Quality of fermented goat milk and powder milk using *Lactobacillus plantarum* and optimizing their antibacterial ability against enterobacteriaceae

Yurliasni, Z Hanum, F Muliawan and A Kardima

Laboratory of Milk Science and Technology, Faculty of Agriculture, Syiah Kuala University, Darussalam- Banda Aceh

Corresponding author: Yurliasni62@gmail.com

Abstract. The purpose of the study was to evaluate the quality of fermented goat milk using *Lactobacillus plantarum* and determine its antibacterial ability against enterobacteriaceae. The quality of fermented milk was evaluated through measurement of pH, lactic acid levels, protein content and total bacteria. The antibacterial ability was measured by inhibitory tests on enterobacteriaceae. The experiment was applied in a completely randomized factorial design with two factors, namely factor A, type of milk (goat's milk and powder milk), factor B, level of *Lactobacillus plantarum* (2.5, 5 and 7.5%) and 3 equal replicates. The data were subjected to analysis of variance, and continued to Duncan’s multiple range test to determine the difference between means at 1 % probability. The results indicated that milk type and *L. plantarum* levels had a very significant effect on pH, lactic acid levels, and the inhibitory zone width. There is an interaction between factors. Meanwhile the type of milk had very significant effects (P <0.01) on protein content and total lactic acid bacteria. In conclusion, fermented goat milk has better quality and antimicrobial ability against enterobacteriaceae than powder milk.

1. Introduction

As a functional food fermented, milk must have a good nutritional quality that can meet some nutritional needs, which can be seen from lactic acid levels, pH, protein content, total lactic acid bacteria (LAB), and the benefits of LAB used as the starter. The use of *Lactobacillus plantarum* as a starter fermentation has some advantages, such as it is a catalase negative, aerobic or facultative aerobics, digesting protein, resistant to acidic conditions and producing lactic acid [1]. This LAB lives normally in the intestine and its role is very useful for maintaining the health of the digestive tract. Chemically, these bacteria will degrade lactose in the growth medium to produce lactic acid. The presence of this acid compound shows beneficial effects by inhibiting the growth of pathogenic bacteria such as *Escherichia coli* [2] and *Salmonella sp* [3]

2. Material and methods

2.1. Material

Fresh goat milk, milk powder and LAB (*L. plantarum*) were used in the experiment to produce fermented milk. *E. coli* dan *Salmonella sp*. were used in enterobacteriaceae inhibition test.
2.2. Methods

2.2.1. Preparation of Fermented Milk. Fresh goat milk was pasteurized at 85°C for 15 m, while powdered milk was reconstituted using 100 mL of boiled water in 12.5 g of powder milk. Then, the milk was inoculated using *L. plantarum* with 3 levels of starter (2.5, 5.0 and 7.5%) then incubated at 37°C for 8 hours.

2.2.2. Lactic Acid Analysis. As much as 18 g of sample, added by phenolphthalen indicator solution of 3 to 4 drops and titrated using 0.1 N NaOH solution until a pink color was formed. the titration volume of NaOH was measured. Then the lactic acid level was calculated using a formula (mL NaOH x 0.009: Sample weight x 100 %) [4].

2.2.3. Protein Analysis. 10 mL of fermented milk was added with 0.4 mL of potassium oxalate solution and 0.5 mL of phenolphthalen solution, then left for 2 minutes. The sample was titrated using 0.1 N NaOH until pink color was formed, then added by 2 mL formaldehyde solution, and left for 1 minute. Then sample is titrated using 0.1 N NaOH, until red color was formed, and the titration volume of NaOH was measured [5].

2.2.4. pH value. The pH was measured by dipping the pH meter electrode, which had been previously calibrated, into the fermented milk sample. The pH sample appeared on the monitor screen was recorded [4].

2.2.5. Total Plate Count. Nutrient agar media (Oxoid) was autoclaved for 15 minute at 15 psi 121°C, then cooled to 45°C. Pour-plate technique or serial dilution was used for plating. Serial dilutions of sterile pepton water made up to 106. As much as 1 mL sample was transferred into any dilution in sequence until dilution 107 and 108. As much as 1 mL of each dilution placed into sterile petri dish in duplicate. Molten agar 45°C was poured into petridishes containing diluted sample. It was then incubated at 37°C for 24 h. Total number of cfu/mL counted on Quebec colony counter.

2.2.6. Antimicrobial Activity Assay. Antibacterial activities against *E. coli* and *Salmonella* were observed using diffusion methods as described by [6]. The selected bacterial test, *E. coli* and Salmonella, were grown in physiological NaCl at 37°C for 24 h until stationary phase, then by pour-plate technique and serial dilution bacterial test were plated on a Vogel Johnson (VJ) Agar media (Oxoid), respectively about 103 cfu/mL. As much as 100 µL of sample was spotted in duplicates onto paper disk on the surface of VJA agar plate containing lawn of test bacteria. The agar plates then incubated at 37°C for 24 h. Diameter of a clear zone was measured by using calipers to determine antibacterial activities.

2.2.7. Experimental Design and Statistical Analysis. The experiment was assigned in a completely randomized factorial design with two factors, namely the type of milk (goat's milk and powdered milk) and starter levels (2.5, 5 and 7.5%). Each treatment was repeated three times. The quantitative data were analyzed by using Two-way analysis of variance (ANOVA) and the differences among treatments means were examined by Duncan Multiple range Test P<0.05 [7]. The total of microorganism cell (cfu/mL) from sample test was converted to logarithmic value before statistical analysis.
3. Results and discussion

3.1. Lactic acid content
The results showed milk types, in this case fresh goat milk and powdered milk, and different levels of starter affected the levels of lactic acid production. Table 1 shows lactic acid content of goat milk was higher than powder milk. This result might be occurred due to the higher concentration of lactose in goat milk compared to milk powder. Lactic acid content was also influenced by the levels of starter used. In accordance with the statement of [8] that higher starter level will produce higher lactic acid levels. [9] also stated that lactose is the main carbon source for lactic acid bacteria in the fermentation process. Furthermore, in another study conducted by [10] they found that lactic acid is one of the main metabolites of lactic acid bacteria.

3.2. Protein content
The protein content in fermented goat milk was higher than in powder milk. Protein concentration in fermented milk is influenced by protein content in raw milk. Raw milk containing high protein tend to produce fermented milk with high protein content as well. LAB produces proteolytic enzymes and digest proteins into simpler compounds [11], thus higher levels of LAB inoculation will affect on milk protein digestion. Meanwhile, protein concentration of powder milk was lower, due to the heating process during processing might damage some of existing protein in raw milk.

Table 1. Milk quality and width of inhibition zone of fermented goat milk and powder milk inoculated different levels of L. Plantarum

| Treatment | Parameters | Lactic acid (%) | Protein (%) | pH | TPC (Log cfu/mL) | Zone width |
|-----------|------------|----------------|-------------|----|----------------|------------|
|           |            |                |             |    | E. coli | Salmonella |
| 2.5%      | Goat Milk  | 1.63±0.03      | 4.29±0.00   | 3.93±0.00^d | 9.12±0.06 | 2.85±0.15  | 2.96±0.20^ab |
|           | Powder Milk | 1.03±0.07    | 2.56±0.13   | 4.09±0.12^a | 8.74±0.17 | 0.71±0.00  | 2.61±0.0^b  |
| 5%        | Goat Milk  | 1.71±0.05      | 4.29±0.00   | 3.93±0.00^d | 9.13±0.09 | 3.23±0.15  | 3.08±0.09^a |
|           | Powder Milk | 1.01±0.05    | 2.81±0.07   | 4.01±0.12^b | 8.90±0.21 | 1.87±0.50  | 0.71±0.00^c  |
| 7%        | Goat Milk  | 1.74±0.09      | 4.23±0.04   | 3.92±0.01^d | 9.22±0.17 | 3.03±0.05  | 3.03±0.06^a |
|           | Powder Milk | 1.41±0.06    | 2.81±0.07   | 3.99±0.21^c | 8.87±0.12 | 1.32±0.61  | 2.60±0.1^b  |
| Avg       | Goat Milk  | 1.64±0.17^a   | 4.27±0.06^b | 3.93±0.00^d | 9.16±0.11^a | 3.04±0.12^a | 3.02±0.09 |
|           | Powder Milk | 1.04±0.21^b  | 2.62±0.31^a | 4.03±0.07  | 8.64±0.22^b | 1.30±0.16^b | 1.97±0.23 |

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

3.3. pH levels
Different levels of L. plantarum inoculation resulted lower pH of fermented goat milk than powdered milk. This was closely related to the concentration levels of starter used in fermentation. Higher level of starter will increase the break down of lactose into lactic acid then decrease the pH of milk. There is no difference between the levels of starter being treated. It is in line with [12] which stated that the pH of fermented milk will be lower when the higher starter levels is used. Previous research by [13] stated that the decrease in pH is also caused by acidification activity which is influenced by the amount and type of organic acid produced.
3.4. Total plate count
Total LAB colonies was higher in goat milk, at each starter level, compare to powder milk (Table 1). Better nutritional quality of goat milk compare to powder milk may result in higher LAB total colony in fermented goat milk. As nutritional needs for LAB growth were sufficiently fulfilled in goat milk. This is supported by the statement of [11] that the growth of microorganisms is determined by several things such as nutrition, temperature, humidity, oxygen, pH and inhibiting substances. The total of LAB colonies were also influenced by pre-treatment of milk which were goat milk was pasteurized while powdered milk has been heated at high temperatures in the manufacturing process.

3.5. Antimicrobial activities
Based on inhibitory tests (Table 1), this study showed that the antibacterial activity of goat milk was significantly better than milk powder that was shown by wider clean zone formed. Based on the starter level, this study showed that a wider clear zone was formed on 5% starter for both testing bacteria. This result was related to the total colonies of LAB, that grow higher in goat milk. According to [14], the ability of an antimicrobial to inhibit microorganisms depends on antimicrobial compounds therefore the greater the antimicrobial concentration, the faster the diffusion occurs and the wider the diameter of the inhibition zone.

4. Conclusion
Goat milk has better quality based on the nutrients produced after fermentation as well as the antibacterial activity against enterobacteriaceae.

References
[1] Salminen S and W A Ouwehand 2004 *Lactic Acid Bacteria Microbiological and functional aspects* Third Edition, Revised and Expanded, (New York: Marcel Decker Inc)
[2] Istiqamah L S N, Hajati E, Damayanti, H. Julendra A A, Sakti T Untari 2013 *Med Pet* 36 pp 14–20
[3] Nouri M F, Rabarizadeh D, Akhmadvand F, Moosakhani E, Sadeqzadeh S, Lavasani and Vishteh K 2010 *J. Biotecnol* 8 32–37
[4] Hadiwiyoto S, 1994 *Teori dan Prosedur Pengujian Mutu Susu dan Hasil Olahannya* (Yogyakarta: Liberty) p 54
[5] Mirnawati 1993 *Pemeriksaan susu dan produk Olahannya* Pusat AntarUniversitas Pangan dan Gizi (Bogor: Institut Pertanian Bogor) p 69
[6] Bonev B J, Hooper and Parisot J 2008 *J. Antimicrob. Chemother.* 61 1295-301
[7] Steel R G D and Torrie J H 1995 *Prinsip dan Prosedur Statistika* (Jakarta: PT. Gramedia Pustaka Utama)
[8] Khalil A A 2006 *J. Biotecnology* 4 206–12
[9] Yusmarini and Raswen E 2004 *J. Natur Indonesia.* 6 104–10
[10] Theron M M and Lues J F R 2011 *Organic Acids and Food Preservation* (United State: CRC Press) p 273
[11] Karinawatie S, Kusnadi J and Martati E  2008 *J. Tek. Pertanian.* 9 121–30
[12] Lengkey H A W, Siwi J A and Roostita L B 2013 Lucrari Stiintifice-Seria Zootehnie 59
[13] Alvorado S B E, Almandarez S E, Martin and Regalado C 2006 *Microbiologia,* 48 206–268
[14] Rastina M dan Wientarsih I 2015 *J.K. H.* 9 185–189