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Stability of Viable Counts of Lactic Acid Bacteria during Storage of Goat Milk Soft Cheese

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The use of goat milk is limited in Indonesia due to lack of good milking practices resulted in disliked goaty smell. One of the method to eliminate this off flavor is by processing the goat milk into soft cheese. The aim of this research was to study the stability of viable starter lactic acid bacteria cultures (*Lactobacillus acidophilus* FNCC-0051 and *L. casei* FNCC-0090) during storage of goat milk soft cheese. Three batches of goat milk soft cheeses were produced with different starter cultures *L. acidophilus* FNCC-0051 (5.0 x 10^6 cfu mL^{-1}); *L. casei* FNCC-0090 (5.0 x 10^6 cfu mL^{-1}); and the mixture of *L. acidophilus* FNCC-0051 (2.5 x 10^6 cfu mL^{-1}) and *L. casei* FNCC-0090 (2.5 x 10^6 cfu mL^{-1}). The goat milk cheeses had white color and soft. The viable lactic acid bacteria in the goat milk soft cheese reached 10^9 cfu g^{-1}, which was stable for 8 weeks at 5 °C. Panelists liked goat milk soft cheeses, especially in term of its aroma. The specific aroma produced could mask the disliked goaty smell.

Key words: goat milk, soft cheese, starter lactic acid bacteria

Susu kambing di Indonesia masih terbatas pemanfaatannya karena bau kambing yang kurang disukai, terutama akibat proses pemerahan yang kurang baik. Salah satu cara untuk mengurangi aroma yang kurang disukai tersebut ialah dengan mengolahnya menjadi keju lunak. Penelitian ini bertujuan menentukan stabilitas kultur starter bakteri asam laktat (*Lactobacillus acidophilus* FNCC-0051 dan *L. casei* FNCC-0090) selama penyimpanan keju lunak susu kambing. Keju lunak diproduksi menggunakan tiga kultur starter yang berbeda, yaitu *L. acidophilus* FNCC-0051 (5.0 x 10^6 cfu mL^{-1}); *L. casei* FNCC-0090 (5.0 x 10^6 cfu mL^{-1}); dan campuran *L. acidophilus* FNCC-0051 (2.5 x 10^6 cfu mL^{-1}) dan *L. casei* FNCC-0090 (2.5 x 10^6 cfu mL^{-1}). Keju yang dihasilkan berwarna putih dan bertekstur lunak. Jumlah bakteri asam laktat pada keju lunak susu kambing mencapai 10^9 cfu g^{-1} dan relatif stabil selama penyimpanan 8 minggu pada suhu 5 °C. Para panelis menyukai aroma keju lunak susu kambing karena mampu menutupi aroma susu kambing yang kurang disukai.

Kata kunci: keju lunak, starter bakteri asam laktat, susu kambing

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The goat milk production has steadily increased in Indonesia since 2000; however, its usage as a healthy drink is limited due to its unpleasant smell. The goat milk is usually consumed by children and adults. Goat milk provides essential nutrients, such as minerals, vitamins and easily digestible proteins with balanced amino acid profile which is important in supporting most body functions (Silanikove *et al*. 2010). However, many people does not like the goaty smell of goat milk resulted in the low consumption of goat milk. The goaty smell comes from medium-chain fatty acid (caproic, caprylic and capric acids) in goat milk fat (Silanikove *et al.* 2010).

In Turkey, Greece, and France, goat milk cheese has been processed commercially. In recent years, cheese becomes popular in Indonesia and it is mainly consumed as a food complement. Most of cheeses in Indonesia are imported products, particularly, natural cheeses made from cow's milk. Goat milk is potential to be processed into cheese products in order to promote the added value of goat milk.

Recently, world-wide interest on functional foods containing probiotic bacteria for health promotion and disease prevention has increased remarkably (Vankerckhoven *et al*. 2008). Cheese product has been developed as a vehicle for probiotic bacteria. In an effort to extend the probiotic product range, a small number of researchers and companies have manufactured cheeses with a high viable count of probiotic cultures. Cheese containing probiotic is a functional food.

Therefore, this study reports the stability of *Lactobacillus acidophilus* FNCC-0051 and *L. casei* FNCC-0090 as starter cultures in goat milk soft cheese during storage, and goat milk soft cheese's chemical and sensory state.

MATERIALS AND METHODS

Starter Cultures. The commercial *L. acidophilus* FNCC-0051 and *L. casei* FNCC-0090 were used as cheese starter cultures. The strains were activated by growing at 37 °C overnight in a sterile de Mann's Rogossa Sharpe Broth (MRSB). The goat milk was heated at 85 °C for 30 min. The two lactic acid bacteria (mL L^{-1}) were inoculated into goat milk then incubated at 37 °C for 6 h.

Goat Milk Soft Cheese Production. Goat milk soft cheese was made from heated Ettawa goat milk (85 °C

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for 30 min) and 5% (v/v) inoculums of the single or mixed strain starter culture was added. Three batches of goat milk soft cheeses were produced with different starter cultures: *L. acidophilus* FNCC-0051 (5.0×10^6 cfu mL^{-1}) (batch 1), *L. casei* FNCC-0090 (5.0×10^6 cfu mL^{-1}) (batch 2), and the mixture of *L. acidophilus* FNCC-0051 (2.5×10^6 cfu mL^{-1}) and *L. casei* FNCC-0090 (2.5×10^6 cfu mL^{-1}) (batch 3). Each batch was made in two replicates. The use of lactic acid bacteria reduced the pH which affected the coagulation time. Heated goat milk was inoculated by starter culture and was incubated at 37 °C for 6 h. The matured milk was added with liquid rennet (0.06 mL L^{-1} of milk) and it was coagulated after 2 h. Coagulated milk (curd) was cutted with cheeseharp into cube form (1x1x1 cm). The curds were cooked at 40 °C for 30 min. The whey was drained and the fresh cheese was salted (2%, w/w). The soft cheese was packed in plastic bag and was stored at 5 °C for 8 weeks.

pH Measurement. The pH change of raw milk, maturated milk, curd, whey, and fresh cheese were measured. The pH of soft cheese was also measured during 8 weeks storage period. The pH was measured according to the standard procedure of AOAC (1995). 10 mL of goat milk and whey were measured directly by pH meter (Thermo). Ten gram of curd and fresh cheese were diluted in 10 mL of aquades before measurement.

Viability of Lactic Acid Bacteria during Processing and Storage of Soft Cheese at 5 °C. To count the viability of starter culture bacteria, twenty gram samples of maturated milk, curd, whey, as well as soft cheese were collected during production. Twenty gram of cheese samples were collected each week at first month and every two weeks at second month to find out the stability of the starter lactic acid bacteria during storage. The viable lactic acid bacteria during processing was only counted for the first batch (*L. acidophilus* FNCC-0051) while during storage was counted for all batches (*L. acidophilus* FNCC-0051, *L. casei* FNCC-0090, and the mixture of *L. acidophilus* FNCC-0051 and *L. casei* FNCC-0090). These experiments were carried out in two replicates. The enumeration of the viable lactic acid bacteria was carried out as described by Burns et al. 2008. Twenty gram of cheese was placed in 180 mL of 2% (w/v) sodium citrate sterile solution. The cheese sample was crushed to bits by stomacher. Decimal dilutions of the homogenates were made in 0.0043% (w/v) KH_2PO_4 solution. 1 mL of maturated milk and whey were directly diluted into 9 mL of 0.0043% (w/v) KH_2PO_4 solution. Appropriate dilutions were pour-plated. Lactic acid bacteria were enumerated (37 °C, 48 h) on de Mann's Rogossa Sharpe Agar (MRSA).

Sensory Quality Test. The acceptance of goat soft cheese was evaluated according to the procedure of Drake' (2007). The cheeses were evaluated based on their aroma, taste, and aftertaste by nine trained panelists. A commercial goat milk cheese was tested to know the position of the produced cheese. The soft cheese samples were placed in saucer coded by three-digit random numbers. Coffee powder and water were served in between to neutralize the sense of smell.

Sensory evaluation used 0-15 cm-line scale (0 = dislike intensely and 15 = like extremely). The panelis scored on the line scale. The scores were obtained by measurement the length from 0 cm to the signed mark. Then, the scores were analysed by ANOVA.

Chemical Composition and Trace Element Analyses. Cheese which has no syneresis during 8-weeks storage was used for chemical and trace element analyses. The chemical analyses were moisture content (gravimetric method, BSN 1992a), ash (BSN 1992a), fat (soxhlet method, BSN 1992a), crude protein (Kjeldahl method, AOAC 1995), and total carbohydrate (by difference). The trace element analyses were done using AAS included As, Pb, Cu, Zn, Hg, and Sn (SNI 01-2980-1992 method, BSN 1992b; 1998a; 1998b).

RESULTS

pH Value and Stability of Viable Lactic Acid Bacteria during Production and Storage at 5 °C. The pH value and viable counts of lactic acid bacteria during cheese production are shown in Table 1. pH reduction was observed during cheese production. The viable count of lactic acid bacteria at cultured milk, curd and whey were 10^8 cfu g^{-1} and it increased one log cycle in soft cheese (Table 1). pH value of soft cheese significantly decreased during storage at 5 °C ($p<0.05$). The lowest pH occurred after 8 weeks storage at 5 °C was 4.37. Viable counts of lactic acid bacteria was stable during storage, which remained constant at 10^9 cfu g^{-1} after 8-weeks storage at 5 °C (Fig 1).

Sensory Evaluation. The average sensory scores by trained panelists are presented in Table 2. There was significant ($p<0.05$) difference in aroma score between the experimental goat milk soft cheese and commercial goat milk soft cheese. Goat milk soft cheese had better aroma (11.33-11.97: like fairly well-like very well) than commercial goat milk soft cheese (6.66: like slighly). Moreover, no significant ($p>0.05$) difference in taste and aftertaste score were observed between the cheeses.

Chemical Composition and Trace Element Content. The selected goat milk soft cheese originated from batch 1, cultured with *L. acidophilus* FNCC-0051 was analyzed its chemical composition and trace element content. The chemical composition of the cheese is shown in Table 3. The experimental cheese had lower fat and protein content as compared to commercial cheeses. Trace element content of goat milk soft cheese is shown in Table 4. As, Pb, Hg, and Sn level were below limit of detection (LoD) of instrument, indicating the cheese was free from heavy metals. However Cu and Zn were detected in the goat milk soft cheese. Cu and Zn as essential trace elements for body function were present at 13.53 and 21.56 ppm, respectively.

Table 1 pH values and total *Lactobacillus acidophilus* FCNN-0051 in goat milk soft cheese during production

Sample	pH	*L.acidophilus* FCNN-0051 (log_{10} cfu g^{-1})
Heated raw milk	6.60±0.00	na
Cultured milk	6.30±0.00	8.45±0.12*
Curd	6.12±0.02	8.85±0.17
Whey	6.15±0.07	8.47±0.09
Soft cheese	5.70±0.00	9.94±0.09

na: no available data
*cfu mL^{-1}

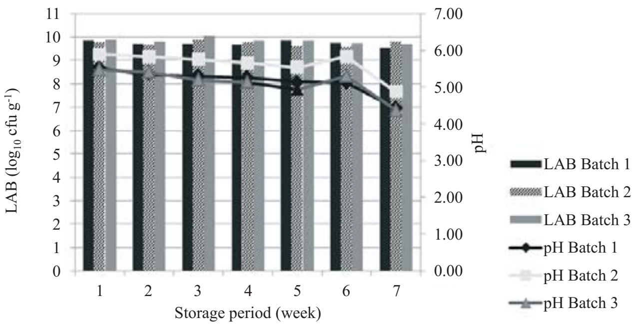

Fig 1 Total viable lactic acid bacteria and pH in goat milk soft cheese with different starter culture during 8-week storage at 5 °C.

Table 2 Sensory properties* of goat milk soft cheese with different LAB starter culture

Samples	Aroma	Taste	After taste
Cheese with *L.acidophilus* FCNN-0051	11.40± 2.38 a)	10.36 ± 2.50a)	9.49 ± 3.36 a)
Cheese with *L. casei* FNCC-0090	11.97± 2.13 a)	10.42 ± 2.95a)	8.56 ± 2.82 a)
Cheese with *L.acidophilus* FCNN-0051 and *L. casei* FNCC-0090	11.33 ± 2.89 a)	10.37 ± 2.69a)	8.92 ± 3.50 a)
Commercial feta cheese	6.66 ± 4.47 b)	8.54 ± 4.09 a)	5.70 ± 3.06 a)

* : 0 = dislike intensely and 15 = like extremely
a,b :different letters in the same column indicate significant differences ($p< 0.05$)

Table 3 Nutritional composition of goat milk soft cheese compared to other cheeses

Component (%)	Experimental goat milk soft cheese *L.acidophilus* FCNN-0051	Commercial goat milk cheese[a]			SNI[b]
		Fresh soft	Feta	Cheddar	
Moisture	52.00 ± 0.67	59.80 ± 6.81	52.30 ± 1.21	41.70 ± 1.76	max. 45.0
Ash	3.17 ± 0.05	1.74 ± 0.97	4.30 ± 0.27	3.60 ± 0.13	max. 5.5
Fat	23.49 ± 0.68	22.50 ± 4.37	25.30 ± 1.06	26.60 ± 1.13	min. 25.0
Protein	15.67 ± 0.03	18.90 ± 5.26	25.10 ± 1.56	30.30 ± 0.56	min. 19.5

[a]Park (1990); [b]BSN (1992) for Cheddar-processed cheese

Table 4 Trace element content of goat milk soft cheese compared to other cheese

Trace elements (ppm)	Goat milk soft cheese *L.acidophilus* FCNN-0051	Cheeses		SNI[c]
		Goat milk cheese[a]	Cow milk cheese[b]	
As	< 0.003	na	na	max. 0.1
Pb	< 0.01	na	na	max. 0.3
Cu	13.53	6.46	na	max. 20.0
Zn	21.6	15.5	28.1	max. 40.0
Hg	< 0.0002	na	0.001	max. 0.03
Sn	< 0.01	na	na	max. 40.01

[a]Park (1990); [b]Gambelli et al. (1999); [c]BSN (1992)
na: no available data

DISCUSSION

In this research, *L. acidophilus* FCNN-0051 and *L. casei* FCNN-0090 were used as starter cultures for manufacturing cheese. Vinderola *et al*. 2009; Ong and Shah 2009 reported that *L. acidophilus* A3 as well as *L acidophilus* 4962, *L. acidophilus* L10 and *L. casei* 279, *L. casei* L26 were able to use for manufacturing cheese. The decrease of pH value was observed during cheese production due to metabolic activity of starter culture in fermenting lactose during growing, shown by higher population of viable lactic acid bacteria. The milk was fermented for 6 hours and pH value was 6.3. Chymosin, which is the most important enzyme in rennet, was activated at pH 6.3. The enzyme helped in hydrolizing peptide bonds, particularly the Phe-Met bond between residues 105 and 106 of κ-casein, which triggered protein coagulation (Egito *et al.* 2007).

The goat milk soft cheeses had white color, soft, and crumbly texture. Goat milk is reported to form a finer curd than cow milk following acidification, which mimics the conditions in the stomach, suggesting it would be more readily digested. During cooking, the temperature of the cheese curds was 40 °C. *L. acidophilus* have optimal growth temperatures of 35-45 °C, while *Lactobacillus casei* was able to grow through a wide range of temperatures (15-45 °C) (Randazzo *et al*. 2004). In suitable temperature, metabolism of viable lactic acid bacteria was optimized. In soft cheese, pH change was attributed to the increase of metabolic activities of bacteria. The culture was in better environmental conditions to multiply in cultured milk affected lower pH value in soft cheese.

The number of viable lactic acid bacteria (10^9 cfu g^{-1}) was relatively stable during 8-weeks storage at 5 °C, indicating that storage at 5 °C did not promote metabolic activity of LAB in cheese. Soft cheese goat milk is a suitable food for the delivery of *L. acidophilus* FCNN-0051 and *L. casei* FCNN-0090 since the culture remained viable. Similar result was reported by Ong *et al*. (2006) at Cheddar cheese inoculated with *L. acidophilus* 4962, *L. casei* 279, *Bifidobacterium longum* 1941 as well as inoculated with *L. acidophilus* LAFTI® L10, *L. paracasei* LAFTI® L26, *B. lactis* LAFTI®B94, survived during manufacturing process and maintained their viability of >$3.2x10^7$ cfu g^{-1} at the end of ripening (6 months, 4 °C). Ong and Shah (2009) also showed the stability of viable counts of *L. acidophilus* 4962 during ripening of cheddar cheese, which was remained viable (>10^8 cfu g^{-1}) at the end of 24 weeks and their viability was not affected by the ripening temperatures (4 and 8 °C).

Sensory properties of goat milk cheese are an important factor for consumer in accepting and for producer in manufacturing and marketing the products (Ribeiro and Ribeiro 2010). In general, goat milk cheese has brighter white color than cow milk cheese due to the low β-carotene in goat milk (Raynal-Ljutovac *et al*. 2008). Lack of the pressing process led to the texture of cheese becomes soft and crumble. The pressing is needed (except for soft cheeses) to achieve form a rind surface.

The goat milk soft cheese was accepted by panelist. Panelist gave better score to the experimental goat milk soft cheese from batch 1, batch 2, and batch 3 than the commercial one, especially in term of its aroma. The goat milk soft cheese had sour aroma that covered the goaty smell. Molecular weight of lactic acid (90.08 g mol^{-1}) which is responsible for sour aroma is lower that caproic acid (116.1 g mol^{-1}), caprylic acid (144.2 g mol^{-1}), and capric acid (172.3 g mol^{-1}) which are responsible for goaty smell, so that easily reach the olfactory epithelium. Smit *et al*. (2005) described that flavour compounds in cheese arise from the action of enzymes from rennet, milk, the starter and non-starter lactic acid bacteria, together with non-enzymatic conversions. Moisture content of the cheese was within the range of semisoft or semihard cheese (45-55%). Fat and protein composition (%) of experimental goat milk soft cheese were lower than commercial fresh soft nor feta cheese due to its milk composition. Heavy metals content (As, Pb, Hg, and Sn) of goat milk soft cheese were below the LoD of AAS, indicating the cheese was free from toxic substances, while Cu and Zn as essential trace elements for body function were present at 13.53 and 21.56 ppm, respectively.

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