

Chemical Characteristics of Goat Cheese with Different Percentages of Mixed Indigenous Probiotic Culture during Ripening

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(Received 10-11-2016; Reviewed 27-12-2016; Accepted 23-02-2017)

ABSTRACT

This research was aimed to study the chemical characteristic of goat cheese that used various concentrations of probiotic starter with combinations of *Lactobacillus rhamnosus* TW2 and *Lactobacillus plantarum* TW14 isolates. The experiment was conducted with a completely randomized design with a 4 x 4 factorial arrangement. The first factor was the concentration of probiotic starter consisted of 4 levels i.e., 2.5, 5.0, 7.5, and 10% v/v. All probiotic concentrations used the same ratio of *L. rhamnosus* TW2 and *L. plantarum* TW14, i.e. 1:1. The second factor was the duration of ripening process consisted of 4 levels i.e., 0 (no ripening), 10, 20, and 30 days at 5°C. The observed variables were cheese chemical properties including water content, protein, fat, ash, pH, total titratable acidity (TTA), free fatty acids (FFA), and proteolysis profile. The results showed that addition of probiotic starter at a concentration of 5% mixture of *L. rhamnosus* TW2 and *L. plantarum* TW14 increased ($P<0.01$) only on ash content. The duration of ripening process up to 30 d significantly increased ($P<0.01$) protein and fat. Combination of probiotic starter concentrations and duration of ripening process increased ($P<0.01$) fat and ash contents. Proteolysis profile showed that protein was degraded into 72 and 52kDa proteins, but no 17kDa protein was found in cheese ripened for 30 d. It is concluded that 30 d duration of ripening at 5°C was the main contributing factor to chemical characteristics of cheese including chemical properties, pH, TTA, FFA, and proteolysis profile of probiotic goat cheese, while the concentration of mixed probiotics affected ash content, TTA and FFA of cheese.

Keywords: *Lactobacillus rhamnosus* TW2, *Lactobacillus plantarum* TW14, cheese, starter, characteristics

ABSTRAK

Penelitian bertujuan untuk mempelajari karakteristik kimiawi keju kambing yang diberi beberapa konsentrasi starter probiotik dengan campuran isolat *Lactobacillus rhamnosus* TW2 dan *Lactobacillus plantarum* TW14. Penelitian menggunakan metode eksperimental dengan rancangan acak lengkap pola faktorial berukuran 4 x 4. Faktor pertama adalah konsentrasi penambahan starter probiotik yang terdiri atas 4 level, yaitu 2,5; 5,0; 7,5; dan 10% v/v dengan rasio 1:1 antara *L. rhamnosus* TW2 dan *L. plantarum* TW14. Faktor kedua ialah lama simpan yang terdiri atas 4 level, yaitu 0 (tanpa disimpan), 10, 20, dan 30 hari pada suhu 5°C. Variabel yang diukur adalah karakteristik kimia keju, yaitu kadar air, protein, lemak, abu, pH, total asam tertitrasi (TAT), asam lemak bebas (ALB), dan profil proteolisis. Hasil penelitian menunjukkan penambahan konsentrasi starter sebesar 5% campuran *L. rhamnosus* TW2 dan *L. plantarum* TW14 meningkatkan ($P<0.01$) kadar abu keju, sedangkan lama penyimpanan sampai dengan 30 hari meningkatkan ($P<0.01$) kadar protein dan lemak keju. Interaksi antara konsentrasi starter campuran dan lama penyimpanan yang berbeda meningkatkan ($P<0.01$) kadar lemak dan abu keju. Profil proteolisis menunjukkan bahwa pemecahan protein terjadi pada protein dengan BM 72 dan 52 kDa, sedangkan protein dengan BM 17 kDa tidak mengalami pemecahan protein selama 30 hari penyimpanan. Dapat disimpulkan bahwa lama penyimpanan sampai dengan 30 hari pada suhu 5°C merupakan faktor utama yang mempengaruhi karakteristik kimiawi keju, yang meliputi komposisi kimia, pH, TAT, ALB, dan profil proteolisis keju kambing probiotik sedangkan konsentrasi campuran probiotik mempengaruhi kadar abu, TTA dan FFA keju.

Kata kunci: *Lactobacillus rhamnosus* TW2, *L. plantarum* TW14, keju starter, karakteristik

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INTRODUCTION

Improving consumers' awareness on the importance of functional food has positively affected the development of healthy food products. Probiotic-enriched food product belongs to a functional food category. Probiotic is defined as a living organism that when it is given in a proper amount around 10^5 - 10^7 CFU/g can give health benefits to the host (FAO/WHO, 2002). Some fermented products that contain probiotic are cheese, yoghurt, concentrate yoghurt, and kefir. Probiotic in cheese functions as a starter and it is within the cheese matrix during the ripening process. The use of probiotic strains as functional ingredients in dairy products offers health benefit to digestive tract in various conditions. *Lactobacillus rhamnosus* TW 2 and *Lactobacillus plantarum* TW 14 that were isolated from Ettawa goat milk (Setyawardani *et al.*, 2011) belong to the group of *in vitro* and *in vivo* probiotics (Setyawardani *et al.*, 2014).

Goat cheese is a product with a potential development as a functional food product because it is the source of macro nutrient substances and contains micro nutrients and bioactive peptide that highly beneficial for health (Korhonen & Pihlanto, 2006). Cheese as a food is believed to have health benefits (Choi *et al.*, 2012) due to the release of bioactive peptides during protein hydrolysis process and temperature treatment or microbial enzyme (Hafeez *et al.*, 2014). The peptide has many benefits for health including antioxidant, antimicrobial, anti-hypertension, and so on (Nagpal *et al.*, 2011).

During ripening, cheese will undergo physical, chemical, or microbiological changes. During the ripening process, the texture becomes smoother, consistency is solidified, the flavor is perfected, and the probiotic metabolite of Lactic Acid Bacteria (LAB) is optimally produced. Those changes are due to LAB interaction in the cheese matrix. Some researchers used probiotic with different strains, such as Himalayan cheese (Mushtaq *et al.*, 2016), Scarmorza cheese (Guidone *et al.*, 2015), and Minash freshal cheese (Dantas *et al.*, 2016).

Temperature and duration of ripening affect the outcome characteristics of cheese. Ripened at different temperatures, cheese will undergo change in pH value, LAB viability, and proteolysis process. The pH of the product tends to decrease because of metabolic activity of probiotic LAB. During proteolysis, peptides and proteins dissolve into simple amino acids that serve in forming the flavor and texture of the cheese. Probiotics can survive during the ripening and processing and does not produce unusual aroma and texture (Albenzio *et al.*, 2013).

This research was designed to study the chemical characteristic of goat cheese produced by using various durations of ripening and concentrations of probiotic starter with mixture of isolates *L. rhamnosus* TW2 and *L. plantarum* TW14.

MATERIALS AND METHODS

This experiment used goat milk derived from Ettawa grade (PE) in breeding and production center of PE goat, PEGUMAS, in Central Java, Indonesia,

while the probiotic culture of *L. rhamnosus* TW2 and *L. plantarum* TW14 were isolated by following the method used by Setyawardani *et al.* (2011). Commercial animal rennet, microbiology media of MRSB (deMan Rogosa and Sharpe Broth), Phosphate Buffer Saline (PBS), and chemicals were collected for laboratory analysis. The equipments were tools for cheese production namely incubator, cheese cloth, cheese presses and pan, chemical (proximate) analysis apparatus such as soxlet, Kjeldahl, oven, and SDS-PAGE Electrophoresis.

Production of Probiotic Starter

Starters from isolates *L. rhamnosus* TW2 and *L. plantarum* TW14 were inoculated separately in MRSB media and incubated at 37°C for 24 h until the cells grows that was indicated by the increased turbidity. Then, 5% (v/v) of the probiotic starter was added to the goat milk and incubated at 37°C for 6 h to produce a 10^8 cfu/mL final concentration of starter that was examined by serial dilutions.

Production of Goat Milk Cheese

The cheese was produced by pasteurizing milk at 63°C for 30 min, then the temperature was decreased to 37°C. The pasteurized milk was then added starter with concentration according to treatments but in a constant ratio of 1:1 (v/v) of *L. rhamnosus* TW2 and *L. plantarum* TW14 and the mixtures were re-incubated at 37°C for 6 h to reach the pH of 6.1. Afterwards, 0.06 ml/L of animal rennet was added and incubated at 37°C for 2 h to form gel. The gel was sliced and rested for 10-15 min for syneresis. Heating of the gel was conducted at 40°C for 30 min and then it was filtered to separate the whey to obtain the remaining matrix called curd, which was ripened at cool temperature (5°C), without a package.

Analysis of Chemical Characteristics of Goat Milk Cheese

Procedure of chemical analysis of cheese. Cheese composition comprising water, protein, and fat contents was analyzed using standard method (AOAC [Association of Official Analytical Chemists], 2006).

Procedure of measuring pH and acidity

The pH of cheese. The pH of cheese produced was measured using glass-electrode pH meter (Hanna Instrument) for 10 g cheese pulverized with 10 mL distilled water (Shakeel-Ur-Rehman *et al.*, 2003).

Free Fatty Acid (FFA). Determination of FFA in the goat cheese was conducted by titration of NaOH 0.1 N (Sudarmadji *et al.*, 2007). First, ten grams of sample was placed in Erlenmeyer tube, added with 50 mL of 96% alcohol and 2 mL of pp. indicator. The solution was titrated with standardized 0.1 N until pink color appeared and rested for 30 s.

Total Titratable Acidity (TTA). Determination of total titratable acidity in goat cheese was aimed to measure the

amount of organic acid in the sample (Sudarmadji *et al.*, 2007).

Proteolysis profile with electrophoresis. One gram of cheese was defatted by extracting with diethyl ether, dissolved in solution containing 1 mL of EDTA (1%), 1 mL of natrium deoxycholic (1%), and 5 mL of urea (50% w/v). The pH of the solution was set at 7.0 and ripened at 4°C for 24 h. Cellulose acetate electrophoresis of casein fraction was measured with electrophoresis unit. Buffer was made of 18 g Tris barbital-sodium barbital buffer (pH 8.6-9.0) and acid blue stain (100 mL *acid blue* colorant with 50% acetic acid). As much as 5 µL of sample was put in well, and placed in an acetic cellulose plate for 10 s. Electrophoresis was performed at 185 volt for 15 min and the plate was soaked in acid blue stain for 25 min and the stain was removed with 5% trichloro acetic acid in three-time washing until the background turned white. The last cleansing was with mixed solution of methanol: glacial acetic acid: cleaning solution with a ratio of 70:30:4 v/v/v. The cellulose plate was dried at 70°C for 8-10 min. The dissolved-casein fraction was measured with the remaining total area.

Experimental Design

The experiment used a completely randomized design with a 4x4 factorial arrangement each with 3 replications. The first factor was the mixed concentration of probiotic starters of isolates *L. rhamnosus* TW2 and *L. plantarum* TW14 with 4 levels i.e., 2.5, 5.0, 7.5, and 10 % v/v. The second factor was the duration of ripening at 5°C i.e., 0 (no ripening), 10, 20, and 30 d.

Statistical Analysis

The obtained data were subjected to analysis of variance (ANOVA), followed by Duncan test for the difference among treatments (*Software* SPSS 17.0).

RESULTS

Cheese produced with the addition of mixed isolates of *L. rhamnosus* TW2 and *L. plantarum* TW14 produce 54.00±1.38% to 65.12±1.93% water content and is included into a soft cheese category (Table 1). The highest water content was observed in unripen cheese with the addition of 2.5% probiotic starter. However, the protein contents of cheese produced with the addition of 2.5% probiotic starter increased when the duration of ripening was 30 d. The averaged fat contents in this research ranged from 4.05±0.50% to 29.03±1.76%. The lowest fat content was observed in the cheese produced by addition of 2.5% probiotic starter without ripening and the highest fat content was observed in the cheese produced by addition of 2.5% probiotic starter with 20 days of ripening. Analysis of variance showed that the addition of starter up to 10% did not significantly affect ($P>0.05$) cheese fat content, but 30 d duration of ripening and its interaction significantly increased ($P<0.01$) this parameter. Duncan test revealed that after 10 d of ripening, cheese had higher fat content and remained high during 30 d of ripening. The increased fat content during 30 d of ripening was related to the ability of the starter used to hydrolyze fat in cheese matrix; therefore it dissolved fat that was detected as cheese total fat.

The average of ash content was 1.74±0.20% to 3.09±0.07%. The lowest level of ash content was observed in cheese produced with the addition of 7.5% probiotic starter with the duration of 10 h ripening. The

Table 1. Average percentages of water, protein, fat, and ash contents of goat milk cheese with different starter concentrations and durations of ripening process (%)

Starter concentration (%)	Duration of ripening process (day)	Variables			
		Water	Protein	Fat	Ash
2.5	0	65.12±1.93 ^A	10.76±1.31 ^A	4.05±0.50 ^A	2.28±0.27 ^C
	10	56.24±2.60 ^B	15.29±1.82 ^B	25.83±3.09 ^B	1.82±0.22 ^A
	20	54.00±1.38 ^B	18.74±1.13 ^B	29.03±1.76 ^B	2.41±0.14 ^A
	30	54.06±0.76 ^B	18.47±0.61 ^B	27.24±0.91 ^B	1.79±0.06 ^{AD}
5.0	0	62.87±0.68 ^A	12.66±0.47 ^A	9.10±0.34 ^A	3.03±0.11 ^{BC}
	10	56.44±1.32 ^B	16.50±0.99 ^B	23.71±1.43 ^B	1.86±0.11 ^B
	20	56.52±0.76 ^B	16.63±0.58 ^B	25.21±0.88 ^B	2.45±0.08 ^{BD}
	30	56.47±2.07 ^B	17.64±1.68 ^B	25.41±2.42 ^B	2.44±0.23 ^B
7.5	0	62.78±2.23 ^A	12.08±1.47 ^A	15.28±1.86 ^A	2.36±0.28 ^{AC}
	10	57.72±1.53 ^B	16.24±1.87 ^B	21.51±2.49 ^B	1.74±0.20 ^A
	20	56.79±2.83 ^B	16.87±2.19 ^B	21.90±2.86 ^B	2.19±0.28 ^{AD}
	30	56.98±2.13 ^B	15.49±1.51 ^B	23.14±2.26 ^B	1.99±0.19 ^A
10	0	61.80±0.63 ^A	13.60±0.45 ^A	16.69±0.56 ^A	2.09±0.07 ^{AC}
	10	55.68±3.13 ^B	16.77±2.40 ^B	25.52±3.65 ^B	1.92±0.27 ^A
	20	56.20±0.82 ^B	16.46±0.62 ^B	25.43±0.97 ^B	2.09±0.75 ^{AD}
	30	56.67±1.70 ^B	17.43±1.37 ^B	23.03±1.81 ^B	2.21±0.17 ^A

Note: Means in the same column with different superscripts differ very significantly ($P<0.01$).

highest level of ash content was found in cheese processed with the addition of 5.0% probiotic starter prior to or without ripening. Analysis of variance showed that the addition of mixed starter of *L. rhamnosus* TW2 and *L. plantarum* TW14 and the duration of ripening significantly significantly increased ash content of cheese. Total ash content upon 30 d of ripening was 3.09% that was lower than 4.37% reported by previous study (Dervisoglu & Aydemir, 2007).

Average pH Value of Cheese

Cheese products from several mixtures of isolate starters of *L. rhamnosus* TW2 and *L. plantarum* TW14 and ripened for 30 d had pH values ranged from 4.97±0.13 to 6.55±0.20 (Table 2). The change in pH during cheese production and ripening process is one of the indications of LAB activity. Observation on cheese pH during ripening showed that the concentrations of probiotic starter did not affect pH value of cheese while the duration of ripening (0, 10, 20, and 30 d) significantly decreased (P<0.05) the pH values of cheese produced. However, there was no interaction effect of concentrations of probiotic starter and the duration of ripening was observed on the pH of cheese produced. Duration of ripening for 30 d decreased the pH of cheese, started from the 10th day to the 30th day. The lowest pH value was found in cheese processed with the addition of 7.5% probiotic starter with the duration of ripening of 30 d, while the highest was found in that processed with 5.0% probiotic starter without ripening process (fresh).

Total Titratable Acidity (TTA)

Total Titratable Acidity (TTA) during 0 to 30 d of ripening showed that the highest average (0.20±0.03)

was found in cheese ripened for 20 and 30 d, regardless of concentrations of probiotic starter. Regardless of the duration of ripening, the highest average of TTA (0.17±0.07) was found in cheese processed with the addition of 2.5% prebiotic starter (Table 3). The concentration of probiotic starter used, as well as the duration of ripening process, significantly (P<0.01) increased the amount of TTA, but there was no interaction effect of those factors on the TTA (P>0.05). Lactic acid is a metabolite product from *L. plantarum* TW14 included in homofermentative LAB that produces dominant lactic acid (Setyawardani *et al.*, 2011). Total titratable acidity increased after more than 10 d of ripening and the highest was found at the 20th d. Ripened for 20 and 30 d, cheese showed the highest bacteria population, namely 10.04 and 10.98 log cfu/g, respectively (unpublished data). The result was supported by the decreased pH level up to the 30th d of ripening. The result of TTA during 30 d ripening was 0.20±0.03 that was lower than 2.38±0.03 reported in traditional Kulek cheese (Dervisoglu & Aydemir, 2007).

Free Fatty Acid (FFA)

The duration of ripening process significantly increased (P<0.01) the FFA in all starter concentrations (Table 4). The lowest FFA level, was 0.19±0.02%, found in unripen cheese and the level started to increase with the duration of 10 d ripening. The duration of ripening of 20 and 30 d had equal 0.52% to 0.57% FFA which started to increase after 10 d. The increased FFA during ripening was also reported in Baladi goat cheese (Tabel *et al.*, 2016).

Table 2. Average pH values of goat milk cheese with different starter concentrations and durations of ripening process

Starter concentration (%)	Duration of ripening process (day)				Average
	0	10	20	30	
2.5	6.51±0.16	5.42±0.20	5.10±0.13	5.03±0.20	5.51±0.64
5.0	6.55±0.20	5.51±0.08	5.15±0.05	5.05±0.00	5.56±0.62
7.5	6.43±0.17	5.52±0.08	5.28±0.28	4.97±0.13	5.55±0.59
10	6.47±0.11	5.53±0.20	5.05±0.50	5.05±0.07	5.43±0.65
Average	6.49±0.15 ^a	5.43±0.16 ^b	5.14±0.16 ^c	4.99±0.12 ^d	

Note: Means in the same column with different superscripts differ significantly (P<0.05).

Table 3. Total titratable acidity (TTA) of goat milk cheese with different starter concentrations and durations of ripening process (%)

Starter concentration (%)	Duration of ripening process (day)				Average
	0	10	20	30	
2.5	0.06±0.03	0.15±0.02	0.23±0.01	0.21±0.01	0.17±0.07 ^A
5.0	0.06±0.01	0.11±0.05	0.19±0.02	0.17±0.02	0.13±0.07 ^B
7.5	0.07±0.00	0.11±0.04	0.15±0.04	0.19±0.03	0.13±0.06 ^B
10	0.07±0.00	0.13±0.01	0.19±0.05	0.21±0.01	0.15±0.07 ^{AB}
Average	0.06±0.012 ^A	0.13±0.04 ^B	0.20±0.04 ^C	0.20±0.03 ^C	

Note: Means in the same row and column with different superscripts differ very significantly (P<0.01)

Cheese Proteolysis Profile

Protein degradation did not occur in unripen cheese in band [1] (Figure 1). Band [2], after 10 days ripening, showed protein degradation and proteolysis of protein with molecular weight of 72, 55, and 43 kDa. Band [2] contained proteins with molecular weights of 34, 17, and 10 kDa, and the protein with molecular weight of 17 kDa was not found during 30 d ripening in all treatments. The process was started with protein hydrolysis with the conversion of water insoluble and soluble peptides into free amino acids.

DISCUSSION

Chemical Characteristic of Goat Cheese

Cheese with high water content has more free water to be used by bacteria, either the beneficial (starter) or the environment contaminant. Cheese with high water content is not favorable because the water weakens the structure of casein tissue, therefore the produced cheese is less solid (Banks, 2007; Yasin & Shalaby, 2013). The higher water content in cheese showed relatively low fat and protein contents (Jung *et al.*, 2013). Water content in cheese decreases during the ripening period because of syneresis process inside the cheese. In 120

days of ripening, water content decreased from 50% to 39% particularly in the inner part that contained more water than the outer side (Dervisoglu & Aydemir, 2007).

Starter and non-starter bacteria in cheese actively dissolve protein and peptide where LAB has a weak proteolysis capacity but proteinase can gradually digest protein into simple peptides and amino acids (Hayaloglu *et al.*, 2004). The use of mixed culture increases soluble protein and releases higher amino acids on the first day after cheese processing. The use of mixed cultures enhances the soluble protein and the release of amino acid that are occurred on the first day after the processing of cheese (Bezerra *et al.*, 2016).

Casein is the most important part in the production of cheese for coagulation to form curd then processed into cheese. The lower proportion of α_{s1} -casein in goat milk resulted in a softer curd milk as compared to those with higher α_{s1} -casein (Thomann *et al.*, 2008). Those differences were related to the processing technique and types of milk, but the effect on nutrient content of cheese product was still unknown (Raynal-Ljutovac *et al.*, 2008).

Mineral content in cheese is observed from the ash, protein, and fat contents, and milk minerals such as calcium, phosphorus, and magnesium are concentrated in curd during coagulation process (Miller *et al.*, 2006). According to Park (1990), ash content of goat milk cheese was higher in hard cheese than soft cheese. The

Table 4. Free fatty acid (FFA) values of goat milk cheese with different starter concentrations and durations of ripening process (%)

Starter concentration (%)	Duration of ripening process (day)				Average
	0	10	20	30	
2.5	0.21±0.06	0.43±0.04	0.72±0.07	0.68±0.08	0.51±0.22 ^A
5.0	0.15±0.01	0.31±0.01	0.47±0.05	0.45±0.09	0.34±0.07 ^B
7.5	0.19±0.02	0.30±0.14	0.38±0.08	0.56±0.16	0.35±0.17 ^B
10	0.21±0.04	0.36±0.03	0.51±0.02	0.60±0.09	0.42±0.18 ^{BC}
Average	0.19±0.04 ^A	0.35±0.10 ^B	0.52±0.14 ^C	0.57±0.12 ^C	

Note: Means in the same row and column with different superscripts differ significantly (P<0.01).

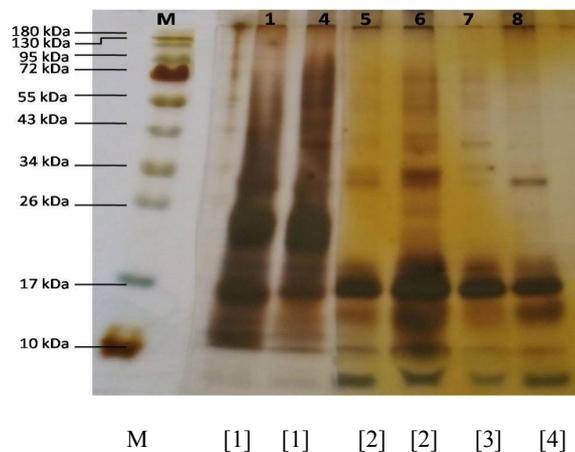


Figure 1. The proteolysis profile of cheese produced with starter *Lactobacillus plantarum* TW14 and *L. rhamnosus* TW2 during 30 days of ripening. M: Marker; [1] unripen goat cheese; [2] goat cheese ripened for 10 days; [3] goat cheese ripened for 20 days; [4] goat cheese ripened for 30 days.

difference was due to varied mineral contents in the milk used for cheese production. A rapid acidification process by lactic acid followed by a rapid whey formation accelerates demineralization process, while a slow acidification with a rapid coagulation can protect mineral content in milk. Concentrations of Ca, P, Na, and Cl were lower in soft cheese than hard cheese. The varied mineral content was assumed due to the different mineral contents in raw material and processing technique (Park, 1990).

The pH of Goat Cheese during Ripening

In addition to starter concentration, milk pH would affect the pH value of cheese product through enzymatic reaction and aggregation that lowered the stability of milk colloid (De Marchi *et al.*, 2009). During ripening, LAB cheese are growing, developing, and producing metabolite in the form of lactic acid that can lower the pH of cheese produced. Low pH value during ripening process plays a role in creating a non-conductive environment for the growth and development of other bacteria particularly pathogenic and spoilage bacteria. The final pH value in the ripened cheese was 4.99 ± 0.12 that was in line with previous finding by D'Amato *et al.* (2008) that cheese pH at the 30th day ripening was 5.1. During the final stage of cheese-making process and the initial stage of cheese ripening, LAB form lactic acids. The pH of cheese produced was also affected by the degree of development of added microbes; therefore different percentages of LAB culture addition would result in varied pH decreases. The pH of cheese produced decreases during 60 days of ripening because lactic acid produced and the release of certain amino acids such as aspartate and glutamate affect the pH of cheese (Sallami *et al.*, 2004). At the final stage of ripening, the pH of cheese did not increase, indicating the metabolic activities of the isolates; therefore lactic acid was still produced and accumulated that eventually decreased the pH of cheese produced.

TTA of Goat Cheese during Ripening

Total titratable acidity plays important role in all milk coagulation phases, including micelle aggregation rate of casein and rennet reactivity. Total titratable acidity also affects syneresis rate and determines how easy the milk will be turned into cheese. To produce cheese with premium quality, milk with low acidity (hypoacid milk) is generally unused because it produces cheese curd that is rheologically unwanted with its paste-like texture (Formaggioni *et al.*, 2001).

Surono (2004) stated that lactic acid resulted from fermentation process was in either dissociated or undissociated form, depended on pH value. Total titratable acidity increased during the ripening process and this result was supported by the values of cheese pH that significantly changed, and cheese made from heated milk would experience pH decrease during ripening followed by the increased total titratable acidity.

FFA Value of Goat Cheese during Ripening

The increased FFA concentration showed an increase in lipolysis activity. FFA served as precursors for flavor components such as methyl ketone, ester and fatty acid lactone, and alcohol (McSweeney & Sousa, 2000). Free fatty acids (FFA) were released during lipolysis along with the proteolysis components and products that were directly contributed to form the compounds that contribute to the flavor of the product. Lipolysis is defined as the accumulation of FFA during cheese ripening in which high FFA is released from triglyceride. Total FFA concentrations, both short-chain and long-chain FFA, were related to the type and amount of lipase during the ripening process and cheese sensory characteristics (Londono *et al.*, 2011). FFA value increased in cheese produced with the 20 days duration of ripening, indicating lipolysis process due to the activities of lipases derived from rennet, milk, or LAB. According to Melilli *et al.* (2004), lipolysis in cheese varied due to the different sources of lipase. Lipase can be derived from pregastric namely rennet paste, from milk, and from microbe. Lipase source is important during lipolysis in cheese. The other source of lipolysis is the use of salt during cheese salting (Lavasani *et al.*, 2012).

FFA increase after lipolysis, and are catabolized into methyl ketones by microbe. Methyl ketones decrease secondary alcohol by reductive bacteria as a mechanism against toxicity (Molimard & Spinnler, 1996). Methyl ketone and its derivatives played significant role as an aroma substance in soft cheese and ripened cheese (Gómez-Ruiz *et al.*, 2002).

Proteolysis Profile of Goat Cheese during Ripening

SDS-PAGE molecular weight between 22 and 18.5 kDa was mostly β -CN peptide such as β -CN (f1-190) and β -CN (f29-207). Fragment κ -CN (f1-105) was dominated by 10-15 kDa peptides. While the smaller one from β -CN fragments such as β -CN (f106-207) and β -CN (f108-207) and some α S2-CN had approximately 14-10 kDa. Cheese ripening is generally followed by the increased level of peptide hydrolysis into smaller peptide and amino acid. Intact protein tends to be lower after cool ripening for 28 days, but it is not significant and indicates a slow proteolysis in cheese (Hekken *et al.*, 2005). The process of milk homogenization increases the proteolysis activities (Juan *et al.*, 2016). The process of proteolysis during cheese ripening is started by the enzyme in milk (plasmin), rennet (pepsin and chymosin), and probiotic LAB. Enzyme activity would lower casein concentration (CN) (α s-1, α s-1, and κ -CN) in forming large-sized peptide and medium-sized peptide.

At the final stage of cheese ripening, primary and secondary proteolysis occur very rapidly, leading to 90% degradation of α s1- and β -casein (Diezhandino *et al.*, 2015).

Peptide was then hydrolyzed by a proteolysis enzyme produced by microbe (LAB starter, nonstarter, and adjunct probiotic) into a smaller peptide and free amino acid (FFA) that produced cheese flavor. The products of proteolysis directly gives cheese flavor or served

as a precursor substance of cheese flavor (Singh *et al.*, 2003).

CONCLUSION

It is concluded that 30 days duration of ripening at 5°C was the main contributing factor to chemical characteristics of cheese including chemical properties, pH, TTA, FFA, and proteolysis profile of probiotic goat cheese, while the concentration of mixed probiotics affected ash content, TTA and FFA of cheese.

ACKNOWLEDGEMENT

The author expressed sincere gratitude to Directorate General of Higher Education for the grant through National Strategic scheme in the year of 2014-2015.

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