Antibacterial Activity of Kefir Grain Levels on Fermented Goat Milk

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Abstract. The purpose of this study was to determine the antibacterial activity of the use of kefir grains in goat's milk. Preliminary tests included the total lactic acid bacteria and yeast. The main research includes syneresis test, total plate count, Escherichia coli and Staphylococcus aureus inhibition test using the disc method. The study used a non-factorial randomized complete design with 3 treatments and 6 replications. Kefir grains presentation were 5%, 10%, and 15%. The results showed that the population of lactic acid and yeast bacteria was $9.3 \times 10^9$ and $1.2 \times 10^7$, respectively. The syneresis values were 5%, 10% and 15% by kefir grain addition 36.60%, 52.07% and 52.05%, respectively. The highest total number of microbes was obtained from the use of 5% yeast (7.67 log cfu.ml$^{-1}$). The highest antibacterial ability was shown in the use of 5% yeast, each of which was 2.19 mm against Escherichia coli and 2.50 mm against Staphylococcus aureus. The results of the study concluded that kefir fermentation in goat milk has not been able to inhibit Escherichia coli and Staphylococcus aureus growth.

1. Introduction
Kefir is a fermented product consist of 0.5%-1.0% alcohol and 0.9%-1.11% lactic acid [1]. There are two different types of kefir, including milk kefir [1] and water kefir [2]. Kefir has a thinner texture than yogurt, but its milk lump is softer and contains CO2 [1]. According to [3] kefir is generally made from cow, goat or sheep milk. While water kefir is made from a mixture of fruits, water, dried fruits such as raisins, small pieces of lemon and granulated sugar [2].

Kefir is one of fermented milk product produced by adding some kefir grain. Kefir grain has white yellowish irregular form, resembling cauliflower’s granule [4]. Kefir grain produces several components namely lactic acid, acetate acid, CO2, alcohol and aromatic compounds that give kefir special feature of sour fresh [5].

Fermented products are affected by starter ability to form lactic acid which is determined by the amount and type of the starter used [6]. [7] explained that the concentration of starter influences kefir pH and antibacterial activity. Based on its activity, antibacterial agent is divided into two types, namely bacteriostatic and bactericidal. Bacteriostatic is an antibacterial agent that fights against pathogenic bacteria (inhibit the growth of bacteria), while bactericidal is an antibacterial agent that kills the bacteria. However some of antibacterial agents become bacteriostatic in low concentration and become bactericidal in high concentration. Pathogenic bacteria are bacteria that can cause disease. Pathogens can be transmitted in a few ways depending on the type. They can spread through...
skin contact, body fluids, and airborne particles having contact with feces and touching a surface touched by an infected person.

The starter concentration shows the strength of bacteria involved in lactose break down. The high addition of starter concentration will result in high level of lactic acid and alcohol as well as the metabolism of microorganism. This study was conducted to determine the characteristics of goat-milk kefir with the addition of different amount of kefir grain by measuring antibacterial ability.

2. Materials and Methods

2.1 Material

The materials used in this research were some liters of goat milk and 15% kefir grain, Staphylococcus aureus and Escherichia coli were also used in this study.

The study was designed with a randomized complete design (RCD) with three levels of kefir grains (A1= 5%; A2= 10%; A3= 15%). Data obtained were analyzed by analysis of variance (ANOVA). If the effect was significant, the treatment differences were tested by the Least Significant Difference (LSD) test.

2.2 Method

Preliminary Research. The total lactic acid bacteria of goat-milk kefir. The calculation of the total lactic acid bacteria was determined by using the method of [8]. The sample volume of 1 mL of kefir was put into 9 mL of sterile distilled water solution with a dilution of 10^-1, 10^-2, 10^-3, 10^-4, 10^-5, 10^-6 then vortexed (Schoot). The dilutions of 10^-5 and 10^-6 were performed with 0.1 mL of de Man Rogosa Sharpe Agar (MRS-Agar, Merck) into a petri dish. The incubation was conducted at 37°C for 24 hours and the growing colonies were calculated by using colony counter (WTW, BZG 30). The calculation was determined by selecting colonies that grew from 25-250 colonies in a petri dish and included as follow:

$\text{Colony/gram (CFU/mL)} = \text{The number of colonies x (1/dilution factor)}$

The total yeast. The yeast test was calculated using pour plate method. There was 0.1 ml of kefir starter that was diluted from 10^-6 up to10^-8, then inoculated in PDA media and incubated at 37 °C for 48 hours. Bacteria colonies were counted by using colony counter. The total of bacteria colonies was stated in log cfu/ml.

$\text{The number of colonies/ ml=the number colonies x 1/dilution x 10}$

Main Research. Total Plate Count. The sterilized tools and materials were cooled. There was 1 ml of sample that was put into a test tube containing 9 ml NaCl 0.85%, conducted aseptically and divortex. This step was conducted up to 10-6 dilutions. Natrium agar was added into petri dishes containing 1 ml diluted sample. Then petri dishes containing the samples were located into incubator in an upside down position at 35 °C for 24-48 hours. The number of bacteria colonies was calculated.

Testing bacterial inhibition activity. The antibiotic activity of kefir goat milk was evaluated using the disk diffusion method as described by the [9]. Antibiotics, ampicillin and penicillin were used to compare bacterial inhibition activity. Paper disc (5mm) was kept in Kefir A (5% of kefir grain), kefir B (10% of kefir grain) and kefir C (15% of kefir grain), and antibiotic for 2 hours. The paper disk with antibiotics and kefir were applied to the agar surface which was previously inoculated with bacterial suspension. These plates were inoculated at 35 °C for 24 hours. The inhibition zone was measured at the end of the fermentation period.

Syneresis. There were 25 grams of kefir sample weighed on a filter paper placed on top of the funnel. Syneresis of whey carried out by gravity and the quantity of whey collected in a flask of known weigh was used as a syneresis value. The drainage time and temperature was 120 minutes and the temperature was 4 °C [10].

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3. Result and Discussion

3.1 Total Plate Count (TPC)

The analysis of variance showed that the addition of kefir grain (%) did not effect (P>0.05). The Total Plate Count (TPC) of goat milk kefir. Figure 1 presents the number of microorganism colonies with the addition of 5% kefir grain ranged 7.40 – 7.67 log CFU/ml-1, while the addition of 10% was 7.60 log CFU/ml-1 which decreased 0.067 of total bacteria. Then the addition of 15% was 7.40 log CFU/ml-1 which decreased 0.2 of total bacteria. This condition shows that the addition of 5% and 10% of kefir grain give an adequate nutrition for microorganism growth. However, the addition of 15% of kefir grain caused most of microorganism in the dead phase due to less nutrition. In the present study, the higher addition of kefir grain will decrease the growth of lactic acid bacteria and yeast. The lower addition of kefir grain will increase the number of bacteria colonies and the higher addition of kefir grain will decrease the number of bacteria colonies in the fermented milk.

The growth of microorganism is influenced by physical and chemical factors including the nutrition in culture media [11]. Microorganism need nutrition such as water, energy source (carbon), nitrogen source, mineral, vitamin and other growth factors. It is in line with [12] who stated that the growth of microorganism in kefir was influenced by physical condition, one of which is temperature applied during the fermentation.

3.2 Syneresis

The syneresis percentage of goat milk kefir with the addition of kefir grain is shown in Figure 2. Note: different superscripts differ significantly (p<0.05).
The analysis of variance showed that the addition of kefir grain (%) had a significant effect (P<0.02) on the percentage of syneresis. Figure 2 presents the percentage of syneresis were 36.60%, 52.07% and 52.05% with addition of 5%, 10% and 15% kefir grain. It is generally found that the higher the addition of kefir grain the higher the syneresis percentage. Wulandari (2010) [13] stated that the higher level of syneresis shows that kefir has poor quality.

Sumardikan (2007) [14] demonstrated that syneresis was caused by the low number of solid content in the fermented milk. Wilkinson (2000) also reported that increasing of milk content is able to increase the strength of gel and viscosity, than reduce the level of syneresis. This condition was occurred due to the fermentation that is not optimal which results in the less nutrition and low binding capacity. The syneresis is probably caused by the change of casein solubility and shrinkage of casein particles [15]. Factors that influence the syneresis of fermented milk include acidity, pH and water holding capacity [16].

3.3 Antibacterial activity
The analysis of variance showed that the addition of kefir grain (%) did not effect (P>0.05) on the inhibiton power against E.coli and S.aureus in the goat milk kefir. Figure 3 presents the inhibition zone formed against E.coli with the addition of 5%, 10% and 15% of kefir grain were 2.19 mm, 1.79 mm and 2.16 mm, respectively. In addition, the inhibition zone formed against S.aureus with the addition of 5%, 10% and 15% of kefir grain were 2.50 mm, 1.85 mm and 2.36 mm, respectively. According to [17] cell wall structure of Gram-negative Bacteria is more complex than that of Gram-positive bacteria. The Gram-negative cell wall is composed of three layers, outer membrane, a peptidoglycan layer and a periplasm, while the Gram-positive bacteria have cell envelopes made of a thick layer of peptidoglycans. [18] also stated that Gram-negative cell wall is relatively complex that enables the bacterium to enter the cell. Chloramphenicol and Tetracycline were used in this study. Chloramphenicol is an antibiotic with a broad spectrum of activity against both aerobic and anaerobic bacteria. The mechanism of action its action is through the inhibition of bacterial protein synthesis by binding with ribosomes [19].

4. Conclusion
The best goat-milk kefir can be produced by the addition of 5% kefir grain based on the Total Plate Count, antibacterial ability and syneresis.
References

[1] Rahman A, Fardiaz S, Rahaju W P, Suliantari and Nurwitri C C 1992 Bahan Pengajaran Teknologi Fermentasi Susu (Bogor: Pusat Antar Universitas Pangan dan Gizi IPB)

[2] Gulitz A, Stadie J, Wenning M, Ehrmann M A, and Vogel R F 2011. International Journal of Food Microbiology. 151(3): 284-288

[3] Kosikowski F and Mistry V V 1982 Cheese and Fermented Milk Foods 3rd Edition (New York : F. V. Kosikowski and Associates)

[4] Guzel ZB, JT Wyffels, AC Seydim, and AK Greene 2005 Int. J. Dairy Tech. 58(1) : 25-29

[5] Otles S and O Cadigi 2003 Pakistan J.Nutrit. 2(1) :54-59

[6] Albaarri A N, and Murti, T. W. 2003. Analisa pH, Keasaman dan Ksadar Laktosa pada Yakult, Yoghurt, Kefir (Semarang: Unika Soegijapranata)

[7] Wijaningsih, W 2008 Aktivitas antibakteri in vitro dan sifat kimia kefir susu kacang hijau vignaradiata) oleh pengaruh jumlah starter dan lama fermentasi (semarang: Tesis Universitas Diponegoro)

[8] Swanson K, Busta E H, Peterson and Jonhson M 1992 Colony Count Methods In: C Vanderzant DF Splittoesser (Eds) Compendium of Methods for The Microbiology Examination of Foods 3rd editionb(The APHA Technical Commite on Microbiology Methods for Foods)

[9] NCCLS 2000 Performance Standards for Antimicrobial Disk Susceptibility Test. 7th Ed (Wayne, National Committee for Clinical Laboratory Standards)

[10] Sahin N, K Yasar and A A Hayaloglu 2008 Food Hydrocolloids 22:1291–1297

[11] Silvia P J 2008 Creativity Research Journal 20, 34–39.

[12] Safitri M and Swarastuti A 2016 J Aplikasi teknologi Pangan 2(2): 87-92

[13] Wulandari E and WS Putranto 2010 J Ilmu Ternak 10(1): 14–16

[14] Sumardikan 2007 Penggunaan Carboxymethylcellulose (CMC) terhadap pH Keasaman, Viskositas, Sineresis, dan Mutu Organoleptik (Malang: Teknologi Peternakan)

[15] Manab A 2008 J Ilmu dan Teknologi Hasil Ternak 52-58

[16] Tamime AY and RK Robinson 1989 Yogurt Science and Technology (London :Pergamon Press)

[17] Pelczar M J dan E C SChan1986 Dasar- Dasar mikrobiologi 1 (Jakarta : Universitas Indonesian Press)