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The Potential of Concentrate of Fermented Milk for Natural Antibacterial

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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 concentrate 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 bacyeria 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 starer, 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 Sirirat and Jelena (2010) and Senel *et al*. (2011). *Whey separation* method (whey reduction) applied in this study was *Berge methode*. One litre of the fermented milk was put in a cheesecloth and hung on a rod 60cm 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 100x15mm 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 50ml of ethanol 96% and 2 ml of pp indicator (phenolphthalein), then titrated with 0.1N 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 x 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 x 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

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	0.828±0.073	0.92	0
Kefir	Goat milk	52.58±13.71	47.42±13.71	1.108±0.146	0.79	0
	Cow milk	42.89±11.62	57.11±11.20	1.006±0.169	0.785	0
F		ns	ns	ns	ns	ns
M		ns	ns	*	ns	ns
TxM		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.

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 (Sirirat 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 goat 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 Chandan, 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 21.09, 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 embden 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% (Budiarsaha 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 the globules of goat milk was 0.73-8.58 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 2. Chemical and physical properties of concentrate and whey of fermented milk

Fermentation	Milk	Concentrate				Whey			
		Amount (%) concentrate (%)	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
		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
	Cow milk								
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	12.82±2.48	0.01503±0.0026
		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±0.294	0.01056±0.0002
	Cow milk								
F		*	ns	ns	ns	ns	ns	ns	ns
M		*	ns	ns	ns	*	ns	ns	ns
FxM		*	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	Concentrate		Whey	
		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	ellowish	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 carotene. Carotene I 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 43-45ºC for yoghurt and room temperature (23-25ºC) 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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References

Attaie, R. and R. L. Richer. 2000. Size distribution of fat globules in goat milk. J. Dairy Sci. 83: 940-950.

Association of Official Analytical Chemists. AOAC. 2006. Official Method of Analysis. 15th edn. Association of Official Analytical Chemists Inc., Virginia USA.

Balthazar, C. F., T. C. Pimentel, L. L. Ferrao, C. N. Almada, A. Santillo, M. A. Benzio, N. Mollakhalili, A. M. Mortazavan, J. S. Nascimento, M. C. Silva, M. Q. Freitas, A. S. Sant'ana, D. Granato, and A. G. Cruz. 2017. Sheep milk : physicochemical chacarteristics and relevance for functional food development. Comprehensive reviews in food science and food safety. 2 16 : 247-262.

Budiarsaha, I. G. M. and I. K. Sutama. 2014. Efisiensi produksi susu kambing Peranakan Etawah. Seminar Nasional Teknologi Peternakan and Veteriner 2001, Balai Penelitian Ternak, Ciawi Bogor.

Chandan, R. C. 2006. Milk composition, physical and processing characteristics manufacturing yoghurt and fermented milk. In: Manufacturing yoghurt and fermented Milk R.C. Chandan (ed). Blackwell Publishing, Victoria, pp. 17-24.

Chen, M.-J., J. Liu, J. Sheu, C. Lin, and C. Chuang. 2006. Study on skin care properties of milk kefir whey. Asian-Aust. J. Anim. Sci. 19: 905.

Chen, Y., P. Hsiao, W. Hong, T. Dai, and M. Chen. 2012. Lactobacillus kefiranofaciens M1 isolated from milk kefir grains ameliorates experimental colitis *in vitro* and *in vivo*. J. Dairy Sci. 95: 63-74.

Ersoz, E., O. Yerlikaya, and M. Acu. 2011. Effect of phenolic compounds on characteristics of strained yoghurts produced from sheep milk. African J. Agricultural Res. 6: 5351-5359.

Guler, Z. and A. C. Gursoy-Balci. 2011. Evaluation of volatile compounds and free fatty acids in set types yoghurt made of ewes', goats', milk and their mixture using two different commercial starter cultures during refrigerated storage. Food Chemistry. 127: 1065-1071.

Gurakan, G. C. and N. Altay. 2010. Yoghurt microbiology and biochemisttry. In: Development and manufacture of yoghurt and other functional dairy product. F. Yildiz (ed). CRC Press, Boca Raton, pp. 21-47.

John, S.M., and S. Deeseenthum. 2015. Properties and benefits of Kefir-A Review. Songklanakarin J. Sci. Technol. 37 (3): 275-285.

Lee, W. and J. Lucey. 2010. Formation and physical properties of yoghurt. Asian-Aust. J. Anim. Sci. 23: 1127-1136.

Leite, A., B. Mayo, C. Rachid, R. Peixoto, J. Silva, V. Pachoalin, and S. Delgado. 2012. Assessment of the microbial diversity of Brazilian kefir grains by PCR-DGGE and pyrosequencing analysis. Food Microbiology 31: 215-221.

Ouwenhand, A. and H. Roytio. 2015. Probiotic fermented foods and healtth promotion. In: Advances in fermented food and beverage:

improving quality, technologies and health benefits. Wilhelm Holzapfel (ed). Woodhead Publishing, Korea, pp. 1-12.
Özer, B. 2006. Production of concentrated products. In: Fermented Milks. A. Tamime (ed.) Blackwell Science Ltd, New York USA. p 128-155.
Özer, B. H. and R. K. Robinson. 1999. The behaviour of starter cultures in concentrated yoghurt (labneh) produced by different techniques. LWT-Food Science and Technology 32: 391-395.
Panesar, P. S. 2011. Fermented dairy products: starter cultures and potential nutritional benefits. Food Nutr. Sci. 2: 47-51.
Park, Y. W. 1994. Hypo-allergenic and therapeutic significance of goal milk. Small Ruminant Res. 14: 151-159.
Park, Y. W. 2009. Bioactive Components in Milk and Dairy Products. Wiley-Blackwell, Lowa 50014-8300, USA.
Raynal-Ljutovac, K., G. Lagriffoul, P. Paccard, I. Guillet and Y. Chilliard. 2008. Composition of goat and sheep milk product: An update. Small Ruminant Res. 79: 57-72.
Senel, E., M. Atamer, A. Gürsoy, and F. Öztekin. 2011. Changes in some properties of strained (Süzme) goat's yoghurt during storage. Small Ruminant Res. 99: 171-177.
Setyawardani, T., J. Sumarmono, A. H. D. Rahardjo, M. Sulistyowati, and K. Widayaka. 2017. Kualitas kimia, fisik and sensori kefir susu kambing yang disimpan pada suhu and lama penyimpanan yang berbeda. Buletin Peternakan 41: 298-306.
Shiby, V. and H. Mishra. 2013. Fermented milk and milk products as functional food. A review. Critical Review in Food Science and Nutrition. Science and Nutrition. 53. 482-496.
Singh, J., A. Khanna and B. Chander 1979. Antibacterial activity of yoghurt starter in cow and buffalo mik. Journal of Food Protection 42: 664-665.
Sirirat, D. and P. Jelena. 2010. Bacterial inhibition and antioxidant activity of kefir produced from Thai jasmine rice milk. Biotechnology 9: 332-337.
Sudarmadji, S., B. Haryono, and Suhardi. 1997. Prosedur Analisis untuk Bahan Makanan and Pertanian. 4 edn. Liberty, Yogyakarta.
Sumarmono, J. and M. Sulistyowati. 2015. Fatty acids profiles of fresh milk, yoghurt and concentrated yoghurt from Peranakan Etawah goat milk. Procedia Food Science 3: 216-222.
Van Den Berg, J. 1988. Dairy Technology in the Tropics and Subtropics, Deen Hag. Pudoc Wageningen.
Wulansari, P. D. 2013. Optimasi pembuatan and karakterisasi concentrated yoghurt berbahan dasar susu sapi and kambing.Thesis Universitas Jenderal Soedirman, Purwokerto.
Yildiz, F. 2010. Development and Manufacture of Yoghurt and Other Functional Dairy Products. CRC Press, Boca Raton 33487-2742, USA.