyme concentration (0.6 - 3.0 U/g protein), temperature (30 - 60°C), reaction time (1.5 - 6 h). The results showed that all objective functions reached the optimal values at treated enzyme concentration, temperature and reaction time of 2.59 U/g protein, 36.14°C and 4.53 h, respectively. Under enzymatic treatment, scanning electronic micrographs (SEM) also showed that the network structure of the experimental products became more uniform. The quality properties of fresh cheese (sensory evaluation score, syneresis, acidity and the total number of lactic acid bacteria) met the CODEX STAN 243-2003 revised 2010 for fresh cheese products.

Keywords: Transglutaminase; MTGase; fresh cheese; cross-linking; milk powder.

1. INTRODUCTION

Since consumers have perceived enzymes to be more 'natural' than chemicals, the use of it to modify the functional properties of foods has attracted food scientists. In the last few years, there are many applications of enzymatic treatment increasing in food technology. Recently, microbial transglutaminase (MTGase) has received much attention for its ability to produce cross-linkages in protein-based products.

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 [1].

Several applications of MTGase in the production of milk and dairy products have been extensively studied. Yuksel and Erdem (2010) investigated the effect of cross-linking formation between milk proteins by MTGase on yogurt properties. The study was conducted on skimmed milk and reconstituted whole milk (14% non-fat solids concentration) with different enzyme treatment conditions. Actually, MTGase is an effective treatment in the production of low-fat yogurt without addition of additives. Furthermore, MTGase contributes to the shelf-life of products. In 2015, Sanli [2]

evaluated the effects of using MTGase on many yogurt properties such as acidity, viscosity, gel strength, and microstructure. The addition of enzyme contributed to the increase in gel durability and the reduction in whey separation of the product. According to Metwally [3], the MTGase is the only covalent binding enzyme that is available to improve the quality of dairy products. Cross-linking reactions can lead to changes in protein properties such as solubility, emulsification, foaming and gel formation. For example, in Quark cheese, the MTGase led to lower hardness, less grain structure and finer texture.

The current domestic raw milk has only been able to fulfill 30-40% of consumption demand, (EU-Vietnam Business Network, 2016) and has met only production of drinking milk. Most of cheese in Vietnamese market today is imported products from other nations. Besides, there hasn't been any studies about the effect of MTGase addition on physico-chemical 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. Under MTGase treatment, effects of factors (enzyme concentration, temperature and reaction time) on responses (hardness, yield, protein content, total solid content and sensory evaluation score) were investigated.

2. MATERIALS AND METHODS

2.1 Materials

Microbial transglutaminase (MTGase, EC 2.3.2.13, Activa® MP) was derived from spore-forming bacteria *Streptococcus mobaraense* was supplied by Ajinomoto, Malaysia. The enzymatic powder has a specific enzymatic activity of 36 units (U) per gram powder.

Freeze-dried yogurt starter culture, a mixed strain of *Streptococcus thermophilus* CHCC 3534 and *Lactobacillus delbrueckii* ssp. *bulgaricus* CHCC 3984, was obtained from Chr. Hansen, Denmark.

Whole milk powder was supplied by Fonterra Ltd, New Zealand.

2.2 Fresh cheese preparation

Whole milk powder (15.44 g) was added to distilled water (100 ml). Then, reconstituted milk (300 ml) was heated (85°C, 30 min) to eliminate bacteria and inactivate enzyme existing in the raw material, then cooled to investigated temperature. Afterward, MTGase was added. The conditions for the enzymatic reaction (enzyme concentration, temperature and reaction time) were optimized by experimental design using a response surface methodology (RSM). After enzymatic treatment, starter culture (5%, w/v) was added at 43±1°C for the coagulation (4-5 h, pH reached to 4.6). The curd was then transferred into a plastic tube ($\phi = 56$ mm) and was slightly pressed to achieve a final height of 2.8 cm. Fresh cheese (M1) was weighed to determine the yield of production and then was stored at 4±2°C.

Control samples (M2) were made from whole milk powder undergoing the same procedure as above without the transglutaminase-treatment stage. To assess the quality of experimental samples (M1), fresh cheese products made from raw milk with and without enzyme treatment (M3 and M4) were also prepared following the above procedure of preparation.

The quality properties (whey separation, titratable acidity, total count of LAB and sensory evaluation) of the samples were determined.

Whey separation. Whey separation (syneresis) was determined according to a method of Dmytrów et al. [4]. Cheese samples (25 g) were weighed and put into zip-lock packages. The whey leached out from samples at 25°C was weighted after 20 hours. Percentage of whey separation (Wh , %) was calculated by the formula:

$$Wh = \frac{m_1}{m_0} \times 100, \quad (1)$$

where, m_1 was the weight of separated whey from sample (g); m_0 was the initial weight of sample (g).

Microstructure observation. Scanning electron micrograph (SEM) was taken according to a method of Lobato-Calleros et al. [5]. 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 taken a photograph.

Fat content (FC), titratable acidity (TA), total lactic acid bacteria count. Fat content (%) and titratable acidity (millimole sodium hydroxide per 100 g of product) of cheese samples were determined in accordance with the ISO 1736:2008/IDF 9:2008 and ISO 11869:2012, respectively. Besides, total lactic acid bacteria count (cfu/g) was determined according to the ISO 15214:1998.

2.3 Design of optimization experiments

Preliminary analyzed results of the dairy treatment process with transglutaminase showed that cheese hardness (Y1), yield of fresh cheese production (Y2), protein content (Y3), total solid content (Y4) and organoleptic properties (Sensory evaluation - Y5) of cheese were determined as technological objects. They were affected by 3 factors: enzyme concentration, (Z1, U/g protein), enzyme-treated temperature (Z2, °C), enzyme-treated time (Z3, hours). A table of factors affecting the technology objects of the dairy treatment process was showed in table 1. A regression analysis of responses was performed on the obtained data (n=3) and was fitted into an empiric second order polynomial model [6]:

$$Y = b_0 + \sum_{j=1}^k b_j x_j + \sum_{j,i=1;i \neq j}^k b_{ji} x_j x_i + \sum_{j=1}^k b_{jj} (x_j^2 - \lambda) \quad (2)$$

where, Y – the predicted response, b0 – the model constant, bj – the coefficients of the

linear effects, bji – the coefficients of interaction between the factors, bjj – the coefficients of the quadratic effects, xi, xj – the coded variables, λ – coefficient, the condition of orthogonal matrix, k – the number of considered variables (k = 3).

The coded variables were determined:

$$x_j = \frac{Z_j - Z_j^o}{\Delta Z_j} \quad (3)$$

The experimental number was determined:

$$N = 2^k + 2k + n_0 = 2^3 + 2 \times 3 + 4 = 18 \quad (4)$$

The value of the star point:

$$\alpha = \sqrt{\sqrt{N \cdot 2^{k-2}} - 2^{k-1}} = 1.414 \quad (5)$$

The condition of orthogonal matrix:

$$\lambda = \frac{1}{N} (2^k + 2\alpha^2) = 2/3 = 0.667 \quad (6)$$

A combination of 18 experiments with variation of the input variables was designed following the Table 2.

Table 1. Levels of actual variables

Variable	Levels					ΔZ
	-α	-1	0	+1	+α	
Z ₁ (U/g protein)	0.10	0.60	1.80	3.00	3.50	0.10
Z ₂ (°C)	23.8	30.0	45.0	60.0	66.2	23.8
Z ₃ (h)	0.57	1.50	3.75	6.00	6.93	0.57

Determination of experimental technological objects:

Determination of cheese hardness (Y1). Cheese hardness was measured by a CT3 Texture Analyzer (Ametek Brookfield, America). Parameters for measurement were: (a) a cylinder force (TA-AACC36) with diameter of 3.6 cm; (b) test speed of 3.0 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 8.0 mm [7, 8].

Yield of fresh cheese production (Y2). The yield (H, %) was determined by a formula:

$$H = \frac{m_1}{m_o} \times 100, \quad (7)$$

where, m₁ was the weight of fresh cheese (g); m_o was the weight of reconstituted milk solution (g).

Protein content (Y_3) and total solid content (Y_4). Protein content (%) and total solid content (%) were determined following procedures of ISO 13580:2005 and ISO 8968-1:2014, respectively.

Sensory evaluation (Y_5). Sensory properties of cheese samples were evaluated by a panel of 07 trained accessors. The sensory test was taken according to the ISO 22935-3:2009 with scale of 05 points using a commercial product as a reference. Evaluated attributes were: appearance, texture and flavour. The sensory evaluation score was counted as the total score of the attributes (15 scores). Sensory assessment sessions were made in individual booth under fluorescent light. The samples were randomly coded with three-digit numbers. The testing room was cleaned without strange odor.

Optimization method

Building the mathematical model of the one-objective optimization problems

Every objective function $f_j(Z)$ ($j = 1 \div 5$) with Z variable vector $Z = \{Z_i\} = (Z_1, Z_2, Z_3) \in \Omega_Z$ ($i = 1 \div 3$) was formed as the one-objective optimization problem. This problem can be stated by determining the root of $Z_j = \{Z_i^{jopt}\} = (Z_1^{jopt}, Z_2^{jopt}, Z_3^{jopt}) \in \Omega_Z$ in order that:

$$\left\{ \begin{array}{l} f_{1(480.5g)} = f_1(Z_i^{1opt}) = f_1(Z_1^{1opt}, Z_2^{1opt}, Z_3^{1opt}) \\ \quad = 480.5g \\ f_{jmax} = f_j(Z_i^{jopt}) = f_j(Z_1^{jopt}, Z_2^{jopt}, Z_3^{jopt}) \quad (8) \\ \quad = \min f_j(Z_1, Z_2, Z_3) \\ j= 2 \div 5, i = 1 \div 3 \end{array} \right.$$

Actually, the appropriate hardness (Y_1) of our cheese (480.5 g) was as nearly similar as that of a reference sample (tvorog “Savushkin Khutorok” – a Russian fresh cheese). Solving the one-objective optimization problems was done by Solver function in Microsoft Excel 2019.

Building the mathematical model of the multi-objective optimization problem

The m-objective optimization problem ($m = 5$) could be simply transformed into the

problem of finding the optimum variable values for a set (9) of m one-objective optimization problems [6]. Determining the root of $Z_j = \{Z_i^{opt}\} = (Z_1^{opt}, Z_2^{opt}, Z_3^{opt}) \in \Omega_Z$ in order that:

$$\left\{ \begin{array}{l} f_{1(480.5g)} = f_1(Z_i^{opt}) = f_1(Z_1^{opt}, Z_2^{opt}, Z_3^{opt}) \\ \quad = 480.5g \\ f_{jmax} = f_j(Z_i^{opt}) = f_j(Z_1^{opt}, Z_2^{opt}, Z_3^{opt}) \quad (9) \\ \quad = \min f_j(Z_1, Z_2, Z_3) \\ j= 2 \div 5, i = 1 \div 3 \end{array} \right.$$

Solving the m-objective optimization problem was done using a response surface methodology (RSM) by Design-Expert software program (version 11.1.0.1).

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 Building mathematical models of dairy treatment process with MTGase

Eighteen (18) cheese samples with input variables (x_1, x_2, x_3) (table 2) were performed following the procedure described in section 2.2. The quality properties of the fresh cheese products (hardness, yield, protein content, total solid content and sensory evaluation score) were practically analyzed. The mean value \pm standard deviation ($n = 3$) were shown in Table 2.

After empirical analysis, the regression equation coefficients were calculated, the significance of the regression equation coefficients was tested based on Student standard, the compatibility of regression equations of results was checked based on the Fisher standard [6]. Analytical results showed the correlation between the variables MTGase concentration (x_1 , U/g protein), reaction temperature (x_2 , °C) and reaction time (x_3 , h) with the target functions (Y_1 – hardness, Y_2 – yield, Y_3 – protein content, Y_4 – total solid content and Y_5 – sensory evaluation score of fresh cheese) were shown in regression equations (10-14).

Table 2. Two-level orthogonal experimental matrix ($k = 3, n_o = 4$) and their experimental responses

Number of experiments		Actual variable			Coded variable			Experimental response*				
N		Z ₁	Z ₂	Z ₃	x ₁	x ₂	x ₃	Cheese hardness, g (Y ₁)	Yield, % (Y ₂)	Protein content, % (Y ₃)	Total solid content, % (Y ₄)	Sensory evaluation score (Y ₅)
2 ^k	1.	0.60	30.00	1.50	-1	-1	-1	139.17 ± 13.87 ^a	21.87 ± 0.57 ^b	12.10 ± 0.20 ^{ab}	28.20 ± 0.26 ^b	9.89 ± 1.69 ^{abcd}
	2.	3.00	30.00	1.50	1	-1	-1	136.67 ± 18.58 ^a	25.28 ± 0.48 ^d	12.12 ± 0.39 ^{abc}	29.61 ± 0.23 ^{de}	11.11 ± 1.54 ^{de}
	3.	0.60	60.00	1.50	-1	1	-1	335.00 ± 16.58 ^c	20.27 ± 0.49 ^a	12.60 ± 0.41 ^{bcd}	25.63 ± 0.58 ^a	9.11 ± 1.54 ^{abc}
	4.	3.00	60.00	1.50	1	1	-1	296.83 ± 31.85 ^{bc}	25.50 ± 0.53 ^d	12.50 ± 0.42 ^{abcd}	29.71 ± 0.31 ^{de}	8.44 ± 1.42 ^a
	5.	0.60	30.00	6.00	-1	-1	1	332.00 ± 22.85 ^c	23.97 ± 0.67 ^c	12.21 ± 0.22 ^{abc}	29.25 ± 0.40 ^{cd}	10.11 ± 1.9 ^{bcde}
	6.	3.00	30.00	6.00	1	-1	1	247.17 ± 25.90 ^b	26.54 ± 0.31 ^f	13.14 ± 0.39 ^{efg}	31.85 ± 0.22 ^g	10.44 ± 1.42 ^{cde}
	7.	0.60	60.00	6.00	-1	1	1	462.33 ± 84.03 ^e	22.77 ± 0.25 ^b	12.31 ± 0.27 ^{abc}	28.10 ± 0.37 ^b	8.89 ± 1.27 ^{ab}
	8.	3.00	60.00	6.00	1	1	1	323.17 ± 35.04 ^c	23.76 ± 0.70 ^c	12.89 ± 0.13 ^{de}	29.11 ± 0.27 ^c	10.89 ± 1.62 ^{de}
2 ^k	9.	0.10	45.00	3.75	-1,414	0	0	487.83 ± 45.18 ^f	22.50 ± 0.87 ^b	12.32 ± 0.18 ^{abc}	27.78 ± 0.19 ^b	9.89 ± 0.78 ^{abcd}
	10.	3.50	45.00	3.75	1,414	0	0	240.00 ± 44.86 ^b	26.38 ± 0.71 ^{ef}	12.64 ± 0.32 ^{cd}	31.65 ± 0.18 ^g	12.56 ± 0.88 ^{fg}
	11.	1.80	23.80	3.75	0	-1,414	0	118.50 ± 12.29 ^a	25.28 ± 0.17 ^d	12.57 ± 0.28 ^{bcd}	30.74 ± 0.26 ^f	10.44 ± 1.33 ^{cde}
	12.	1.80	66.20	3.75	0	1,414	0	353.83 ± 15.18 ^{cd}	22.67 ± 0.70 ^b	13.66 ± 0.17 ^h	28.22 ± 0.20 ^b	9.89 ± 1.05 ^{abcd}
	13.	1.80	45.00	0.57	0	0	-1,414	436.67 ± 18.06 ^{ef}	24.56 ± 0.58 ^{cd}	12.02 ± 0.07 ^a	29.81 ± 0.12 ^e	11.56 ± 1.74 ^{ef}
	14.	1.80	45.00	6.93	0	0	1,414	413.00 ± 54.21 ^{de}	26.57 ± 0.23 ^f	13.01 ± 0.22 ^{def}	31.79 ± 0.36 ^g	10.78 ± 1.30 ^{df}
n _o	15.	1.80	45.00	3.75	0	0	0	615.00 ± 32.25 ^g	27.35 ± 0.42 ^f	13.73 ± 0.21 ^h	33.78 ± 0.21 ⁱ	13.44 ± 0.73 ^g
	16.	1.80	45.00	3.75	0	0	0	705.67 ± 63.29 ^h	26.55 ± 0.22 ^f	13.62 ± 0.09 ^{gh}	33.85 ± 0.21 ⁱ	12.67 ± 1.58 ^{fg}
	17.	1.80	45.00	3.75	0	0	0	699.50 ± 56.33 ^h	27.15 ± 0.19 ^f	13.46 ± 0.42 ^{fgh}	33.12 ± 0.08 ^h	13.33 ± 1.50 ^g
	18.	1.80	45.00	3.75	0	0	0	809.17 ± 61.83 ⁱ	27.07 ± 1.05 ^f	13.49 ± 0.09 ^{fgh}	33.42 ± 0.26 ^{hi}	13.11 ± 1.27 ^g

*Superscripts in each column indicate the significant differences ($p < 0.05$)

Regression models in terms of actual factors

$$Y_1 = 679.86 + 74.60x_2 - 130.07x_1^2 - 193.95x_2^2 - 99.60x_2^2 \quad (10)$$

$$Y_2 = 26.98 + 1.47x_1 - 0.75x_2 + 0.58x_2 - 0.64x_1x_2 - 1.21x_1^2 - 1.44x_2^2 - 0.65x_2^2 \quad (11)$$

$$Y_3 = 13.51 + 0.16x_1 + 0.19x_2 + 0.22x_2 + 0.20x_1x_2 - 0.48x_1^2 - 0.16x_2^2 - 0.46x_2^2 \quad (12)$$

$$Y_4 = 33.39 + 1.21x_1 - 0.83x_1 + 0.66x_2 - 1.68x_1^2 - 1.80x_2^2 - 1.14x_2^2 \quad (13)$$

$$Y_5 = 13.11 + 0.56x_1 - 0.42x_1 - 0.91x_1^2 - 1.44x_2^2 - 0.94x_2^2 \quad (14)$$

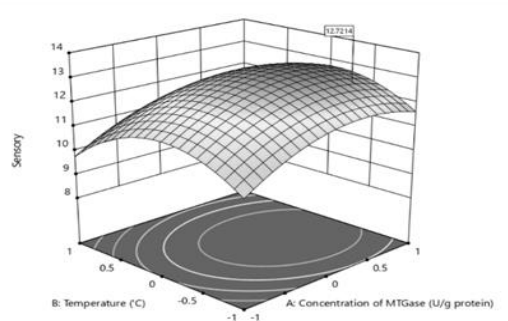
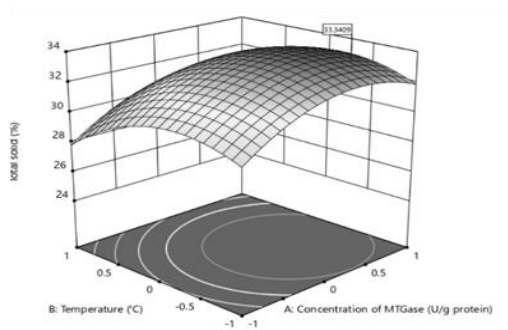
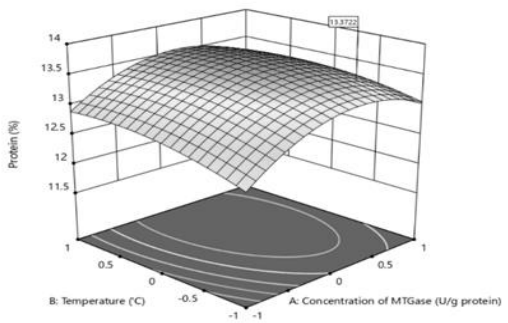
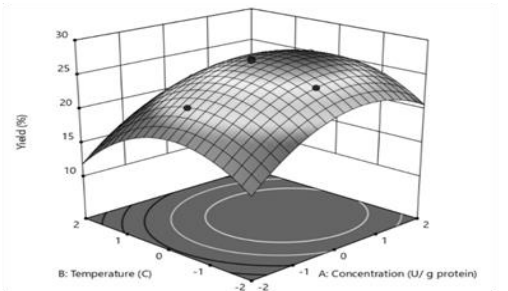
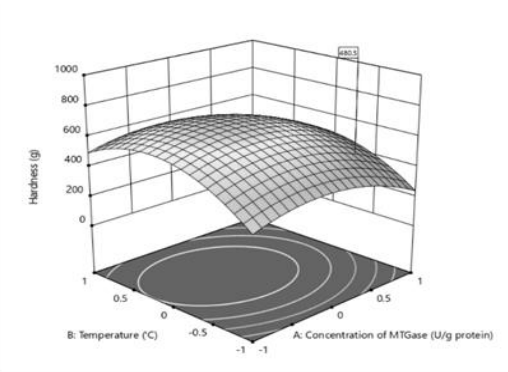
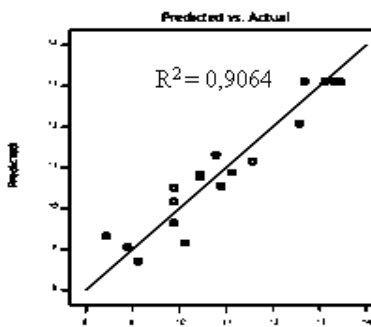
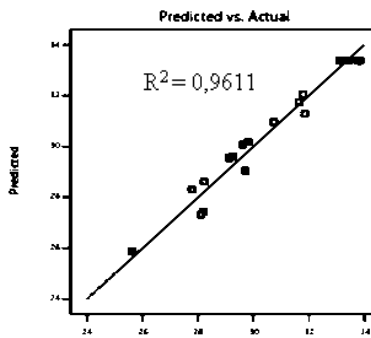
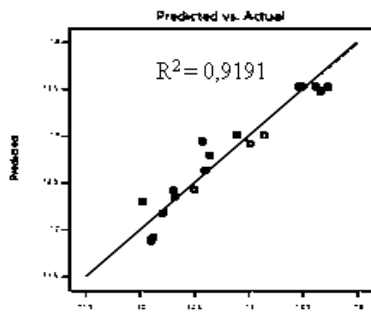
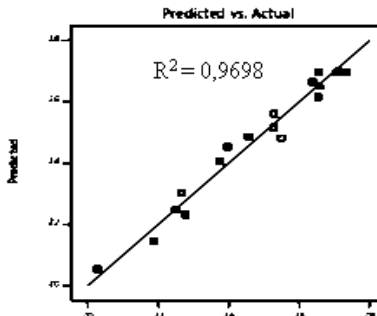
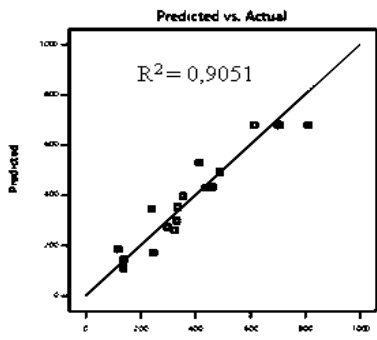


Figure 1. Predicted vs. actual values of the response functions from Y1 to Y5 forward top to bottom.

Figure 2. Response surface plots and contours of temperature and enzymic concentration versus response functions from Y1 to Y5 forward top to bottom.

Obviously, all above equations showed the correlation between variables and responses (equations 10-14). Except for the interaction between (MTGase concentration) and (reaction time) on Y2 (yield) and Y3 (protein content), all variables were not interactive to each other on responses. The polynomials well explained response variations ($R^2 > 0.9$, p -value < 0.05). The predicted values for all response functions are close to the experimental values ($R^2 > 0.9$, Figure 1). These correlations demonstrated that the models were applicable.

3.2 Determination of technological values dairy treatment process with MTGase

The analytical results (Figure 2, Table 3) predicted that there was no defined condition to satisfy all five response functions. Respectively, the maxima of yield (Y2), protein content (Y3), total solid content (Y4), sensory evaluation score (Y5) were reached at ranges of MTGase concentration (Z1 from

2.01 to 2.71), temperature (Z2 from 41.54 to 52.18 oC), reaction time (Z3 from 3.61 to 4.37 hours).

The multi-objective optimized values of a set of five response functions (8-12) were statistically defined with a desirability of 0.913 as follow variables: MTGase concentration (Z1), reaction temperature (Z2) and reaction time (Z3) of enzymatic treatment were 2.59 U/g protein, 36.14°C and 4.53 h, respectively (Table 4).

Furthermore, following above multi-objective optimized variables, practical experiments were done to verify the predicted values. Obviously, verified and predicted values were insignificantly different (Table 4). Thus, the mathematical model was fitted to practical results.

According to the solving of multi-objective optimization problem, the technological process of manufacture of fresh cheese was presented in Figure 3.

Table 3. The solution of each (single) response function

Response function	Optimized value	Variable		
		Z ₁ , U/g protein	Z ₂ , °C	Z ₃ , h
Y ₁ – Hardness, g	480.499	2.94	41.53	3.30
Y ₂ – Yield, %	27.465	2.71	42.53	3.61
Y ₃ – Protein content, %	13.610	2.01	52.18	4.22
Y ₄ – Total solid content, %	33.776	2.20	41.54	4.37
Y ₅ – Sensory evaluation score	13.223	2.18	42.86	3.84

Table 4. The multi-objective optimized response of verified samples

	Hardness (Y ₁), g	Yield (Y ₂), %	Protein content (Y ₃), %	Total solid content (Y ₄), %	Sensory evaluation score (Y ₅)
Optimized (predicted) values	480.500	27.396	13.372	33.341	12.721
Verified values	471 ± 14.91	26.93 ± 0.62	13.76 ± 0.40	32.78 ± 0.70	11.81 ± 1.01

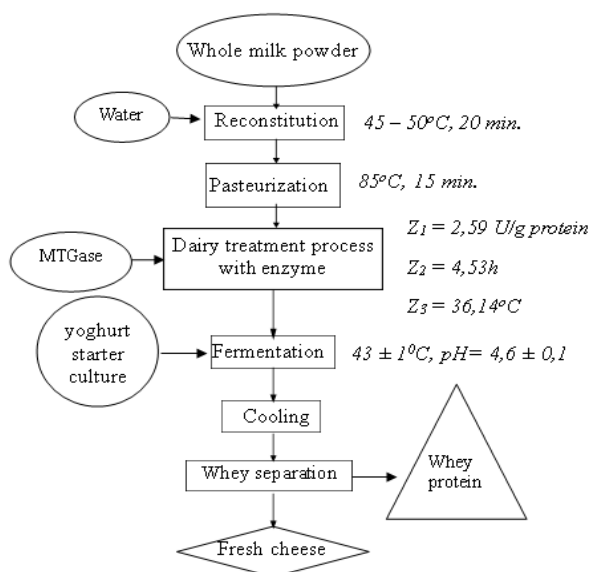


Figure 3. Flowchart of technological process of manufacture of fresh cheese

3.3 Quality properties of fresh cheese

Fresh cheese sample was produced under the multi-objective optimized condition of MTGase treatment above (Figure 3). Practically, the protein, lipid and total solid contents (%) of this cheese were 13.76, 16.00 and 33.20, respectively. As a result, whole milk powder (raw material) caused the high fat content of the cheese.

To characterize the influence of MTGase on quality properties of fresh cheese, four samples under optimized condition above were done. Two of the samples were made from whole milk powder with (M1) and without MTGase (M2). The others were made from raw milk with (M3) and without MTGase (M4). The microstructure property of the samples was determined (Figure 4).

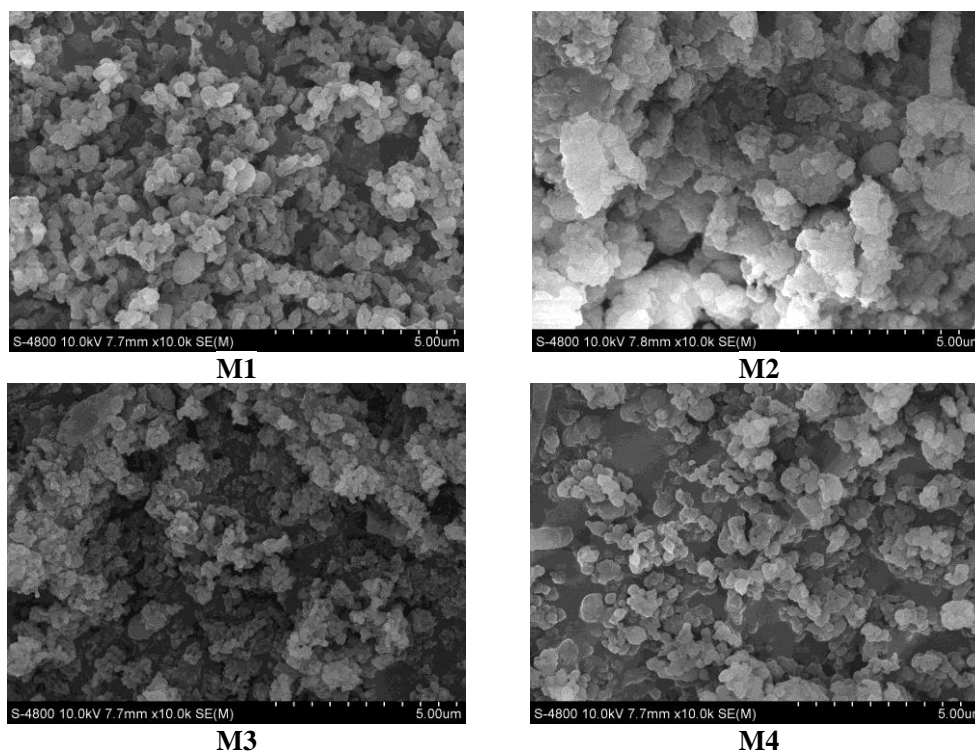


Figure 4. SEM images of samples

Obviously, the microstructural components of M1 were smaller than those of M2 products (Figure 2). It seems that M1 had more sporous structure than M2 cheese. Interestingly, the similar phenomenon was found in cheese made from fresh milk (M3 and M4). The changes in microstructure of fresh cheese manufactured with MTGase could be

explained by the creation of cross-linking between protein molecules. In the sample without enzyme treatment, the components were separated by large gaps, whilst the microstructure of the enzyme-treated gel was more homogeneous. Thus, MTGase-treated cheese contained not only a collection of small constituents linked together but also smoother

networks with smaller constituent chains and gaps. Basically, cross-linking by MTGase helps prevent phase separation. Actually, this phenomenon was similar to a research of Schorsch C. et al. (2000). In that study, a comparison of casein micelles microstructure, with and without MTGase treatment, was done. In this study, fresh cheese samples (M3, M4, which made from fresh milk), had less tight structure, smaller particle size and fewer pores than others made from whole milk powder (M1, M2).

SEM images (Figure 3) of M1 and M2 samples made from whole milk powder at all magnification showed that the microstructure of components in M1 samples was smaller than those in M2 products. Similarly, in M3 and M4 samples made from fresh milk, SEM

images also show smaller particle sizes in enzyme-treated samples. The change in microstructure of fresh cheese manufactured with MTGase can be explained by the creation of cross-linking between proteins.

The quality properties of cheese samples (whey separation, titratable acidity, total count of LAB and sensory evaluation score) were determined (Table 5). The results showed that the fresh cheese samples (M1) had less whey separation than that in the sample without enzyme treatment (M2). The titratable acidity recorded in fresh cheese samples treated with MTGase (M1, M3) was lower than that of similar samples without enzyme (M2, M4). This can be explained by the fact that some alkaline products (NH₃) can be formed during cross-linking [9].

Table 5. *Quality properties of fresh cheese samples with/without MTGase*

Sample	Whey separation (%)	Titratable acidity, (°T)	Total count of LAB, (LgCFU/g)	Average sensory evaluation score
M1	0	11,76±0,16 ^a	8,05±0,03 ^a	14.00±0,48 ^a
M2	0,89±0,11	11,97±0,54 ^a	8,20±0,05 ^b	13.28±0,56 ^a
M3	0	13,67±0,6 ^b	8,17± 0,01 ^b	13.71±0,42 ^a
M4	0	14,68±0,27 ^b	8,31± 0,02 ^c	13.29±0,42 ^a

The total count of LAB in cheese samples treated with enzyme (M1, M3) was lower than that of fresh cheese samples without enzymic treatment (M2, M4). According to Ozer et al. [10], there was no toxic side effect of MTGase on LAB. The only possible effect was the delayed growth of LAB due to the low-molecular-weight peptides and/or amino acids necessary for the growth of *Str. thermophilus* was cross-linked together by MTGase and became unavailable. Similar results were found in the studies of Bonisch [11] Karzan [12] and Lorenzen [13].

The sensory evaluation scores of cheese samples made from powdered milk (M1, M2) were not different from those of cheese samples made from fresh milk (M3, M4).

These results (Figure 4, Table 5) showed that the ability to produce fresh cheese products from whole milk powder was entirely

feasible. The quality properties of fresh cheese (sensory evaluation score, syneresis, acidity and the total number of lactic acid bacteria) met the CODEX STAN 243-2003 revised 2010 [14] for fresh cheese products.

4 CONCLUSION

The application of the response surface methodology was effective in optimizing the parameters for the microbial transglutaminase activity in fresh cheese production. The enzymatic treatment under optimized conditions (MTGase concentration of 2.59 U/g protein at 36.14°C and enzyme treatment time was 4.53 hours) improved product recovery efficient, protein content and sensory evaluation of products due to the high-molecular-weight polymers formed during the cross-linking reaction.

SEM images also showed that the samples undergoing with MTGase treatment

had more homogeneous microstructure network with smaller elements than those of the fresh cheese samples without MTGase.

The results of quality properties' analysis showed that the quality of fresh cheese samples made from whole milk was within

the permitted level. The application of researching technological process to produce fresh cheese using MTGase can limit the use of other additives such as stabilizers, thickeners, gelatin, agar, etc.

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