CHEMICAL AND FREE FATTY ACID COMPOSITION OF GOAT MILK CHEESE RIPENED WITH *Lactobacillus acidophilus* AND EXTRACT RABBIT STOMACH AS COAGULANT

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Abstract

Rabbit stomach is waste from rabbit slaughtering that has the potential to be used as an alternative source of rennet for cheese production. The purpose of this study was to determine the effect of ripening by using *Lactobacillus acidophilus* on the chemical composition and free fatty acids (FFA) profile of goat milk cheese with extract of rabbit stomach as coagulant. *Lactobacillus acidophilus* (1%) was inoculated into pasteurized goat milk and incubated for 1 h, rennet extract up to 3% was added and incubated for 25 min. Cheese was stored at 4°C for 42 d. The moisture, fat, protein and ash content were analyzed at day 0, 21 and 42, whereas the FFA of goat milk cheese were analyzed on day 0 and 42. There were significantly differences in the moisture, protein, and ash content in the goat milk cheese on 21 and 42 days of ripening, but there was no significant difference in the fat content. The long-chain saturated (C16:0 and C18:0) and unsaturated (C18:2) FFA were the most abundant in goat milk cheese, and there were no significant differences between pre or post ripening. The concentration of short chain fatty acid (C6:0, C8:0 and C10:0) and the overall concentration of FFA in goat milk cheese after 42 days ripening was higher compared to those pre-ripening.

Keywords: Chemical composition, Free fatty acid, Goat milk cheese, Rabbit stomach

1. Introduction

The development of the cheese industry in Indonesia is usually constrained by the low quality of milk from farmers and also milk coagulant that is still imported. As is known that plants rennet often cause a bitter taste in the cheese. Therefore, the number of natural cheese industry in Indonesia is very limited. Rabbit stomach is waste of rabbit slaughtering can be used as an alternative in the cheese production. According to [1], the stomach of young rabbits represented most of the lipolytic activity of the whole digestive tract, and contained almost half of the total proteolytic activity.

Cheese ripening clearly involves a very complex series interrelated events, resulting in the development of the flavour and texture characteristic of the cheese variety. The most complex of these biochemical events, proteolysis, is caused by agents from a number of sources: residual coagulant (usually chymosin), indigenous milk enzymes, starter, adventitious non-starter microflora and, in many varieties, enzymes from secondary flora [2].

Lipids in foods may undergo hydrolytic or oxidative degradation. However, in cheese, oxidative changes are very limited due to the low oxidation/ reduction potential. However, triglycerides in all cheese varieties undergo hydrolysis by the action of indigenous, endogenous and/or exogenous lipases, which result in the liberation of fatty acids in cheese during ripening [3].

Free fatty acids contribute to the flavour characteristics of many types of cheese, either directly or indirectly, since they precursors for the formation of several aroma components, e.g. methyl ketones, lactones and aliphatic and aromatic esters [4]. Lipolytic agents in cheese generally originate from the milk, the coagulant (in the case of rennet paste) and the cheese microflora (starter, nonstarter and adjunct microorganisms). Milk contains a indigenous lipase, lipoprotein lipase (LPL), with a molecular mass of 55 kDa, that exists in milk as a homodimer [3]. The lipoprotein lipase (LPL) activity, although lower in goat than in cow milk, is more bound to the fat globules and better correlated to spontaneous lipolysis in goat milk. The regulation of spontaneous lipolysis differs widely between goats and cows. Goat milk lipolysis and LPL activity vary considerably and in parallel across goat breeds or genotypes, and are low during early and late lactation, as well as when animals are under fed or receive a diet supplemented with protected or unprotected vegetable oils [5].

The profile of free fatty acid during ripening

in goat milk cheese with a coagulant *Cynara cardunculus* extract has been studied by [4], but so far has not been studied in extract of rabbit stomach as coagulant. Therefore, the aim of this study was to determine the effect of ripening by using *Lactobacillus acidophilus* on the chemical composition and fatty acids profile during ripening in cheese produced from goat milk with extract of rabbit stomach as coagulan

2. Materials and methods

2.1. Rabbit stomach extraction

Rennet extraction was performed by modified procedure according to [6]. After removing the internal contents, rabbit stomach was washed with tape water internally while their veins and fat contents were removed externally, cut in small size and weighed. For stomach extraction was prepared by using mixture of acetic acid (1.5%) and NaCl (5%) solution. The pieces of stomach was mixed with solution mixture, covered and stirred overnight at room temperature. After stirring, the mixed was filtered with cheese cloth, and measured the pH value. NaOH 1.0 N was added, when pH of rennet extract less than 5.6 until reaches pH 5.6.

2.2. Goat milk cheese processing

Goat milk cheeses were manufactured according to the modified procedures of [7]. After pasteurization, the goat milk was cooled to 33–35°C, added 1% *Lactobacillus acidophilus* starter and incubated for 1 hour at 37°C. Extract of rabbit stomach (3%) was added to the milk and then allowed to coagulate for 30 min. The curd was cut using knives and allowed to heal for 5 minutes. The second cutting for 3 min, and the curds was cooked at 39°C for 10 min assuring for a firm curd. After draining whey, curds were moulded , pressed and brined in 18% salt solution for 4 hours. After removed from brining, curd was packed in aluminium foil and placed in plastic container in refrigerator for 21 and 42 days.

2.3. Chemical and fatty acid profile analysis

Cheese samples were analyzed for moisture by oven at 105° C. The ash content of cheese samples was determined by dry ash method and their total protein contents were determined by measuring total nitrogen using the Kjeldahl method and converting it to protein content by multiplying by 6.38 [8]. Fat content of cheese samples was analyzed by Babcock method [9] .

Free fatty acid concentrations of cheese samples were quantified using a gas chromatography (Shimadzu GC-2010). This procedure is based on AOAC Official Method 969.33/963.22 [10]). GC condition: Rtx-5 column (oven temperature 180º C, hold for 2 min; increase to 270ºC at 10ºC/min, hold for 4 min and total of running time 15 min. Carrier gas He 2.43 ml/min, flow rate of air 190 ml/min and flow rate H_2 80 ml/min. Injector temperature is 290ºC and temperature of flame ionization detector (FID) is 290ºC. The peak of sample chromatogram having the same retention time with the retention time of standard is the peak of fatty acid.

2.4. Statistical analysis

The data of chemical and fatty acids profile (3 replications) were statistically analyzed by One Way ANOVA and Duncan's multiple mean comparison using SPSS 12.0 [11].

3. Results

The moisture content of goat milk cheese after 42 day ripening was lower ($p<0.05$) than before ripening, whereas the protein and ash content after 42 days ripening were higher ($p<0.05$) than before ripening. During ripening, there was no significant differences in the fat content of goat milk cheese (Table 1).

Table 1. Chemical composition of goat milk cheese during ripening

Chemical composition (%)	Ripening (day)		
	0	21	42
Moisture	51.38 ± 5.44^{a}	44.48 ± 6.66^{a}	35.52 ± 2.10^{b}
Protein	15.37 ± 1.89^{a}	16.42 ± 0.60^{a}	20.42 ± 0.92^{b}
Fat	22.70 ± 4.25^{a}	21.83 ± 2.02^{a}	20.86 ± 0.86^{a}
Ash	8.40 ± 0.92^{a}	9.08 ± 0.74^{a}	10.80 ± 0.77^{b}

The different letter in the same row indicate significantly different ($p<0.05$)

Profile of free fatty acid of goat milk cheese before and after ripening was given in Table 2. The caproic, caprilic, capric acid and total of free fatty acid of goat milk cheese after 42 day of ripening were higher than before ripening ($p<0.05$). There were no significant changes during 42 day of ripening in the content of lauric, myristic, palmitic, palmitoleic, stearic, linoleic, linolenic and eicosanoic acid.

4. Discussion

The moisture content of goat milk cheese in

Table 2. Free fatty acid profile of goat milk

Fatty acids (%)	Pre-ripening (day 0)	Post- ripening (day 42)
Caproic, C6:0	0.17±0.08[a]	0.95±0.03[b]
Caprilic, C8:0	1.39±0.06[a]	1.67±0.04[b]
Capric, 10:0	5.73±0.22[a]	6.72±0.11[b]
Lauric, C12:0	2.60±0.23[a]	2.86±0.23[a]
Myristic, C14:0	7.91±0.58[a]	8.29±0.10[a]
Palmitic, C16:0	22.94±2.00[a]	23.72±1.14[a]
Palmitoleic, C16:1	1.38±0.04[a]	1.38±0.03[a]
Stearic, C18:0	37.07±1.93[a]	35.30±0.10[a]
Linoleic, C18:2	18.67±0.84[a]	17.58±0.17[a]
Linolenic, C18:3	1.11±0.13[a]	1.10±0.17[a]
Eicosanoic, C20:0	0.38±0.01[a]	0.36±0.01[a]
Total free fatty acid	19.98±2.3[a]	31.92±6.06[b]

The different letter in the same row indicate significantly different ($p<0.05$)

this study was similar to the previous study by [12]. However the protein and fat content of goat milk cheese in this study were lower, and ash content was higher than goat milk cheese according [12]. That goat milk cheese that produced from whole milk during 3 month ripening in refrigerator have TS 47.7% (moisture = 52.30%), fat 25.6%, protein 22.5% and ash 3.70% in goat milk. Vacuum-packed Kashar cheese during ripening for 5 – 90 days has moisture content of 41.46 – 42.55% and fat 26.35 – 26.83% [13]. The composition of the original milk from which cheese is manufactured can vary according to nutritional, genetic and physiological factors [14].

Different with the other study by [15], as ripening progressed of cheese that placed in airtight plastic containers without primary packaging, moisture and protein contents continuesly decreased whereas the total ash increased and the fat content remaining stable. When cheese is made with rennet, so many minerals bound in casein micelle, so that when the moisture content of cheese decrease, the minerals will increase. Conversely, if t

the cheese is made with acid, causes a progressive transfer of the calcium and the inorganic phosphate from micelle to the aqueous phase, that is, the coloidal calcium phosphate in milk is dissolve entirely by pH around 5.0 [16]. In this study, goat milk cheese was packed in aluminium foil as primary packaging and placed in non airtight plastic container, so that the moisture content more evaporated, and the protein seemed increased. There was no different in the fat content of the goat milk cheese in this study during ripening, althought the moisture content decreased after ripening. According to [17], water in cheese is found as either free or bound to the protein since fat, the other major component, is hydrophobic.

One should emphasise that the fat present in goat milk is a rich source of short chain fatty acids (SCFA – C6:0, C8:0, C10:0), which are synthesized *de novo* in the mammary gland [5]. In this study, the content of caproic, caprilic and capric of goat milk cheese were similar to the goat milk at 30 days after parturition i.e. 1.73%; 1.91 and 5.50%, repectively [18]. At the end of maturation, oleic (C18:1), butyric (C4:0), and palmitic (C16:0) acids were the most abundant [19] and all FFA significantly increased during maturation of a raw goat milk cheese. The long-chain saturated palmitic and stearic (C16:0 and C18:0) and unsaturated (C18:2) FFA in this study were the most abundant in fresh and ripened cheese, and there were no significantly different in after and before ripening. According to [20], saturated (stearic and palmitic) and unsaturated (oleic and linolenic) FFA and also SCFA (capric) were present at the highest concentration at all stages of ripening (7–35 days) in Serra cheese. However, the other study by [4], the FFA that showed the highest fractional increase by 68 days of ripening in caprine milk cheese they were C4 :0, C8:0, C10:0, C14:0 and C18:1.

Differences in fatty acid composition variations during the cheese ripening stage due to many factors. According to [14], nature of the animal's forage diet, i.e. botanical composition, maturity stage and preservation mode, strongly influences the milk composition in fatty acids, vitamins and carotenoids. Similarly, the nutritional composition of milk also depends on species, breed, parity, production yield and stage of lactation of the animals. Those FFA in cheese were result from lipolytic activity of residual rennet of rabbit stomach and *Lactobacillus acidophilus* starter and maybe from the milk. Lipolysis in cheese is catalysed by lipases from various source, particularly the milk and cheese microflora, and, in varieties where this coagulant is used [3].

5. Conclusion

There was no changes in the fat content during ripening of goat milk cheese by using *Lactobacillus acidophilus* and extract of rabbit stomach as coagulant. However the protein and ash content increased as decreasing of the moisture content

during ripening. The long-chain saturated (C16:0 and C18:0) and unsaturated (C18:2) free fatty acids were the most abundant in goat milk cheese, and there were no change in post and pre- ripening. The concentration of short chain fatty acid (C6:0, C8:0 and C10:0) and the overall concentration of FFA in goat milk cheese increased during ripening. Further research is required to preserve milk clotting enzymes from rabbit stomach in small-scale production systems, and genetic engineering for enzyme production on an industrial scale.

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