Fermented Goat Milk and Cow Milk Produced by Different Starters of Lactic Acid Bacteria: Quality Studies

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Abstract: Lactic acid bacteria (LAB) is widely used as culture starters in dairy fermentation. The aim of this study was to investigate the quality of fermented goat milk and cow milk, as well as the viability of LAB in the same products. Fermentations were performed with pasteurized goat milk or cow milk added with skim milk (18% of solids) using three separately different starters; yoghurt starter (a combination of Streptococcus thermophilus FNCC-0040 and Lactobacillus bulgaricus FNCC-0041), single starter of Lactobacillus acidophilus FNCC-0029 and Lactobacillus casei FNCC-0051. The parameters observed were pH, acidity, nutritional quality including protein, fat and lactose content and product’s viscosity. Acidity, pH and viability of LAB were also monitored during storage at refrigerated temperature (4 °C) for 28 days. Results show that the different LAB starters did not affect the pH, acidity, lactose and protein content. Differences on LAB starters affected fat content and viscosity. The highest score of viscosity (30.00 Pa·s ± 7.02 Pa·s) was observed on products fermented by yoghurt starters, followed by products obtained using starter of L. acidophilus (17.7 ± 11.4) and L. casei (8.62 ± 0.35). Protein content, acidity, pH and viscosity were not significantly different between products obtained from goat milk and cow milk. Fat content in fermented goat milk was higher (5.03% ± 0.62%) than in fermented cow milk (3.52% ± 0.37%), however, lactose content was higher in fermented cow milk (5.16% ± 0.40%) than in fermented goat milk (4.53% ± 0.35%). Total LAB concentration in fermented cow milk during storage was 8.03 ± 0.52 log10 cfu/mL, while in fermented goat milk was 7.81 log10 cfu/mL ± 0.67 log10 cfu/mL. There was a 10.83% decrease in LAB viability in fermented cow milk and 11.40% in fermented goat milk after 28 days of storage. In conclusion, quality of fermented milk is affected by the starters applied, raw milk source and storage period.

Key words: Lactic acid bacteria, fermented milk, goat milk, cow milk, viability.

1. Introduction

The population of goat in Indonesia has been growing around 4.6% per annum over the past 10 years [1]. Goats are preferred by local farmers due to the better suited to the tropical condition and native environment of this country. Goats are widespread in Indonesia, but especially concentrated in Java. The total production of goat milk in 2010 was 281,328 ton, whereas, cow milk had a total production of 840,000 ton. Worldwide, the average production of goat milk and cow milk from 2006 to 2009 were 13.1 million ton and 542.1 million ton, respectively [2]. Two main breeds of goat in Indonesia are Kambing Kacang and Peranakan Etawah goats. In terms of dairy production, Peranakan Etawah goat is recently becoming as an alternative to dairy cows. Goat’s milk could improve human nutrition by different ways: (1) substituting cow milk for feeding malnourished people in rural areas in the developing countries [3]; (2) treating people with cow’s milk allergies and gastro-intestinal disorders, a significant segment in developed countries [4-6] and (3) filling the gastronomic needs of specific consumers mainly in developed countries [7].
Fresh milk is the main ingredient in fermented milk manufacturing. Fermented milk with lactic acid bacteria (LAB) has a high nutritional value and bioavailability of proteins. Accordingly, fermentation conserves the milk nutrients and inhibits the growth of pathogenic bacteria [8]. It also increases the nutritional value through the active cultures in significant numbers providing distinct health benefits beyond conventional nutrition [9]. Approximately 400 different fermented milk products are consumed around the world. Nowadays, fermented milk products such as yoghurt, kefir, buttermilk and acidophilus milk are gaining an increased attention. Yoghurt culture usually contains *Streptococcus thermophiles* (ST) and *Lactobacillus delbrueckii* subsp. *bulgaricus* (LB) [10]. Moreover, some yoghurt products also contain additional bacteria, especially from intestinal origin, including genus of bifidobacteria and lactobacilli that are referred as probiotic bacteria [11].

A number of health benefits associated with probiotics has been claimed [12-15] and more than 90 milk products containing one or more groups of probiotic organisms are available worldwide. Probiotic food is defined as food containing live microorganisms which actively enhance the health of consumers by improving the balance of microflora in the intestine [16]. *Lactobacillus acidophilus* (LA) and *Lactobacillus casei* (LC) are examples of LAB species applied as probiotic bacteria. To provide health benefits, the suggested concentration for probiotic bacteria is $10^7$ cfu/g in the product [17].

 Whilst the application of probiotics in fermented cow milk has been globally recognized, the use of probiotics and LAB for goat milk fermentation has never got a broad attention. It is assumed that the application of LAB as starters in goat milk fermentation would increase the nutritional quality of the products. The aim of this study was to investigate the quality of fermented goat milk and cow milk, as well as the viability of LAB’s during the storage of the products at refrigerated temperature.

### 2. Materials and Methods

#### 2.1 Fresh Milk and Bacterial Starter

Raw materials of goat milk and cow milk were obtained from local dairy farm in Yogyakarta (Indonesia) and stored under refrigerator before being used for fermentation. The quality of fresh milk was evaluated with alcohol 75% test and methylene blue reduction time (MBRT) test. Alcohol test was performed by mixing equal amounts of fresh milk and alcohol 75%, and followed by detecting precipitation. MBRT was tested by adding 1 mL of methylene blue into 10 mL of fresh milk, followed by incubation at 37 °C and monitored at intervals up to 6 h. The time required (h) to become colorless is the MBRT.

The yoghurt starter cultures used were: *Streptococcus thermophiles* (ST) from the Food Nutritional Culture Collection (FNCC) 0040, and LB from FNCC-0041. The probiotic cultures used were: LA FNCC-0029 and LC FNCC-0051. To maintain the viability, pure cultures were stored in skim milk with glycerol 20% (1:1, v/v) at -80 °C. Bacterial cultures were grown overnight (12 h), and cultures at this stage of growth were used for the fermentations.

#### 2.2 Fermentations

Fresh milk from goat and cow were added with skim milk powder to obtain 18% of total solids (TS) and pasteurized at 80 °C for 10 min. After cooled, 500 mL of heat-treated milk was separately inoculated with: (1) 4% (v/v) yoghurt starters (ST:LB = 1:1); (2) 4% (v/v) probiotic culture of LA and (3) 4% (v/v) probiotic culture of LC. Fermentations were conducted at 42 °C for yoghurt and 37 °C for probiotic cultures. Fermentations were stopped after 5 h and the products were stored at 4 °C.

#### 2.3 Physicochemical Analysis of Fermented Products

Six parameters were measured after fermentation: protein, fat and lactose contents, acidity, pH, and viscosity. Protein was analyzed based on
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Macro-Kjeldahl method [18]. Fat analysis was carried out based on Babcock method [19]. Lactic acid value was measured using a potentiometric pH-meter (Hanna model) [20]. Titratable acidity was measured as percent (%) of lactic acid by titrating 0.1 N of NaOH using phenolphthalein as indicator [20]. Titratable acidity and pH were measured hourly during the fermentations. Viscosity measurements were performed according to Tuncturk [21] by using Brookfield digital rheometer model DV III (Brookfield Engineering Laboratories Inc., Massachusetts, USA). All viscosity measurements were expressed in Pascal second (Pa·s), performed in triplicate and averaged.

2.4 Cell Viability during Storage

Cell viability of LAB during storage in refrigerated temperature (4 °C) was measured every seven days throughout 28 days by measuring total plate count (TPC) on Mann Rogosa Sharpe (MRS) agar after samples were diluted up to 1/10^9 with sterilized 0.9% NaCl. The plates were incubated for 48 h at 42 °C for yoghurt starters and at 37 °C for probiotic cultures.

2.5 Data Analysis

Data of fresh milk quality presented in Table 1 was statistically analyzed using paired $t$-test, with statistical significance accepted at $P < 0.05$. Data of the fermented quality products (Table 2) were analyzed statistically using SAS programs [22]. Treatment means were compared by Duncan’s new multiple range test and the level of significance was determined at $P < 0.05$.

3. Results and Discussion

3.1 Fresh Milk Quality

The fresh milk quality of either goat milk or cow milk was analyzed and presented in Table 1. There was a significant difference in total solids (TS) comparing goat milk and cow milk. Cow milk had TS of 11.82% and goat milk had higher TS of 14.99%. Table 1 also shows that goat milk has a higher percentage of fat, protein and lactose, contributing for the higher TS. Widodo et al. [23] have previously reported a higher concentration of fat and protein in goat milk compared to cow’s milk. Data of cow milk presented here are in agreement with previous studies [24, 25], and within the range of Indonesian National Standards of cow milk [26]. Meanwhile, the goat milk quality was also in agreement with previous reports [24, 27, 28] and also in line with the goat milk quality according to Thai Agricultural Standard [29], suggesting that the quality of goat milk produced in Indonesia is comparable with that produced from different countries.

Table 1 explains that both cow milk and goat milk had a good microbiological quality as seen by MBRT which was more than 2 h and having negative result at alcohol 75% test. Indonesian National Standard on fresh cow milk requires MBRT score of between 2 h to 5 h [26] to suggest that microbiological quality of fresh milk is at acceptable level. The quicker the time (less than 2 h) required to neutralize methylene blue, the worse microbiological quality of the fresh milk [30]. This fresh milk was then pasteurized and used for fermentation using selected starter cultures.
Table 2  Physico-chemical quality of fermented cow milk and goat milk using different LAB starters.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Milk</th>
<th>Starter culture</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ST and LB</td>
<td>LA</td>
</tr>
<tr>
<td>Protein (%)</td>
<td>Cow</td>
<td>4.04 ± 0.24</td>
<td>3.38 ± 0.23</td>
</tr>
<tr>
<td></td>
<td>Goat</td>
<td>3.34 ± 0.07</td>
<td>3.43 ± 0.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3.69 ± 0.42**</td>
<td>3.41 ± 0.17**</td>
</tr>
<tr>
<td>Fat (%)</td>
<td>Cow</td>
<td>3.60 ± 0.00</td>
<td>3.80 ± 0.00</td>
</tr>
<tr>
<td></td>
<td>Goat</td>
<td>5.40 ± 0.28</td>
<td>4.50 ± 0.99</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.50± 1.05</td>
<td>4.15± 0.70</td>
</tr>
<tr>
<td>Lactose (%)</td>
<td>Cow</td>
<td>4.87 ± 0.30</td>
<td>5.33 ± 0.52</td>
</tr>
<tr>
<td></td>
<td>Goat</td>
<td>4.48 ± 0.59</td>
<td>4.61 ± 0.49</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.68 ± 0.44**</td>
<td>4.97 ± 0.59**</td>
</tr>
<tr>
<td>pH</td>
<td>Cow</td>
<td>4.35 ± 0.02</td>
<td>4.55 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>Goat</td>
<td>4.21 ± 0.23</td>
<td>4.32 ± 0.18</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.28± 0.16**</td>
<td>4.44± 0.19**</td>
</tr>
<tr>
<td>Acidity (%)</td>
<td>Cow</td>
<td>1.02 ± 0.04</td>
<td>0.92 ± 0.13</td>
</tr>
<tr>
<td></td>
<td>Goat</td>
<td>1.12 ± 0.17</td>
<td>1.07 ± 0.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.07 ± 0.12**</td>
<td>1.00 ± 0.13**</td>
</tr>
<tr>
<td>Viscosity (Pa·s)</td>
<td>Cow</td>
<td>24.00 ± 1.41</td>
<td>27.50 ± 3.54</td>
</tr>
<tr>
<td></td>
<td>Goat</td>
<td>36.00 ± 1.41</td>
<td>8.00 ± 0.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30.00± 7.02</td>
<td>17.75± 11.44</td>
</tr>
</tbody>
</table>

ns: non significant;
a, b: different superscript on the same row shows significant differences \((P < 0.05)\).

3.2 Fermented Products Quality

Fermentations of goat milk and cow milk were conducted for 5 h and the quality of the fermented milk was then assessed. Yoghurt was produced with a combination of ST and LB, whereas, other fermented milk was produced by using LA and LC as starters. Table 2 shows that fermented goat milk and cow milk had different fat and lactose content and pH \((P < 0.05)\), but no differences on protein, acidity and viscosity were observed. Meanwhile, differences on viscosity \((P < 0.05)\) were affected by different starter cultures (Table 2), suggesting important roles of the starters on the viscosity. The highest viscosity (30.00 Pa·s) was observed at products fermented using yoghurt starters (ST and LB), followed at products fermented by LA at 17.7 Pa·s. The lowest viscosity (8.62 Pa·s) was obtained at products fermented by LC (Table 2). The effect starter culture on the viscosity of yoghurt and other fermented milk has previously been reported [21, 31, 32]. Tuncturk [21], Rawson and Marshall [32] reported that yoghurt’s viscosity was associated with the ability of starters to produce exopolysaccharides (EPS). ST is a high-EPS producer and milk fermented with ST had a higher viscosity than the milk fermented with other starter culture [31, 33]. Further study by Skriveret et al. [33] proved that a combination of ST and LB resulted in yoghurt products with a higher viscosity than products fermented with single strains.

Protein content of fermented cow milk ranged between 3.38% and 4.04%, whereas on fermented goat milk ranged between 3.34% and 3.96% (Table 2). Protein content presented no difference between the products. Final protein content at fermented products was influenced by the level of skim milk powder addition. Protein content at fresh cow milk was 2.87%, whereas, fresh goat milk was 3.54%. Total solid sat fresh goat milk and cow milk was 11.82% and 14.99%, respectively (Table 1). Since fresh milk was fortified with skim milk powder to obtain 18% of total solids, there were different skim milk powder amount sadded at goat milk and cow milk.
Fat content of the fermented cow milk was 29.04% lower than fermented goat milk, whereas, lactose content was 11.93% higher than fermented goat milk. Fat content in fermented milk was linear with that concentration in fresh milk, which had a higher fat content in goat milk than in cow milk (Table 1). According to Ceballos [34], the basic composition of goat milk and cow milk was fairly similar, with certain differences, with greater or lesser extent. Goat milk normally provides a higher proportion of TS than cow milk, as well as protein fat and mineral contents. Although, when the latter are expressed as dry matter content, the differences tend to disappear. Since TS of cow milk was lower than goat milk, the addition of skim milk powder affected the composition between the fermented products.

3.3 Acidity and pH of Fermented Milk during Fermentation

Acidification is an essential parameter of fermentation process. Organic acids (lactic acid and citric acid) were produced by LAB during fermentations by degradation of lactose and resulting in curd formation with a particular flavor [35]. The level of acidity in fermented milk was monitored by measuring pH and titratable acidity. Fig. 1 shows that time course of fermentations is significantly affected by acidity (Fig. 1a) and pH ($P < 0.05$) (Fig. 1b). A decrease on pH was followed by a gradual increase of the acidity during the fermentations. Titratable acidity of cow milk and goat milk before the fermentations were $0.349\% \pm 0.08\%$ and $0.32\% \pm 0.05\%$, respectively, whereas, at the end of the fermentations increased to $0.82\% \pm 0.27\%$ and $1.15\% \pm 0.16\%$, respectively (Fig. 1a). The average of pH at the beginning of the fermentations on cow milk and goat milk yoghurt were $6.27 \pm 0.49$ and $6.18 \pm 0.19$, respectively, whereas at the end of fermentation decreased to $4.79 \pm 0.56$ and $4.16 \pm 0.08$, respectively (Fig. 1b). Differences of milk and starters did not affect significantly the pH and acidity of the products (Table 2).

The fermentations were carried out for 5 h. The average pH at the final fermented cow milk and goat milk were $4.79 \pm 0.56$ and $4.16 \pm 0.08$, respectively. The minimum level of pH for the fermented milk suggested by Chandan [10] was 4.6 or lower. The present data show that final pH of fermented goat milk was below 4.6, suggesting a rapid acidifying in goat milk during fermentation. Meanwhile a slower acidification was observed in fermented cow milk with a final pH of 4.79 (Fig. 1a). The acidification occurred due to LAB activities, degrading lactose for organic acids production. LAB strains have the ability to ferment lactose into lactic acids resulting in an increase of acidity and decrease in pH of fermented milk. Although a more rapid acidification occurred in goat milk than in cow milk, a final pH and acidity of those two fermented milk did not differ significantly. Also the lactose content was not different between cow and goat milk (Table 1). The Indonesian National Standard of acidity of fermented milk are between 0.5 and 2.0 [24], suggesting that the acidity of the products produced in this study are in accordance with the national standard.

3.4 LAB Viability, pH and Acidity during Storage

After the fermentations, the products were stored at refrigerated temperature. During storage, the quality of the products was monitored measuring the pH, acidity and cells viability. Fig. 2 shows that the acidity and pH during the storage were relatively stable, although an increased of the acidity and decreased in pH was observed at the first week of storage. There was no significant differences on pH and acidity at fermented cow milk and goat milk during storage for 28 days (Fig. 2). The increase in the acidity during storage of fermented cow milk and goat milk were $10.83\%$ and $12.86\%$, respectively, whereas, the decreased in pH during storage was $11.29\%$ and $9.15\%$, respectively (Fig. 2).

Total LAB in fermented cow milk was $8.03 \pm 0.52$
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4. Conclusions

Quality of the fermented milks was partially affected by the source of raw milk quality, the addition of skim milk powder and starter cultures used for fermentation. Final fat content in fermented goat milk was associated with fat content in fresh milk, while final lactose content in fermented cow milk was due to

log_{10} \text{cfu/mL}, while the goat milk was $7.81 \pm 0.67$ log_{10} \text{cfu/mL} (Fig. 3). Overall, final LAB concentration in fermented cow milk and goat milk products was within the recommended probiotics level (7 log cfu/mL) [36-39]. There was a decrease of 10.83% in LAB viability on fermented cow milk after 28 days storage in refrigerated storage and 11.40% in fermented goat milk (Fig. 3), while the total amount did not show significant differences ($P < 0.05$).

Among three starters treatments, LA presented the highest viability. The minimum level of LAB in the product ($10^7$ cfu/mL), was maintained during 14 days, but after a prolonged period of storage, the LAB viability in most of the products was under the minimum requirement. According to Birollo et al. [37], on the basis of minimum value of $10^7$ cfu/mL, the shelf life of yoghurts at 6 °C was longer than 60 days.

LAB viability was affected by pH and acidity. Ng et al. [40] reported that LA showed good survival with high ability to survive at low pH caused by organic acids accumulation. In another study, Beal et al. [41] reported that the concentration of LB in yoghurt was higher than pH 4.8, indicating that LB is more resistant to acidic conditions while the ST growth was stopped at pH 4.8. The ST concentration decreased between pH 4.8 and pH 4.4. When products presented a pH less than 4.4, the activity of the starter ST and LB was stopped and the viability decreased.

4. Conclusions

Quality of the fermented milks was partially affected by the source of raw milk quality, the addition of skim milk powder and starter cultures used for fermentation. Final fat content in fermented goat milk was associated with fat content in fresh milk, while final lactose content in fermented cow milk was due to
skim milk addition to the fresh milk to make 18% total solids. Milk fermented with yoghurt starter culture (a combination starter culture of ST and LB) yielded a higher viscosity than milk fermented with a single LA and LC. The viability of yoghurt starter cultures was affected by temperature of storage and the acidity of the products. After 28 days of storage, the viability of probiotic starters in fermented cow milk was higher than that of fermented goat milk.

Acknowledgments

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[26] Indonesian National Standards on Fresh Milk (SNI 01-3141-1998), National Standardization Committee, Jakarta, Indonesia, 2007. (in Indonesia)


