

Texture, hedonic test and fatty acid profile of goat cheese with *L. plantarum* TW14 and *L. rhamnosus* TW2 isolates stored at different temperature conditions

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ABSTRAK

Penelitian bertujuan untuk mengetahui tekstur, kesukaan dan profil asam lemak kesukaan pada keju kambing yang disimpan dingin dan beku selama 60 hari. Starter yang digunakan berupa probiotik *L. plantarum* TW14 dan *L. rhamnosus* TW2 rasio (1:1/v/v). Penelitian dilakukan secara faktorial menggunakan dua faktor, sebagai faktor pertama adalah suhu (dingin; beku) dan sebagai faktor kedua adalah lama penyimpanan (0; 15,30,45 dan 60 hari) dan dilakukan 3 kali ulangan. Hasil penelitian memperlihatkan bahwa keju yang disimpan dalam refrigerator/suhu dingin mempunyai kekerasan tekstur rata-rata 374.46 ± 77.69 gf dan 221.66 ± 38.46 gf pada keju yang disimpan pada suhu beku dan berbeda sangat nyata ($P < 0.01$). Kesukaan keju untuk atribut rasa, tekstur dan kesukaan keseluruhan tidak berbeda nyata baik suhu maupun, lama penyimpanan ($P > 0.05$) tetapi untuk atribut aroma berbeda sangat nyata pada keju yang disimpan suhu yang berbeda ($P < 0.01$). Keju yang disimpan selama 60 hari pada penyimpanan dingin menunjukkan asam lemak terbesar yaitu asam lemak jenuh *Saturated Fatty Acids* (SFA) dengan rata-rata 64.34 % dan asam lemak tak jenuh *Unsaturated Fatty Acids* (USFA) sebanyak 16.76%. Selama penyimpanan 60 hari, persentase SFA relatif tetap sedangkan persentase USFA asam oleat meningkat.

Kata kunci : keju, suhu, kekerasan, sensori, asam lemak

ABSTRACT

The purpose of this research was to investigate texture, hedonic test and fatty acids profile of goat cheese stored at cold and frozen temperatures for 60 days. Cheese was manufactured from goat milk with addition of probiotics bacteria *L. plantarum* TW14 and *L. rhamnosus* TW2 with a ratio of (1:1/v/v). Treatments were arranged in a factorial design, employing two factors, i.e. temperature (cold; frozen) and storage time (0; 15,30,45 and 60 days). Each treatment was repeated three times. Results showed that goat cheese stored at cold temperature has an average hardness-texture of 374.46 ± 77.69 gf while that of the cheese stored at frozen temperature has 221.66 ± 38.46 gf, which were significantly different ($P < 0.05$). In term of flavor, texture and overall acceptability, there were no significant effects ($P > 0.05$) of storage temperatures and storage time. However, the taste of cheese stored under cold and frozen temperatures was highly significant different ($P < 0.01$). Fatty acids composition of cheese stored for 60 days in a cold storage showed that the highest fatty acid content was SFA which was 64.34% and USFA was 16.76%. During the 60-day storage, the SFA percentage of was relatively stable while the

percentage of oleic acid USFA increased.

Keywords: cheese, temperature, hardness, hedonic, fatty acids

INTRODUCTION

Goat cheese is a type of cheese that is commonly developed in goat milk-producing countries in particular countries with year-round tropical climate (Ribeiro and Ribeiro, 2010). The development of goat cattle as milk producer in Indonesia has been widely practiced, because the goat milk has advantages compared to cow milk. Several advantages of goat milk are more medium level of fatty acid chains, more fatty globules with smaller diameter, and the softer curd produced from the goat milk, so that it is best used as a raw material for cheese (Park and Haenlein, 2013).

Probiotic is a group of bacteria that is beneficial to health, especially to digestive tract. A great number of studies have proven the benefits of probiotics for health and it is applied within functional food and drinks (Albenzio *et al.*, 2015; Mushtaq *et al.*, 2016; Aguilar-Toalá *et al.*, 2017). Cheese is one of the best probiotics carrier according to several studies because cheese has a buffering capacity which plays a role in protecting probiotic bacteria in cheese matrix (da Cruz *et al.*, 2009). *L.plantarum* TW14 and *L.rhamnosus* TW2 are two types of probiotic bacteria that come from goat milk (Setyawardani *et.al.*, 2011) with characteristics of excellent probiotic, both in vitro and in vivo (Setyawardani *et al.*, 2014) which are applied in cheese (Setyawardani *et al.*, 2017).

Cheese is usually stored at room or cold temperature, whereas at cold temperature, cheese has longer shelf life compared to when it is stored at room temperature. During cold storage, the metabolism process goes more slowly so the changes go slowly, and the viability of probiotic LAB can still be maintained up till 10^8 cfu/g for 30-day storage (Setyawardani and Sumarmono, 2015).

It is uncommon to store cheese at frozen temperature, however, storing cheese at frozen temperature is still needed for certain purposes, such as distributing cheese across regions/islands or countries which may take long delivery time (Kuo and Gunasekaran, 2009; Karimi *et al.*, 2011). Frozen storage is needed to maintain cheese quality during a very long period of distribution and sales. Studies on frozen storage on cheese are still limited, possibly because as

long as cheese is stored frozen, there is only insignificant change in cheese quality. On the other hand, there is a possibility of change quality in cheese stored in cold storage, which may lead to a decreased quality. When cheese is stored in frozen temperature, the ice matrix will be formed which influence the texture and chemical composition or LAB metabolism will change that allows cheese lipolysis and proteolysis process (Calzada *et al.*, 2014; Juan *et al.*, 2015; Bezerra *et al.*, 2016). When cheese is stored cold or frozen, change in the texture occurs, in such that it will influence the physical characteristics and hardness. Furthermore, the fatty acid changes and cheese hedonic is also influenced by cheese storage. Fresh cheese would have a different hedonic level compared to previously frozen cheese. Therefore, the objectives of this research were to investigate texture, hedonic characteristics and fatty acids profile of goat cheese fatty acids with *L.plantarum* TW14 and *L.rhamnosus* TW2 isolates stored under cold and frozen temperature conditions.

MATERIALS AND METHODS

Goat milk was obtained from Etawah crossbred goats farm at Gumelar, Banyumas, Central Java. The milk was stored under a cold temperature until it was ready for use. The cheese manufactured from goat milk was stored at frozen and cold temperature, and it was stored for 0, 15, 30, 45 and 60 days. Every treatment was repeated three times.

Starter Preparation

Starter preparation was done following the procedures described by Setyawardani *et al.* (2017) Two isolates, *L.plantarum* TW14 and *L.rhamnosus* TW2, were isolated from Etawah Crossbred goat milk (Setyawardani *et al.*, 2011). One ose of isolates from each sample was taken and planted in De Man-Rogosa-Sharpe Broth (MRSB) media (Difco Laboratories, Michigan USA) then incubated for 24 hours at 37°C until ready to use.

Cheese Manufacture

Cheese manufacture was conducted

according Setyawardani *et al.* (2017). The goat milk was pasteurized at 72°C for 15 seconds, and then cooled down to 37°C. Starter, consisted of two isolates i.e. *L.plantarum* TW14 and *L.rhamnosus* TW2 of 5% with a ratio of (1:1) v/v, was added, and the milk was incubated at 37°C until the milk pH decreased to about 6.1. Then, a total of 0.6 ml/L rennet was added, mixed thoroughly, and the milk was left undisturbed for two hours to allow coagulation. The curd or coagulum was cut and heated at 40°C for 10 minutes, continued with whey separation using cheese-cloth. The curd was pressed to complete the whey separation, and stored under cold and frozen temperatures.

Cheese Texture

The cheese hardness was rated using texture profile analysis (Buriti *et.al*, 2007) of TA-XT21 equipment (Stable Micro Systems, Haslmere, England). The cheese samples were cut into cylindrical form of 3.0 cm height and 2.4 cm diameter. The ready-for-test cheese was taken out of freezer/cold, and placed at room temperature for sample conditioning. The pressure ratio used was 20% from the height of initial sample at 2 mm. The cheese hardness was rated using Texture Expert with Windows program 1.20.

Hedonic Test

Hedonic test was conducted by 30 semi-trained panelists using 1 to 9 scale score sheet. Sensory parameters included taste, flavor, texture, and overall acceptability. The cheese samples were taken out of cold, cut out with the size of 1.5x1.5x1.5 cm (Ong *et al.*, 2007). Sample evaluation was conducted with a scoring to evaluate the like and not-like level on attributes of taste, flavor, texture and overall acceptability. The previous samples which were adapted for 1 hour at room temperature were placed on a tray and each given a three-digit-number code. Coffee powder was provided to neutralize after sensory evaluation for flavor attribute. Members of the panel were asked to determine their level of hedonic on every cheese sample without comparing to other samples. The texture attribute was tested using hands to evaluate cheese texture.

Measuring Fatty Acid with Gas Chromatography

Fatty acids contents of cheese were determined following the procedures of AOAC

(2006). Analysis with gas chromatography was based on partition of components of fluid between the moving phases in terms of gas and static phase, in the form of solid and fluid which do not easily vaporize, attached on the inert supporting materials. The phases were started by hydrolyzing the fat into fatty acid, and then transformed it into an ester form which evaporates better. In this method, the transformation was conducted by way of methylation, until fatty acid ester methyl (FAME) was formed. Next, the FAME was analyzed using gas chromatography. Every component was identified by comparing its retention time against the standard on the same analysis condition. The retention time was calculated on a recorded paper as a distance from a line, when a solvent peak occurred till the center of the treated component peak. Determination of component content in the samples can be done with either external or internal standard technique. The area of each component is directly proportional to the total component in the sample. To minimize the error from injection volume, sample preparation, dilution, etc., it is better to use internal standard technique. In addition, the correction on detector response and interaction among components in the sample matrices must be conducted when it was passing the columns.

Statistical Analysis

The data collected, including quantified data of sensory evaluation, was presented as means \pm standard deviation. Procedure of general linear model was employed, followed by Duncan's Multiple Range Test for means comparison.

RESULTS AND DISCUSSIONS

Cheese Texture

The cheese texture is among parameters of cheese physical qualities that influence consumers' acceptance. The cheese texture changed during storage, which was influenced by chemical composition, in particular fat in the dry matter and moisture in the nonfat substances (Zheng *et al.*, 2016). Table 1 shows the effects of storage temperatures on cheese hardness.

The water content directly influences the firmness, higher water content creates a softer cheese texture and showed that there was an increase in proteolysis. In addition, the content of mineral and sodium in the cheese in the form of ionic will influence the texture (Guggisberg *et al.*, 2017). The cheese stored for 60 days at cold

Table 1. Cheese Goat Texture (hardness) Stored at Different Temperature for 60 Days of Storage (gf)

Temperature	Storage time (days)					Average
	0	15	30	45	60	
Frozen	275.95± 9.82	200.50± 6.36	196.30± 4.94	209.05±16.61	226.50±66.60	221.66±38.46 ^a
Cold	227.40±32.10	373.20±43.41	379.95±89.44	368.30±67.88	473.45±14.90	374.46±77.69 ^b

Different superscript in the same column show highly significantly different (P < 0.01)

temperature had a higher texture hardness, and there was a significant difference (P < 0.01) compared to the cheese stored at frozen temperature. Length of storing did not influence the cheese hardness. Storing at cold temperature increased cheese hardness, since the water evaporated during the ripening (Delgado *et al.*, 2011). Storing cheese at frozen temperature created a lower hardness compared to that of cheese texture stored at cold temperature. Ice crystals that got into cheese matrix during the frosting has weakened the bonds between proteins making the cheese texture softer and when the cheese was conditioned at room temperature before the rating began, the water was out/evaporated due to thawing process, therefore the cheese texture became softer. The low water content (the percentage of water in the material other than fat) is correlated with higher hardness and endurance, however, the springiness and cohesiveness of the cheese is lower (Zheng *et al.*, 2016). Proteolysis influences the establishment of flavor and off-flavor compound in the cheese, by releasing the peptide and free amino acids which thereafter a secondary reaction occurs. Splitting the protein tissue leads to a change of cheese matrix structure which will influence the resulting texture. (Delgado *et al.*, 2011). The hardness of cheese texture at frozen storage was lower than that at cold storage could happen due to a decrease of phosphate calcium content during the formation of colloidal cheese due to low pH, wherein pH 4.7 would result in stable colloidal cheese (Diezhandino *et al.*, 2016).

Cheese Hedonic Test

The cheese hedonic rated by the semi-trained panelists was flavor, aroma, texture, and overall cheese hedonic using a scoring scale (Table 2). Goat cheese stored at different temperatures and length storage time for 60 days has the level of cheese hedonic, texture and overall hedonic

relatively similar (P > 0.05), as rated by 25 semi-trained panelists. The level of hedonic towards cheese flavor ranged between 4.96 and 6.64 (goaty – cheese flavor) The cheese texture received the scores between 4.92 and 6.20 (soft-hard cheese) while the scores for overall cheese hedonic were 4.40-7.04 (less preferred- like). The cheese produced had white color, a typical of color of goat cheese and had a fresh aroma. In addition to being influenced by LAB the cheese flavor and texture was also generated from several complex compound produced from the process of proteolysis, lipolysis, and glycolysis during the storing. The typical cheese flavor and aroma produced from lipolysis process, proteolysis and degradation further amino acid by starter culture and non-starter culture bacteria lactose acid. The proteolysis product contributed directly in the cheese flavor or acted as a precursor compound of cheese flavor. The cheese received aroma scores between 4.40 and 7.26 (goaty- cheese flavor) in which the cheese aroma stored in cold temperature had a higher average score and significantly different (P < 0.01) compared to the cheese stored at frozen temperature. In cold storing, bacteria metabolism still occurred but slowly, therefore, the proteolysis and lipolysis process occurred slowly. In addition to the above, the enzyme produced by probiotic LAB could influence the biochemical process and might result in the cheese smell and aroma. The formation of cheese aroma and flavor was influenced by proteolysis process, which showed matrix casein hydrolysis in small peptide dissolved, and free amino acid which had a role on cheese sensory and texture. The above process was regulated by milk endogen enzyme, coagulants, and enzymes produced by starter and non-starter bacteria (Albenzio *et al.*, 2013). The sour taste of cheese came from citric acid, the bitter taste came from quinine and the salty taste came from NaCl (Albenzio *et al.*, 2013). Cheese

Table 2. Hedonic Test Goat Cheese Stored at Different Temperatures for 60 Days of Storage (Score)

Temperature	Storage time (days)	Taste	Flavor	Texture	Overall preference
Frozen	0	4.96	4.76 ^a	5.08	4.40
	15	5.60	4.40 ^a	4.92	5.40
	30	5.32	4.92 ^a	5.28	5.20
	45	5.52	5.28 ^a	6.00	5.32
	60	5.00	5.32 ^a	5.60	5.52
	Average	5.25	4.94	5.37	5.17
Cold	0	4.68	7.28 ^b	6.00	5.72
	15	5.72	5.84 ^b	5.28	5.36
	30	6.64	6.48 ^b	6.72	7.04
	45	6.16	5.76 ^b	6.20	5.68
	60	5.08	5.00 ^b	5.32	5.16
	Average	5.65	6.07	5.90	5.79

Different superscript in the same column show highly significantly different ($P < 0.01$)

texture stored at frozen received an average hedonic score of 4.94, while the cheese stored at cold temperature received an average score of 6.07. The cheese texture stored at cold temperature is harder than the cheese stored at frozen temperature. The addition of probiotic LAB *Lactobacillus casei* resulted in cheese with low pH values and high proteolysis indexes, with rigidity tended to decrease during storage (Dantas *et al.*, 2016). The above result is in accordance to the review on cheese texture hardness using texture profile analysis (TPA). The hardness on cheese was due to a low water content therefore making the cheese dryer on a lower pH. Changes in pH and calcium concentration did not adversely affect the cheese's cohesive characteristic although there was a change of cheese hardness (Soodam *et al.*, 2015). It has been reported that, biochemical changes during ripening contributed to the changes in textural characteristics of cheese (Andronoiu *et al.*, 2015).

The overall hedonic towards the cheese was an average score of 4.40-7.04 ((less preferred-like)) different treatment of temperature and storing did not influence cheese hedonic ($P > 0.05$). Hedonic towards a product is consumer assessment which is influenced by several attributes which contribute to the hedonic, among others: the sense of taste used to taste flavor and the sense of smell to rate the cheese aroma, and

the skin to feel the cheese texture.

Fatty Acid Profiles

The best result of cheese texture hardness and the test hedonic of aroma attribute by the panelists was the goat cheese stored at cold temperature which was stored for 60 days. Fatty acid profiles from the best result was then analyzed using a gas chromatography. Fatty acids in the identified cheese was the cheese stored at cold temperature, according to the result of physical quality and the level of consumer hedonic. The profile result of cheese fatty acids showed 26 types of fatty acids, in which the said fatty acids were dominated by palmitic fatty acid (C16:0) for saturated fatty acid and oleic fatty acid (C18:1n9c) for 60 days storage (Table 3).

Oleic acid belongs to acid group with the highest amount of acid and carries a characteristic of mammal milk. (Butler *et al.*, 2011; Mayer and Fiechter, 2012). During cheese storing, there was the same of tendency in the short, medium and long chain fatty acid group (Figure 1). The general average for the 60-day storage was the fatty acid with C2-6 of 3.77%, chain C7-11 of 9.98% and chain ≥ 12 of 70.88%.

Research data on cheese stored for 60 days at cold storage showed that the highest fatty acid was SFA i.e. an average of 64.34% and USFA of 16.76%. During the 60-day storage, the SFA

Table 3. Fatty Acids Profile of Goat Cheese Stored at Cold Temperature for 60 Days (%)

Fatty Acids	Storage Time (days)				
	0	15	30	45	60
Fat total	23.53	26.76	27.91	27.39	28.50
Caproic Acid, C6:0	4.74	4.16	3.39	1.61	1.46
Caprylic Acid, C8:0	1.60	1.96	1.91	2.08	1.67
Capric Acid, C10:0	5.23	6.84	6.72	6.79	5.33
Undecanoic Acid, C11:0	0.05	0.06	0.06	0.04	0.04
Lauric Acid, C12:0	1.89	2.80	2.80	2.39	1.87
Tridecanoic Acid, C13:0	0.04	0.05	0.05	0.05	0.04
Myristic Acid, C14:0	5.24	6.16	6.13	5.84	5.15
Myristoleic Acid, C14:1	0.06	0.08	0.09	0.05	0.05
Pentadecanoic Acid, C15:0	0.62	0.55	0.55	0.67	0.70
Palmitic Acid, C16:0	18.15	19.15	19.77	18.40	18.56
Palmitoleic Acid, C16:1	0.63	0.72	0.73	0.61	0.70
Heptadecanoic Acid, C17:0	0.57	0.52	0.55	0.71	0.77
Stearic Acid, C18:0	13.20	11.17	11.60	12.77	13.71
Elaidic Acid, C18:1n9t	3.89	2.47	2.39	2.86	2.41
Oleic Acid, C18:1n9c	22.12	20.11	21.61	20.67	24.54
Linolelaidic Acid, C18:2n9t	0.20	0.16	0.17	0.15	0.16
Linoleic Acid, C18:2n6c	3.24	2.53	2.81	2.28	2.42
Arachidic Acid, C20:0	0.18	0.17	0.17	0.15	0.18
Cis-11-Eicosenoic Acid, C20:1	0.58	0.47	0.51	0.72	0.71
Heneicosanoic Acid, C21:0	0.06	0.05	0.05	0.07	0.07
Cis-11,14-Eicosadienoic Acid, C20:2	0.05	0.04	0.04	0.04	0.05
Behenic Acid, C22:0	0.06	0.05	0.05	0.06	0.06
Arachidonic Acid, C20:4n6	0.14	0.15	0.16	0.13	0.13
Lignoceric Acid, C24:0	0.03	0.02	0.02	0.04	0.03
Cis-5,8,11,14,17-Eicosapentaenoic Acid, C20:5n3	0.04	0.03	0.04	0.06	0.08
Cis-4,7,10,13,16,19-Docosahexaenoic Acid, C22:6n3	0.02	0.03	0.03	0.04	0.04
Total fatty acids	82.64	80.48	82.41	79.27	80.94

percentage was relatively stable while the percentage of oleic acid USFA increased. The composition of fatty acids significantly determines the resulting fat, in which its characteristic depends on the length of carbon chain, the number of unsaturated bonds, whether it is of cis or trans geometrical configuration (Markiewicz-Kęszycka *et al.*, 2013).

The content of monounsaturated fatty acid (MUFA) in the cheese stored for 60 days was relatively lower, i.e. C14:1 of 0.05-0.09 %; C16:1 of (0.61 – 0.73%), this fatty acid contributed as precursor of CLA formation (Markiewicz-Kęszycka *et al.*, 2013). MUFA did not cause a build up of cholesterol as in SFA, and neither did it create rancid smell as in PUFA. MUFA had a

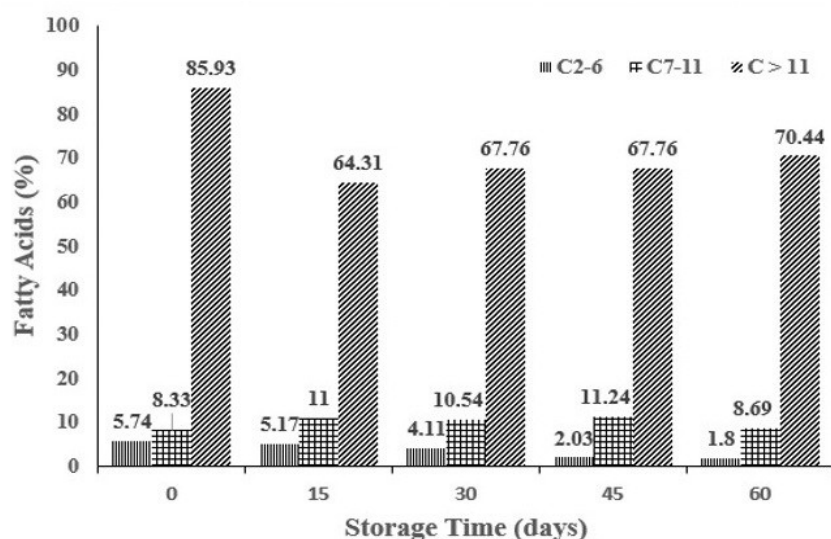


Figure 1. Percentage of Short, Medium and Long Chains Fatty Acids Cheese Kept at Cold Storage Up to 60 Days

positive effect on the controlling High-Density Lipoprotein (HDL), which acted to transport cholesterol from blood vessel wall to liver, which would be degraded by bile acids, then taken out. At the same time, MUFA would lower LDL concentration (Markiewicz-Kęszycka *et al.*, 2013).

CONCLUSION

The hardness of cheese stored in cold temperature was higher than the cheese stored at frozen temperature, and this is in accordance with the rate on texture hedonic by sensory. The highest proportion of cheese fatty acid stored at cold temperature for 60 days was SFA with an average of 64.34% and USFA of 16.76%, in which USFA was dominated by oleic acids and was a characteristic of mammal milk. Therefore, it is recommended to store probiotic-containing cheese under cold temperature for 60 days due to its texture and fatty acid profile.

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