The Potential of Concentrate of Fermented Milk for Natural Antibacterial

Putri Dian Wulansari*, Novia Rahayu and Firgian Ardigurnita

Faculty of Agriculture, Universitas Perjuangan Tasikmalaya, Tasikmalaya, 46115, Indonesia

ABSTRACT

The objective of the study was to investigate the potential of concentrated fermented milk (yoghurt and kefir) from cow milk and goat milk as a natural antibacterial ingredient. Completely randomized factorial design 2x2 was used in this research. The first factor was the type of fermentation (yoghurt and kefir) and the second factor was type of milk (cow milk and goat milk). The parameters measured were composition, inhibitory test, chemical and physical test. The results showed that the type of milk had significantly affected (P<0.05) on amount of whey and antibacterial activity. Type of fermentation and milk type have a significant effect (P<0.05) on concentrate. Concentrated fermented goat milk has the higher resistance to S. aureus bacteria than concentrated fermented cow milk on the inhibitory test. The type of fermentation and milk type has no significant effect on the composition (moisture content and total solids) and chemical characteristics (pH, lactic acid and free fatty acids) of the concentrate and whey. The conclusion of this research is goat milk yoghurt concentrate was potential to used as a natural antibacterial material.

Keywords: Concentrate, Cow milk, Goat milk, Kefir, and Yoghurt

Introduction

Yoghurt is a product of fermented milk using Streptococcus thermophilus and Lactobacillus bulgaricus bacteria. On the other hand, kefir consists of acetic acid bacteria, yeast and several types of lactic acid bacteria such as Lactobacillus kefiranofaciens and Lactobacillus parakefirin and yeast (Yildiz, 2010; Leite et al., 2012; John and Deeseenthum, 2015). Previous studies showed that fermented milk had many benefits for health such as preventing the infection in digestive tract, lowering blood cholesterol level and reducing lactose intolerance (Shiby and Mishra, 2013; Panesar, 2011; Ouwenhand and Roytio, 2015). Yoghurt and kefir are widely used as the natural antibacterial in cosmetic products. Cow milk and goat milk are the prominent ingredients of yogurt and milk, and both milk have different composition and characteristics (Park, 2009).

Yoghurt or kefir concentrate is the semi-solid dairy product of yoghurt and kefir, from which the whey have been removed to extend the storage time and to be more palatable to consumers. The characteristics of concentrate are soft texture, semi-solid, white in colour and a more acidic flavour (Özer, 2006). Yoghurt and kefir in the concentrate form are assumed to have a higher concentration of antibacterial activity due to the more active ingredients in the solid component. Accordingly, yoghurt and kefir concentrate can be used as the alternative natural antibacterial. The objective of the research is to analyse the potential of the fermented milk concentrate as the natural antibacterial. The significance of the study is to investigate the potential extent of yoghurt and kefir concentrate as the natural antibacterial for one of the ingredients of cosmetic products.

Materials and Methods

Research design

The experimental research method used a completely randomized design (CRD) in 2x2 factorial design. The first factor was the type of fermentation, i.e. yoghurt and kefir, and the second factor was the types of milk cow milk and goat milk obtained from the dairy farm Assalam, Tasikmalaya, West Java. Each treatment was repeated six times.

The procedure of yoghurt making

The procedure of yoghurt making was modified according to the study by Sumarmono and Sulistyowati (2015). The process started from making the yoghurt starter. The starter bacteria used in this process was the dry yoghurt starter (freeze-dried) Yogermet® (made in Canada) that contains L. bulgaricus, S. Thermophilus and L.acidophilus. One litre of milk was pasteurized at
72°C for 15 seconds, then the temperature was lowered to 43°C and 5 g of dried starter was added. The mixture was stirred and closely sealed for 8 h incubation at 43-45°C. The yoghurt making in the study was identical to that of the yoghurt starter, using the starter that was previously made. As much as 10% v/v yogurt starter was added to the milk, stirred and closely sealed for 16 h incubation at 43-45°C.

The procedure of kefir making

The procedure of making kefir was the modification of the study by Setyawardani et al. (2017). The starter bacteria used for kefir was the dry starter (freezer dried) Yogourmet® (made in Canada) that contained L. Lactis, L. diacetullactis, L. acidophilus and yeast. One liter of milk was pasteurized at 72°C for 15 seconds. The temperature was reduced to room temperature (23-25°C). This process did not use kefir grains, but kefir starter which had been made using the dry kefir starter. The procedure of making kefir was identical to that of kefir starter. As much as 10% v/v dried kefir was added to the milk, then the mixture was and closely sealed for 48-h incubation at room temperature (23-25°C).

Procedure of making concentrate

The procedure of making concentrate was the modification of the study by Sirrat and Jelena (2010) and Senel et al. (2011). Whey separation method (whey reduction) applied in this study was Berge method. One litre of the fermented milk was put in a cheesecloth and hung on a rod 60 cm above the ground for 2 hours at room temperature (23-25°C). The substrate remains on the cheesecloth was the concentrate of fermented milk while the substrate dripping out of the cheesecloth was the whey product. The concentrate yield was weighed as the data of total concentrate data and calculated by percentage from the initial weight of the fermented milk (one liter).

Inhibitory power test

The procedure of inhibitory power test was the modification of the study by Singh et al. (1979). The method used in the test was disk diffusion method. The nutrient was prepared in a 100x15 mm petri dish. When the nutrient was solid, it was divided into four compartments and inoculated with 0.1 ml 10⁻⁷ S. aureus. The paper disk was dipped in the 10 g sample for ≤30 seconds, then the paper disk was swabbed on the media that were previously inoculated with S. aureus. The positive control used was the chloramphenicol antibiotic disk Sanbe® by imbedding the empty paper disk into the 150 mg chloramphenicol antibiotic solution. The zone of inhibition was measured once after 24-hour inhibition. The diameter of zone of inhibition was the average of two different measuring positions in mm.

Chemical properties test

The chemical properties included water content and total solid (%) which were measured using the standard Association of Official Analytical Chemists (AOAC, 2006).

Free fatty acid test

FFA kefir was measured by the titration of NaOH 0.1 N. As much as 10 ml of yoghurt and kefir concentrates were placed in the Erlenmeyer flask, added with 50 ml of ethanol 96% and 2 ml of pp indicator (phenolphthalein), then titrated with 0.1 N NaOH (standardized) until the color turned pink and remained up to 30 second. The amount of fatty acid (%) was calculated with the formula = (ml NaOH for titration × 0.1 (the normality of NaOH) x molecule weight of 100 fatty acid) divided by (sample weight in gram) x 1000 (Sudarmadji et al., 1997).

Lactic acid test

The amount of lactic acid was measured using 0.1N NaOH titration. As much as 20 g of sample was placed into an Erlenmeyer flask, added with 20 ml of aquadest and 2-3 drops of pp indicator until the color turned pink and remained up to 30 seconds. The amount of lactic acid (%) was calculated with the formula = (ml NaOH for titration × 0.1 (normality of NaOH) x 0.9 x 100) divided by (sample in gram x 1000) Sudarmadji et al., 1997).

pH value test

The pH value was determined using pH meter that was calibrated using pH 4 buffer. The measurement was conducted by placing the pH electrode meter into the 10 ml of sample (AOAC, 2006).

Data analysis

The obtained data were subject to ANOVA analysis, followed by Duncan’s multiple range test if any effects were found in the treatments. SPSS version 16 was used in the analysis with tolerance being 5%.

Result and Discussion

Water content, total solid and inhibition test

Based on Table 1, the different types of fermentation and milk did not affect the total solid and water content of concentrate yield of the fermented milk (P>0.05). It was in line with the previous study that the type of milk did not significantly affected the total solid of the yoghurt concentrate despite the different total solid between cow milk and goat milk (Wulansari, 2013). The average water content and total solid of the concentrate of fermented milk were 47.35% and 52.65%, respectively. It was double the average of the previous studies, i.e. 23-25% (Özer and Robinson, 1999), 23.3-26.61% (Özer, 2006) and 21.41-22.23% (Ersoz et al., 2011). The higher average total solid of fermented milk in this study
was due to the extended incubation time and the processing of yoghurt concentrate and kefir concentrate. The increased incubation time resulted in the decreasing pH and a more solid curd which mitigated the whey extraction; therefore, the concentrate contained more total solid (Siri Rat and Jelena, 2010).

The type of milk significantly affected (P<0.05) the inhibition power of the fermented milk concentrate (Table 1). It was in line with Singh et al. (1979) that the type of milk significantly affected the inhibition power. The diameter of inhibition power of the concentrate made of goat milk concentrate was bigger than that of cow milk, i.e. 1.1215 mm vs 0.917 mm. The inhibition diameter of positive control of chloramphenicol (chemical antibiotic) on S. Aureus bacteria in this study was 1.82 mm. The concentrate of fermented goat milk had a 60% inhibition diameter of chloramphenicol.

The biggest inhibition diameter of whey made of cow milk was 0.92 mm, or 50% of the chloramphenicol. Chen et al. (2006) and Chen et al. (2012) stated that whey could serve as the natural antibacterial and for skin treatment. The inhibition diameter of whey was smaller than that of the concentrate. Yoghurt and kefir from milk fermentation did not have inhibition diameter (Table 1). The result showed that turning fermented milk into concentrate could trigger the inhibition power which allowed the concentrate to serve as the natural antibacterial.

The characteristics of concentrate and whey of the fermented milk

The type of milk, the type of fermentation and their interaction significantly affected (P<0.05) the amount of concentrate yielded (Table 2). Goat milk yoghurt produced the highest concentrate, i.e. 65.7% compared to the yoghurt concentrate from cow milk, cow milk concentrate and goat milk concentrate. Cow milk produced more whey than goat milk with the average of 62.4% and 36.4%, respectively (Table 2). Wulansari (2013) stated that the amount of goat milk concentrate was higher than that of cow milk.

The amount of concentrate and whey yield was correlated to the total solid of each type of milk. Goat milk produced a higher concentrate than cow milk, partly because the total solid of goat milk was higher than cow milk, i.e. 18.1% vs 13.4%. The other chemical and physical properties of milk also affected the amount of concentrate yield (Chand, 2006; Raynal-Ljutovac et al., 2008).

The different type of fermentation and type of milk did not affect pH level of the concentrate and whey of the fermented milk (P>0.05). The concentrate from goat milk yoghurt produced the lowest pH, i.e. 3.21 while the whey of goat milk kefir was 3.31 (Table 2). The pH value of fermented milk was 4.47-4.57 (Wulansari, 2013; Setyawardani et al., 2017). The concentrate yield was not for consumption because the considerably low pH made the concentrate organoleptically too acidic. However, the concentrate could be harnessed as the natural antibacterial, particularly S. aureus which are commonly found on the skin surface. The function of low pH was to prevent the development of pathogen bacteria (Yildiz, 2010).

The different type of fermentation and type of milk did not affect the amount of lactic acid of the concentrate and whey from the fermented milk (P>0.05). The concentrate and whey of goat milk yoghurt produced the highest lactic acid, i.e. 19.15 vs 21.05, respectively (Table 2). The amount of lactic acid in this study was similar to 20.14 from goat milk concentrate (Wulansari, 2013). The amount of lactic acid in yoghurt was sugar/lactose in the milk which was turned into pyruvate through the emden meyerhoff pathway (EMP) with dehydrogenase enzyme (Gurakan and Altay, 2010). Goat milk concentrate produced the highest lactic acid because of the higher level of lactose in goat milk compared to cow milk, namely 4.8% vs 4.7% (Van Den Berg, 1988), even up to 5.08-5.21% (Budiarsoha and Sutama, 2014).

In line with Guler and Gursoy-Balci (2011), this study found that different types of fermentation and milk did not affect free fatty acid of the concentrate and whey of the fermented milk (P>0.05). The highest fatty acid yielded from the concentrate and whey of goat milk yoghurt was 0.03 vs 0.02 (Table 2). Free fatty acid is the fatty acid released during the lipid hydrolysis. Goat milk produced a higher fatty acid than cow milk because of the broader surface of the globules of fat for hydrolysis. Attaie and Ritcher (2000) stated that fatty acid of goat milk was 0.73-8.56 µm with 90% particles less than 5.21 µm; therefore, the surface area was broader than that of cow milk with globule diameter of 0.92-15.75 µm.

**Table 1. Water content, total solid and inhibition power**

| Fermentation | Milk       | Water content (%) | Total solid (%) | Inhibition power |
|--------------|------------|-------------------|-----------------|-----------------|
|              | Concentrate (mm) | Whey (mm) | Fermentation milk (mm)|
| Yoghurt     | Goat milk  | 50.49±15.14      | 49.51±15.49    | 1.175±0.174    | 0.885 | 0   |
|             | Cow milk   | 43.45±15.22      | 56.54±15.22    | 0.82±0.073     | 0.92  | 0   |
| Kefir       | Goat milk  | 52.58±15.71      | 47.42±13.71    | 1.10±0.146     | 0.79  | 0   |
|             | Cow milk   | 42.89±11.62      | 57.11±11.20    | 1.00±0.169     | 0.785 | 0   |
| F           | ns         | ns                | ns              | ns              | ns    | ns  |
| M           | ns         | ns                | ns              | *               | ns    | ns  |
| TxM         | ns         | ns                | ns              | ns              | ns    | ns  |

**= Highly significant (P<0.01); * = significant (P<0.05); ns = non significant;*** = Whey and starter product are not subject to statistical analysis.
Table 2. Chemical and physical properties of concentrate and whey of fermented milk

| Fermentation | Milk     | Concentrate | Whey       |
|--------------|----------|-------------|------------|
|              |          | Amount (%)  | pH | Lactic acid (%) | Free fatty acid (%) | Total whey (%) | pH | Lactic acid (%) | Free fatty acid (%) |
| Yoghurt      | Goat milk| 56.7±38.12  | 3.21±0.39 | 19.15±2.97 | 0.02604±0.006 | 35.2±7.91 | 3.37±0.72 | 21.09±8.33 | 0.01697±0.0011 |
|              | Cow milk | 27.8±39.83  | 3.57±0.34 | 18.46±3.53 | 0.02461±0.008 | 64.3±35.59 | 3.24±0.44 | 13.93±1.14 | 0.0144±0.0014 |
| Kefir        | Goat milk| 48.5±18.94  | 3.25±0.28 | 17.87±3.57 | 0.02385±0.071 | 37.6±53.54 | 3.13±0.47 | 12.62±2.48 | 0.01503±0.0026 |
|              | Cow milk | 37.5±53.19  | 3.32±0.29 | 14.76±3.13 | 0.02142±0.008 | 60.5±94.18 | 3.27±0.518 | 11.60±2.94 | 0.01056±0.0002 |
|             |          |             | * | ns | ns | ns | ns | ns | ns |
|             |          |             | * | ns | ns | ns | ns | ns | ns |
|             |          | * | ns | ns | ns | ns | ns | ns | ns |
** = Highly significant (P<0.01); * = significant (P<0.05); ns = non significant.

Table 3. Physical properties of the concentrate and whey of fermented milk

| Fermentation | Milk     | Color of the concentrate | Consistency | Colour of whey | Whey clarity          |
|--------------|----------|--------------------------|-------------|----------------|-----------------------|
| Yoghurt      | Goat milk| white                    | low         | white          | Many unfiltered curd particles |
|              | Cow milk | slightly yellow           | high        | white          | Many unfiltered curd particles |
| Kefir        | Goat milk| white                    | low         | yellow         | Clear                 |
|              | Cow milk | Yellowish                | high        | yellowish      | Clear                 |
* Based on descriptive analysis
The effect of the type of milk and fermentation on the physical properties of the concentrate and whey of fermented milk is presented in Table 3 using descriptive analysis. The color and the consistency of the fermented milk were affected by the type of milk. The concentrate of goat milk was white while the cow milk was yellowish. Similarly, Özer (2006) stated that the difference was due to the physical properties of the yoghurt concentrate that was not affected by both physical and chemical properties of the milk. The concentrate of fermented milk was whiter in color because goat milk does not contain carotenoids. Carotene in cow milk resulted in the yellowish color in the concentrate (Balthazar et al., 2017).

The concentrate of goat milk had a lower consistency than the cow milk because goat milk had a smaller particle (Park, 1994) and smaller diameter of the globules of fat than those of cow milk. The diameter of globules of fat in goat milk was 0.73-8.58 µm, and the 90% of the particles was less than 5.21 µm, compared to 0.92-15.75 µm in cow milk (Attaie and Richter, 2000).

Color and clarity of the whey of the fermented milk concentrate were affected by the type of fermentation. Whey yielded from yoghurt was white while whey kefir was yellowish. Whey kefir was more translucent than that of yoghurt due to the curd particle in yoghurt whey. Lee and Lucey (2010) stated that the physical properties and the microstructure of the fermented milk was affected by the temperature during incubation. The incubation temperature in the present study was different from yoghurt and kefir, namely 43-45°C for yoghurt and room temperature (23-25°C) for kefir.

Conclusions

The types of milk and fermentation affected the inhibition power of Staphylococcus aureus. The concentrate of fermented goat milk had a higher inhibition power on Staphylococcus aureus compared to that of cow milk. The concentrate of fermented goat milk had the potential of natural antibacterial.

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