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Physical Chemical Properties and Antibacterial Activity of Kefir of Goat Milk Combined with Red Dragon Fruit Juice

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Abstrak

Kefir merupakan minuman probiotik hasil fermentasi susu yang bermanfaat bagi kesehatan diantaranya membantu menjaga kesehatan pencernaan. Buah naga kaya akan kandungan gizi dan mempunyai berbagai aktivitas biologis. Penelitian ini bertujuan untuk mengevaluasi sifat fisika kimia dan efek antibakteri dari kefir susu kambing yang dikombinasi jus buah naga merah terhadap bakteri *Salmonella typhi*. Kefir dibuat dari fermentasi susu kambing dengan starter biji kefir 6% ditambah jus buah naga merah dengan variasi konsentrasi 10%, 15%, 20%. Hasil fermentasi menunjukkan nilai pH kefir susu kambing sebesar 3,84 sedangkan pH kefir dengan kombinasi jus buah naga merah antara 3,51-3,80 dengan kadar total asam sebanyak 0,74-1,11%. Diameter zona hambatan kefir susu kambing tanpa kombinasi jus buah naga merah yaitu 9,0 mm dan kefir susu kambing dengan kombinasi jus buah naga merah dengan konsentrasi 10%, 15%, 20% berturut-turut yaitu 10,2; 10,7; 12,0 mm. Kefir susu kambing dengan penambahan jus buah naga merah 20% memiliki aktivitas antibakteri paling baik. Penambahan jus buah naga merah dalam susu kefir mempengaruhi organoleptis, sifat fisika kimia dan juga meningkatkan aktivitas antibakteri kefir.

Abstract

Kefir is a probiotic drink made from fermented milk that is beneficial for health, including helping to maintain digestive health. Dragon fruit is rich in nutritional content and has various biological activities. This study was purposed to evaluate the physical chemical properties and antibacterial activity of goat milk kefir with combination of red dragon fruit juice against Salmonella typhi. Goat milk kefir with 6% kefir grain plus red dragon fruit juice with concentration variation of 10%, 15%, 20%. The fermentation results showed the pH value of goat milk kefir was 3.84 while the pH of kefir with combination of red dragon fruit juice ranged from 3.51-3.80 by producing total acid of 0.74-1.11%. The diameter of the inhibition zone of goat milk kefir without the addition of red dragon fruit juice is 9.0 mm and goat milk kefir with the addition of red dragon fruit juice with concentrations of 10%, 15%, 20% were 10.2; 10.7; 12.0 mm respectively. The goat milk kefir with 20% red dragon fruit juice combination had the greatest antibacterial activity. The addition of red dragon fruit juice in goat milk kefir affected its organoleptically, chemical physical properties and increased the antibacterial activity.

Sejarah Artikel
Diterima :
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Kata kunci:
Kefir, derajat keasaman, *Salmonella typhi*, susu fermentasi

Keywords:
Kefir, pH value, *Salmonella typhi*, fermented milk

Introduction

Salmonella typhi is a facultative bacterium that can cause 11 million cases of typhoid fever in humans and 16,815 deaths worldwide each year (Stanaway et al., 2019). The spread of *S. typhi* in the community can occur through unsanitary food and water contamination (Crump, 2019). *Salmonella typhi* infection enters the body through the mouth, usually by ingesting water or food contaminated with feces. Susceptible people, such as children, are relatively more easily infected. The body's natural defenses are provided by the gastric acid, which will prevent *S. typhi* from adhering to the lining of the small intestine and, subsequently, to the reticuloendothelial system (Wain et al., 1999). Natural immunity in the body will help to block the infection of typhoid fever. Therefore, it is necessary to increase the body's immunity as an antidote to infections in general, including typhoid fever.

Kefir is a fermented milk that has a distinctive taste similar to yogurt (Rosa et al., 2017). Kefir is naturally fermented using a mixture of bacteria and yeast inoculum contained in kefir grains. Kefir is a probiotic beverage that has health benefits, including maintaining digestive health and strengthening the immune system (Farag et al., 2020). In addition, kefir can be modified or fortified with fruit or alternative substrates, allowing for the creation of new functional beverages that provide options for customers (Karina et al., 2018).

The red dragon fruit (*Hylocereus polyrhizus*) is a fruit with reddish purple pulp. This purple-red colour is associated with betacyanin compounds. This fruit is easy to obtain, safe to consume, and has a delicious sweet taste. Each 1 gram of dragon fruit contains 12.97 grams of carbohydrates, 1.45 grams of protein, and 2.65 grams of fibre. Dragon fruit is also rich in dietary fibre, vitamin C, vitamin A, minerals, and phytoalbumin (Ali Jaafar et al., 2009). Variations in the nutritional content of dragon fruit are influenced by the place of growth, the time of harvest, and the method of processing. In addition to being eaten fresh, dragon fruit pulp can be used to make juice, jam, jelly, syrup, ice cream, and yogurt (Nurul & Asmah, 2014).

The red dragon fruit has some biological properties, including the antioxidant ability to bind free radicals (Wain et al., 1999). It also improves the digestive process, prevents colon cancer and diabetes, and lowers cholesterol levels and high blood pressure. Additionally, dragon fruit lowers levels of blood pressure and cholesterol, prevents hyperglycaemia and colon cancer, and enhances the digestion process (Agustina et al., 2021; Suhartati & Roziqin, 2017). The biological properties are associated with its phenolic and betacyanin content (Wain

et al., 1999). In this study, red dragon fruit juice was added to goat milk kefir to increase the benefits obtained. It was expected that the kefir would have antibacterial activity in preventing typhoid infection due to infection in the gastrointestinal tract as well as make the colour of the kefir more attractive. Therefore, this study was aimed at evaluating the physical chemical properties of kefir of goat milk combined with red dragon fruit juice and determining the antibacterial activity of the kefir against *S. typhi*.

Method

Materials & Equipments

The materials used are fresh goat milk purchased from the Faculty of Animal Science, Universitas Gadjah Mada. Kefir grains were obtained from "Keluarga Kefir" Yogyakarta, a kefir producer located on Jalan Sorosutan Yogyakarta. Red dragon fruits were obtained from a farm in Pantai Baru Bantul, *S. typhi*, and nutrient agar media (Oxoid). The equipment used were analytical balance (Ohaus), incubator (Binder), biosafety cabinet (Monmouth), autoclave, potentiometer, micropipette (Soccorex), yellow tips, blue tips, blender, and glassware.

Procedures

1. Sterilization

All tools were first sterilized. Glassware was sterilized using an oven at 170°C for 2 hours. Plastic tools were sterilized at 120°C for 15 minutes using an autoclave.

2. Preparation of red dragon fruit juice (RDFJ)

The red dragon fruit pulp, separated from the skin, is cut into small pieces and pureed using a blender. Next, the red dragon fruit juice was filtered to separate the seeds.

3. Preparation of the goat milk kefir with a combination of red dragon fruit juice

Fresh goat milk was pasteurized at 60-70°C for 30 minutes and then cooled to room temperature. Composition of kefir formula is presented in **Table 1**. Each 6 grams of kefir grain added 10 g, 15 g, or 20 g of red dragon fruit juice, then mixed with 100 ml of goat milk and stirred evenly. Next, the kefir was incubated at 30°C for 24 hours. When clumps are formed, the kefir then filtered to get the kefir seeds back. For making goat milk kefir (without combination), no red dragon fruit juice was added. Each kefir was replicated 4 times.

Table 1. Composition of kefir formula of combination of goat milk and red dragon fruit juice

Composition	Kefir of goat milk	Kefir of goat milk and red dragon fruit juice		
		10%	15%	20%
Kefir grain (g)	6	6	6	6
Red dragon fruit (g)	-	10	15	20
Goat milk (mL)	Until 100	Until 100	Until 100	Until 100

4. Organoleptically observation

All goat milk kefir products, either without addition or with the addition of RDFJ 10%, 15%, or 20%, were tested organoleptically, including odor, colour, taste, and viscosity. The results were then compared to commercial goat milk kefir products on the market and referred to SNI Yogurt.

5. Acidity degree (pH)

Before use, the pH meter is calibrated first. Calibration was done by standardizing the electrode in a buffer solution at pH 4 and pH 7. The electrode was then dipped into the sample until a pH value was fixed.

6. Total acid measurement

Ten ml of the kefir was put into a 100 ml volumetric flask and added with distilled water. Then it was homogenized and filtered. Ten ml of filtrate was put into an Erlenmeyer flask and added with 3 drops of pp indicator. Then titrated with 0.1 N NaOH solution using potentiometric titration. The titration end point was then determined. Total acid content was determined from the calculation formula:

$$\text{Total Acid content (\%)} = \frac{\text{Volume NaOH x N NaOH x 90}}{\text{sample volume (mL)}} \text{ x } 100\% \quad (1)$$

7. Antibacterial activity test by diffusion method

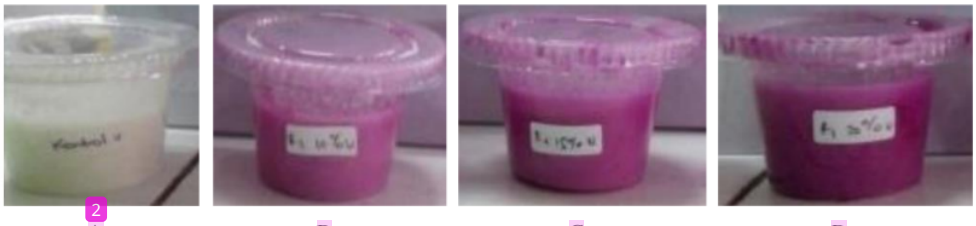
A total of 100 μl of *S. typhi* bacterial suspension, equivalent to 10^8 CFU/mL, was spread on the surface of nutrient agar media. Then, 4 wells were made on the media agar. Then, each well was filled with 100 μl of goat milk kefir, kefir with 10%, 15%, and 20% RDFJ addition. Petri was then incubated for 24 hours at 37°C. Furthermore, the diameter of the inhibition zone formed in the wells was measured using a ruler. Antibacterial activity testing was conducted in 3 replicates (Nugroho & Mulyaningsih, 2022).

8. Data analysis

Data inhibition zone diameters were analysed statistically using one-way ANOVA with a confidence level of 95%.

Results and Discussion

Goat milk kefir had a white colour like milk, while goat milk kefirs combined with RDFJ were purplish pink to purple (**Figure 1**). All the kefir, single or combination with RDFJ, had typical kefir odor, and had a sour taste. The addition of RDFJ caused the odor and taste of kefir to become sourer and sharper. The odor and taste of kefir with 20% RDFJ were sharper than with 15% and 10%. The sour taste was probably caused by enzymatic reactions that convert the lactose substrate in goat's milk into lactic acid, organic acids, and slightly alcoholic due to the fermentation process. The viscosity of goat milk kefir was more viscous than kefir of goat milk combined with RDFJ. The higher the concentration of RDFJ, the less viscosity of the kefir. The addition of RDFJ increased the water content of kefir and causing the viscosity decreased. The overall organoleptic properties of kefir resemble commercial kefir on the market, as shown in **Table 2.**



A B C D

Figure 1. Appearance of goat milk kefir (A), kefir of goat milk combined with red dragon fruit juice 10% (B), 15% (C), dan 20% (D).

Table 2. Organoleptic properties of the kefir of combination of goat milk and red dragon fruit juice

Organoleptic	Commersial kefir	Kefir of goat milk	Kefir of goat milk and red dragon fruit juice		
			10%	15%	20%
Colour	white	white	purplish pink	purple	purple
	typical kefir	typical kefir	typical kefir	typical kefir	typical kefir
Taste	sour	sour	sour	sour	sour
Viscosity	viscous	viscous+++	viscous++	viscous	viscous

Table 3. The result of acidity degree and total acid content of the kefir

Kefir	pH value (mean± SD)	% Total acid content (mean± SD)
Goat milk	6.50 ± 0.012	-
Kefif of goat milk	3.84 ± 0.016	0.77 ± 0.09
Kefir of goat milk & 10% RDFJ kefir	3.80 ± 0.086	0.72 ± 0.07
Kefir of goat milk & 15% RDFJ kefir	3.64 ± 0.051	0.83 ± 0.07
Kefir of goat milk & 20% RDFJ kefir	3.51 ± 0.009	1.11 ± 0.06

The pH and total acid content of goat milk kefir with and without RDFJ added are displayed in **Table 3.** The total acid content of the three kefirs met the requirements of total acid content according to SNI yogurt, which is 0.5-2%. On the other hand, goat milk kefir without RDFJ had a pH of 3.84 and a total acid concentration of 0.77%. Both the pH and total acid concentration decreased with the addition of RDFJ. The pH will decrease and the total acid content will increase as the RDFJ addition concentration increases. Kefir added with 10%, 15%, and 20% RDFJ resulted pH values were 3.80, 3.64, and 3.51, respectively. The obtained total acid concentration was 1.11%, 0.83%, and 0.72%, in that order. The amount of vitamin C or ascorbic acid in dragon fruit may be the cause of the lowering in pH and rise in total acidity, as well as the addition of RDFJ. According to reports, the vitamin C content of red dragon fruit ranges from 63.71 to 132.95 mg (Ali Jaafar et al., 2009).

In general, the antibacterial activity of goat milk kefir with 10%, 15%, and 20% RDFJ addition was greater than that of goat milk kefir. Table 4 shows the inhibition zone diameter of goat milk kefir with the addition of RDFJ is greater than that of goat milk kefir.

Table 4. The result of antibacterial effect of goat milk kefir with and without red dragon fruit juice

Kefir	Diameter of inhibition zone (mm) ± SD
Kefir of goat milk	9.0 ± 0.05
Kefir of goat milk & RDFJ 10% kefir	10.2 ± 0.05
Kefir of goat milk & RDFJ 15% kefir	10.7 ± 0.05*
Kefir of goat milk & RDFJ 20% kefir	12.0 ± 0.01*

*significantly different with negative control

The fermentation process in goat milk kefir uses the sugar lactose as an energy source. While in kefir with the combination of RDFJ, the energy source comes from lactose from goat's milk and sugars contained in red dragon fruit. Lactic acid bacteria (LAB) may utilize red dragon fruit's carbohydrates and other substances for energy during fermentation (Nurul & Asmah, 2014). The higher energy source utilized by LAB is likely to cause an increase in the metabolic process of LAB, thus increasing lactic acid products, organic acids, and other compounds. The addition of carbohydrates also creates a good environment for LAB growth and can be a source of energy for these bacteria (Arfianty et al., 2017). The increase in lactic acid content is due to the activity of LAB that breaks down lactose and other sugars into lactic acid. The greater the concentration of RDFJ added, the smaller the pH of the kefir formula and the higher the total acid content.

The addition of RDFJ lowers pH value possibly due to vitamin C content in RDFJ, which is acidic. Carbohydrates in RDFJ may also increase LAB metabolism producing lactic acid and

other organic acids that act a significant role in reducing pH value. LAB fermentation results in the accumulation of organic acids, such as lactic acid, propionic acid and acetic acid. Acetic acid and propionic acid are major antagonists against fungi such as *Aspergillus fumigatus* and *Aspergillus nidulans*, with their inhibitory potential decreasing with increasing pH (Garrote et al., 2000). The cytoplasm of cells becomes acidified when the external pH is low. Undissociated acid molecules passively migrate across the membrane because they are hydrophobic (Cabo et al., 2002). Consequently, the undissociated acid molecules alter the permeability of the cell membrane, resulting in disruption of the substrate transport system. The antimicrobial activity of kefir is possible from BAL producing low molecular weight antimicrobial compounds and extracellular polysaccharides and bacteriocins (Yang, 2000). Bacteriocins are small peptides or proteins that can generally kill target bacteria by acting on the membrane or cell wall (Mokoena, 2017). Some bacteriocins are involved in dissipating the proton gradient across the bacterial cytoplasmic membrane; others inhibit cell wall biosynthesis or also create "pores" that lead to metabolite loss metabolit (Nishie et al., 2012). Some antimicrobial compounds from LAB have been identified, namely reuterin, reuterocyclin, and reutericin isolated from *Lactobacillus reuteri* (Engels et al., 2016).

The study's findings revealed that the inhibitory zone diameter and total acid value were inversely correlated with pH value. The overall acid content of kefir increases with decreasing pH, and the inhibition zone diameter increases with decreasing pH. The antibacterial effect of the kefir of goat milk and 20% RDFJ had a pH value of 3.51 with a total acid content of 1.11% had the largest inhibition zone diameter and was the best antibacterial formula.

Conclusion

Kefir of goat milk combined with RDFJ at tested concentrations affected the physical chemical properties of the kefir, including colour, taste as well as pH and total acid content. Kefir of combination of goat milk with 20% RDFJ showed the lowest pH value, the highest total acid content, and the strongest antibacterial activity against *S. typhi*.

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