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Research Article

Antioxidant and Antibacterial Activities of Curd and Whey Kefir Produced from Etawa Goat Milk

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Abstract
Kefir is a fermented drink with many benefits. Kefir contains antioxidant compounds that can inhibit free radical activity. In addition, kefir contains lactic acid bacteria, which provide good antibacterial activity and improve the health of the digestive tract. This research aimed to determine the antioxidant and antibacterial activities of kefir made from Etawa goat milk. The methods used in this research included fermentation of Etawa goat milk with 10 % kefir seeds at 37 °C and a fermentation time of 2 days. Antioxidant activity was tested using 1,1-diphenyl-2-picrylhydrazyl (DPPH), and antibacterial activity was tested using agar diffusion. The peptide molecular weights in kefir were analyzed using SDS-PAGE. The results showed that the antibacterial and antioxidant activities in Etawa goat's milk curd kefir has a higher activity than whey kefir. The antioxidant activity of curd has an IC_{50} of 43.99 ppm, which is a powerful antioxidant. Curd's antibacterial activity forms a clear zone of 7 mm against *Bacillus cereus* and is included in the solid antibacterial category. In *Escherichia coli*, a clear zone of 6 mm was formed and included in the moderate antibacterial category. The results of SDS-PAGE gel visualization found three prominent bands measuring 10, 15, and 33 kDa, respectively.

Keywords: etawa goat milk, curd and whey, antioxidant, antibacterial

1. INTRODUCTION

Kefir is a fermented milk product that is popular in many countries because kefir is a probiotic drink that contains many functional substances that are good for health [1]. Kefir differs from other fermented kinds of milk in that it is formed from the fermentation process of kefir grains, which contain a mixture of lactic acid bacteria and yeast. In addition, kefir seeds also contain lactic acid bacteria such as *Lactobacillus brevis, L. helveticus, L. kefir, Leuconostoc mesenteroides, Kluyveromyces lactis,* and *K. marxianus* [2]. The microorganisms that make up the kefir seeds have antibacterial activity, which can assist the digestive system and inhibit pathogenic microorganisms [3]. Kefir has a therapeutic effect because it contains bioactive components and can potentially maintain body health, such as by helping prevent colon cancer and lowering cholesterol levels in the blood. It also has antibacterial properties and can ward off free radicals by functioning as an antioxidant [4].

Free radicals can form inside the body or come from outside the body. Free radicals are formed in the body due to metabolic processes, such as digesting food and using oxygen. Free radicals may come from smoke exposure to radiation, toxic substances, and heavy metals (pollution) [5][6]. Sources of antioxidants are needed to treat cells exposed to free radicals. The exploration of natural sources of antioxidants continues with the growing human awareness of the dangers of free radicals [7]. Therefore, there is increasing worldwide interest in finding natural, food-based antioxidants capable of protecting the human body from free radical attack and inhibiting the development of many chronic diseases [8].

Kefir can be made from various raw materials, such as cow, soy, and goat. During kefir processing, two products are formed, whey and curd [9]. In addition, Etawa goat milk is hydrolyzed with enzymes or by fermentation containing bioactive peptides. Bioactive peptides are a collection of 2–50 amino acids bound to each other by peptide bonds, which have health benefits. Bioactive peptides can be released from their parent proteins through hydrolysis by proteolytic enzymes or fermentation processes [10]. Bioactive peptides produced during fermentation have antihypertensive, antibacterial, antioxidant, antimicrobial, and anti-inflammatory activities. Bioactive peptides also have good functions for body health, such as biological activity in the digestive, immune, and nervous

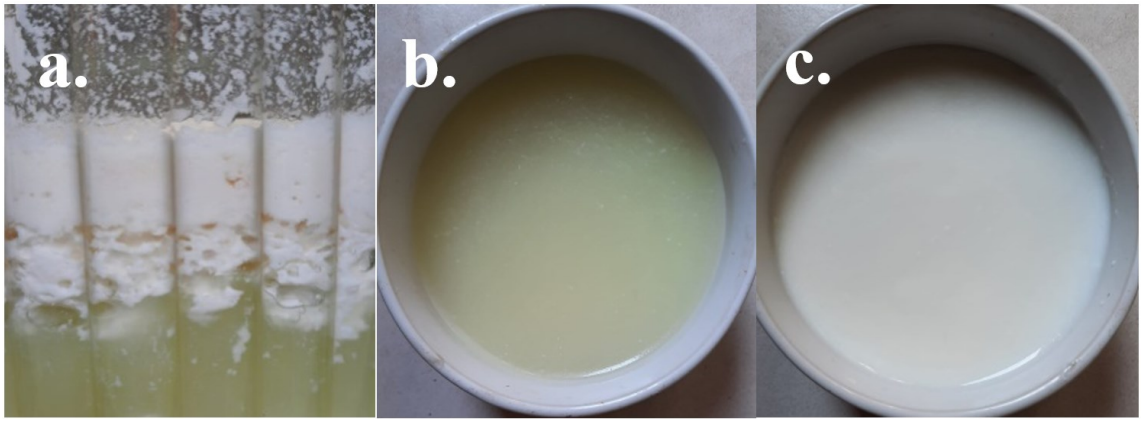

Figure 1. Etawa goat milk kefir (a) whey and curd before being separated; (b) whey and (c) curd.

systems [11].

Producing kefir using sheep's milk as a raw material has obtained antioxidants ranging from 18.39 to 72.41% [12]. Apart from that, it is also known that goat's milk kefir has antibacterial properties against *Propionibacterium acnes* bacteria, forming an inhibition zone between 8.17 to 9.50 mm [13]. Other research reports that goat's milk kefir shows inhibitory activity against *Staphylococcus aureus* bacteria [14], and it is known that goat's milk kefir has antibacterial potential against the pathogens *E. coli* ATCC 8739 and *Salmonella enteric subsp. enterica serovar Typhimurium* ATCC 14028 [15]. Based on the background of the description above, the antioxidant and antibacterial activity of Etawa goat's milk kefir fermentation products and the molecular weight of kefir peptides were identified in this research.

2. MATERIALS AND METHODS

2.1. Materials

Etawa goat milk taken from the Telaga Risqi farm East Metro Lampung, kefir grains (UIN Sunan Gunung Djati Bandung), 1,1-diphenyl-2-picrylhydrazyl (DPPH), NaCl (Merck), *B. cereus* and *E. coli* bacteria cultures (Laboratorium of Microbiology Lampung University), Nutrient Agar media (Merck), and chloramphenicol (Forma mart) were used in this work.

Table 1. IC_{50} value of Etawa goat milk kefir.

Sample	IC_{50} (ppm)
Ascorbic acid (control)	5.443
Whey	46.01
Curd	43.99

2.2. Methods

2.2.1. Etawa Goat Milk Kefir Production

Etawa goat milk kefir is made by pasteurizing it at 72 °C for 15 s, then leaving it at room temperature until it reaches 30 °C [16]. Etawa goat milk pasteurized is taken in as much as 1 L and added with 10% kefir seeds. The kefir milk is fermented at 37 °C for 48 h [17].

2.2.2. Antioxidant Test

The antioxidant test was carried out using the DPPH method. 5 mL of the sample was dissolved in 5 mL of methanol, which was used as the mother liquor. For a 100 ppm primary solution, a series of samples were made with 100, 80, 60, 40, and 20 ppm concentrations. A sample solution with each concentration was put into a test tube, and 2 mL of 40 ppm DPPH was added. The sample solution was vortexed until homogeneous and incubated for 30 minutes in a dark room. The color change from purple to yellow was then observed. Then, the absorbance value of the sample solution was measured with a UV-Vis spectrophotometer at a wavelength of 512–520 nm. The blank solution was 2 mL of methanol and 2 mL of 40 ppm DPPH [18].

2.2.3. Antibacterial Test

The antibacterial test was carried out using the agar diffusion method. A suspension of *E. coli* and

B. cereus bacteria was put into 0.9 mL of NaCl, then vortexed and equalized to the McFarland standard. The agar media that had been prepared was added to 0.1 mL of the bacterial suspension and then levelled with a swab. Then, put the disc dripped with samples and a positive control on it and incubate it for 24 h at 37 °C. The positive control used was chloramphenicol at 2.5 mg/mL. The clear zone around the paper disc indicates a positive test. The clear zone formed was measured and compared with the antibiotic chloramphenicol [4].

2.2.4. Peptide Weight Analysis

SDS-PAGE electrophoresis can be used to determine the molecular weight of the bioactive peptides being analyzed [19]. SDS PAGE electrophoresis (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) The fermented protein samples were denatured with buffer (1 M Tris-HCl pH 6.8, 20% SDS, NO_3) with a protein-buffer ratio of 2:1, boiled at 90 °C for 10 min, and centrifuged for 5 min. An electrophoresis apparatus was prepared. Polyacrylamide gel was prepared from a solution of acrylamide (48%), bisacrylamide (1.5%), stacking buffer (Tris-HCl 0.5 M pH 6.8), resolving buffer (Tris-HCl 1.5 M pH 8.8), 10% SDS, APS, and TEMED as catalysts. After the bottom gel (resolving gel) is formed, stacking gel is inserted at the top, and a mold is made to place the sample protein. Sample electrophoresis was carried out at 150 V for 60 min. The staining protein is Coomassie Blue. The staining results are washed in a destaining solution [20].

3. RESULTS AND DISCUSSIONS

3.1. Etawa Goat's Milk Kefir

Etawa goat milk kefir is a probiotic drink that has many health benefits. In this research, processed milk kefir products were made using Etawa goat milk as raw material. The results obtained from making Etawa goat milk kefir with 10 % kefir seeds for 48 h at 37 °C form two parts, i.e., whey and curd. The results of making Etawa goat milk kefir can be seen in Figure 1.

Figure 1 shows that after 48 h of fermentation, Etawa goat milk kefir forms two parts: the lower layer (the clear phase) is called whey, and the top layer (the solid phase) is called curd. The stage of making kefir goes through a fermentation process to produce a solid called curd and a transparent layer in the form of a liquid called whey [21].

3.2. Antioxidant Activity

The antioxidant activity test in this research used the DPPH method, indicated in IC_{50} values, to ascertain the antioxidant capacity of whey and curd kefir in Etawa goat milk. The antioxidant activity can be divided into five classes based on the IC_{50} value, according to Molyneux [22]: very strong ($IC_{50} < 50$ ppm), strong ($50 < IC_{50} < 100$ ppm), moderate ($100 < IC_{50} < 150$ ppm), weak ($150 < IC_{50} < 200$ ppm), and very weak ($IC_{50} > 200$ ppm). Whey and curd in this research showed IC_{50} values ranging from 43.99 to 46.01 ppm, which included very potent antioxidants. Table 1 displays the findings of antioxidant testing on fermented milk products made from goat whey and curd from Etawa.

The results in the table show that curd has higher antioxidant activity because it has a higher total lactic acid value than whey. In this research, whey and curd had IC_{50} values between 43.99–46.01 ppm, including powerful antioxidants. High total lactic acid can increase antioxidant activity due to the formation of organic acids produced by lactic acid bacteria and yeast, which are synergistic by providing H^+ ions to free radicals [23]. Fermented milk contains natural antioxidants, which are useful

Table 2. Diameter of clear zone whey and curd kefir against *Bacillus cereus* bacteria.

Sample	Clear Zone Diameter (mm)			Average (mm)
	I	II	III	
Whey	4	4	4	4
Curd	7	8	6	7
Chloramphenicol	24	24	24	24

Table 3. Diameter of clear zone whey and curd kefir against *Escherichia coli* bacteria.

Sample	Clear Zone Diameter (mm)			Average (mm)
	I	II	III	
Whey	3	3	3	3
Curd	7	6	5	6
Chloramphenicol	24	24	24	24

for human health because they are safer and more easily absorbed by the body than synthetic antioxidants [7]. Another research used various kinds of milk, such as cows and donkeys. It was found that all fermented kefir products containing amino acids from the breakdown of milk protein by lactic acid bacteria can produce antioxidant activity that is good for health [24].

3.3. Antibacterial Activity

This study examined the whey and curd kefir's antimicrobial properties in Etawa goat milk. At this point, the antibacterial test aims to ascertain whether whey and curd can inhibit both Gram-positive and Gram-negative bacteria. The agar diffusion method was used to conduct the antibacterial test. *E. coli* was employed for Gram-negative bacteria and *B. cereus* for Gram-positive bacteria in the bacterial solution. A clear zone forming around the disc is a sign of antibacterial action. The formed clear zone denotes the region where bacterial growth is inhibited. The results of testing the antibacterial activity of whey and curd kefir in Etawa goat milk with a suspension of *B. cereus* and *E. coli* bacteria can be seen in Tables 2 and 3.

Based on the data in Table 2, the diameter of the clear zone between whey and kefir curd against *B. cereus* bacteria is 4 and 7 mm. As for Table 3, the diameter of the clear zone between whey and kefir curd against *E. coli* bacteria is 3 and 6 mm. Based on these results, it can be seen that the antibacterial activity of whey and kefir curd against *E. coli* was weaker compared to *B. cereus* bacteria, characterized by the formation of a smaller clear zone diameter. This is because *E. coli* is a group of Gram-negative bacteria that have more complex walls. The cell wall of Gram-negative bacteria has as much as 10 % peptidoglycan and several peptide cross-links. The outer layer consists of proteins, lipoproteins, and lipopolysaccharides. This complex *E. coli* cell wall makes it more difficult for antibacterial compounds to penetrate [25]. The ability of goat's milk kefir to have antibacterial activity using 5% seeds gave clear zone results of 2.19 mm against *E. coli* bacteria and 2.50 mm against *S. aureus* bacteria [13].

The inhibition zone, which is antibacterial activity, is categorized into three criteria. The first criterion is an inhibition zone with a weak category with a zone area of 0–3 mm; an obstacle zone with a moderate category with a zone area of 3-6 mm; and the third criterion is an inhibition zone with a strong category with an area of inhibition above 6 mm [26]. Based on the results obtained in this research, it can be concluded that the antibacterial activity of curd forms a clear zone of 7 mm against *B. cereus* and is included in the solid antibacterial category. In *E. coli*, a clear zone of 6 mm was formed and included in the moderate antibacterial category.

3.4. Whey and Curd Kefir Bioactive Peptide Weights

Determination of the peptide molecular weight

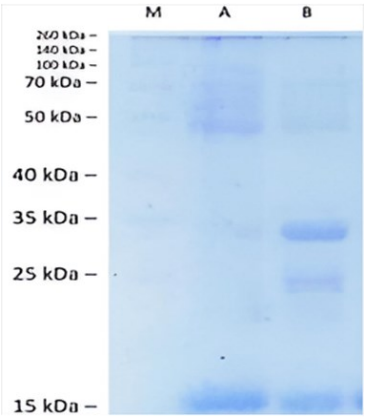

Figure 2. Etawa goat milk kefir peptide weight (M) spectra multicolor broad range protein ladder 26634 for (A) whey; and (B) curd.

was carried out on the whey and curd kefir fractions using the SDS-PAGE method. The results of the analysis of the molecular weight of Etawa goat's milk kefir peptides using SDS PAGE are presented in Figure 2.

Figure 2 shows that the Etawa goat's milk kefir peptide weight intensity in whey and curd has three prominent bands measuring 10, 15, and 33 kDa. Whereas in Etawa goat's milk curd, one other band has a reasonably high peptide intensity, namely the 24 kDa band. This shows that Etawa goat's milk kefir curd has more varied peptides and that the curd has more molecular weight variations. The weight of other peptides with very low intensity in whey and curd of Etawa's milk appears in the 50–100 kDa range. The peptide weighs 10–38 kDa in fermented goat milk yoghurt and has constituent amino acids such as valine, lysine, glutamate, methionine, and alanine [27]. The peptide weight between 10–70 kDa belongs to the water-soluble protein group and is generally a glycoprotein group obtained from protein hydrolysis in goat's milk [20], while the band detected at 50–80 kDa weight belongs to the lactoferrin group obtained from goat's milk kefir Etawa [28].

4. CONCLUSIONS

Antibacterial and antioxidant activities in Etawa goat's milk curd kefir is higher than whey kefir. Curd's antibacterial activity against *Bacillus cereus* was included in the category of intense antibacterial activity while the antibacterial activity against *Escherichia coli* was included in the moderate antibacterial category. The antioxidant activity of curd has an IC_{50} of 43.99 ppm, categorized as a very strong antioxidant. The molecular weight of kefir peptides in whey and curd has three prominent bands, namely 10, 15, and 33 kDa. In curd kefir, there is one band weighing 24 kDa, which has a reasonably high intensity.

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Conflicts of Interest

The author(s) declare no conflict of interest.

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