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Antibacterial activity of colostrum kefir against foodborne pathogen bacteria

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Abstract. Colostrum is the first fluid produced from the mammary gland 24 to 72 hours after the postpartum. Here, we have the psychochemical characteristics of fermented colostrum that fermented with kefir grain for 24 and 48 hours and its activity against eight foodborne pathogens. *Bacillus cereus*, *Bacillus pumilus*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, *Salmonella* Typhi, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* were used as test strains, and antibacterial activity was investigated based on inhibitory zones using disc diffusion methods. Psychochemical characteristics and activity varied according to colostrum kefir type and fermentation time. The widest and the strongest antimicrobial activity is obtained after at least 48 hours of fermentation for all colostrum kefir, although the traditional kefir fermentation method is for 18-24 hours at 25 °C. For the type of colostrum kefir K1, K2, P1, and P2 after fermentation for 48 h, it shows that antimicrobial activity increase along with the increasing of titrable acidity and decrease in pH value.

Keywords: Antimicrobial activity, Colostrum kefir, Foodborne pathogens, Fermentation time, Probiotics

1. Introduction

Along with the rapid development of web-based and mobile-ready electronic commerce, the fast-paced national and international trades in foodstuffs around the globe present new challenges to food safety systems, particularly in developing nations. The most commonly known bacterial pathogens associated with foodborne diseases worldwide are *Salmonella enterica*, *Campylobacter jejuni*, *Escherichia coli*, *Listeria monocytogenes*, *Cronobacter sakazakii*, *Vibrio cholerae*, and *Vibrio parahaemolyticus*. Outbreaks and prevalence of foodborne diseases are not only a major burden on global healthcare systems but also result in a substantial negative impact on economic growth and social stability.

Foodborne diseases occur due to the ingestion of bacteria, viruses, or parasites that multiply in the

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intestine or consumption of non-communicable agents such as toxins and chemicals and cause disease [1]. A common symptom of foodborne illness is diarrhea, which is sometimes accompanied by nausea and vomiting. Several factors contribute to these food diseases, such as lack of personal hygiene from food handlers, no supply of clean water and an unclean environment. Bacteria, viruses, and parasites can cause foodborne diseases. Most foodborne illnesses such as cholera, typhoid fever, hepatitis A, dysentery and food poisoning are associated with acute gastrointestinal symptoms such as diarrhea and vomiting. Generally, cholera is a foodborne disease for *Vibrio cholera* infection in the digestive system. The symptoms include severe acute watery diarrhea sometimes accompanied by vomiting. *V. cholera* can be easily isolated from seafood, such as shellfish and oysters. Typhoid fever is caused by *Salmonella* Typhi bacteria, and symptoms of infection are fever with the body increasing to 39 °C to 40 °C, headaches, loss of appetite, and stomach pain. Also, typhus is easily spread from contaminated food and water or infected humans or carriers.

Hepatitis is a disease that affects the liver. This is caused by a virus called the hepatitis A virus (HAV) that can be transmitted through contaminated food and drink. Symptoms of the disease include jaundice, dark urine, anorexia, malaise, and extreme fatigue. Dysentery is an infection that causes severe diarrhea containing mucus and bloody stools, vomiting blood, or in some cases, serious death if no treatment is given. Examples of organisms that cause dysentery are *Entamoeba histolytica*, a parasite that causes amoebic dysentery and *Shigella dysenteriae* causes bacillary dysentery.

Diseases caused by enteric pathogens can be prevented so that they do not have a more severe impact on certain groups. One of them is by adopting a control strategy to kill food pathogens and prevent waste caused by spoilage. The strategy includes physical, chemical, and biological methods, whether applied alone or in combination. Several non-thermal handling methods have been carried out, such as the use of high hydrostatic pressure, pulsed electric fields, oscillating magnetic fields, photodynamic effects, and high voltage arcs and plasma streamers. However, this technology cannot always guarantee food safety. The use of chemicals in food is also a common way to preserve food. The purpose of adding various chemicals or additives in food preservation is to control pH, as an antimicrobial and antioxidant agent. Natural antimicrobials have received increasing attention over the past decade, for example, essential oils [2]. Testing the antibacterial activity of essential oils against *Listeria monocytogenes*, *Salmonella* serovars, *Escherichia coli* O157: H7, *Shigella dysenteriae*, *Bacillus cereus*, and *Staphylococcus aureus* have been well demonstrated in various in vitro studies [2]. However, because essential oils have many antimicrobial effects, bacteria tend to develop resistance to them. Also, essential oils can affect organoleptic properties. Therefore, it is necessary to choose the right essential oil according to the type of food; it is also recommended to be combined with other physical methods, such as heating, high hydrostatic pressure, and pulsating electric fields against pathogenic bacteria [3].

Besides the use of natural ingredients, it currently also has been widely used antibiotics to prevent and treat bacterial infections. Many diseases can be effectively treated with the use of antibiotics. However, antibiotics may not kill all pathogens during infection, and excessive use of antibiotics or abuse also causes resistance to the victim. Many infections are resistant to antibiotics in Indonesia, the United States, and throughout the world [4]. Increased strains that are resistant to antibiotics have caused increased health problems and losses to society, including increased mortality, length of stay, health care costs, etc. [5]. Foodborne pathogens that are resistant to antibiotics such as *Salmonella*, *Campylobacter*, and *E. coli* have been common. For example, in a European Antimicrobial Resistance Surveillance Network (EARS-Net) report, *E. coli* resistance to cephalosporins and combined resistance to cephalosporins, fluoroquinolones and aminoglycosides increased significantly between 2013 and 2016 throughout Europe [6]. In another report by the National Antimicrobial Resistance Monitoring System (NARMS) conducted in 2009-2011, it showed that 67% of all *Salmonella* Typhimurium were resistant to ciprofloxacin, and nearly 25% *Campylobacter* tested resistant to ciprofloxacin [5]. These results show that antimicrobial resistance is a challenge for the food industry. Therefore, monitoring and monitoring of antimicrobial resistance need to be

continued; and alternative strategies are needed to control foodborne pathogens, for example, by using probiotics.

According to United Nations of Food and Agriculture Organization and WHO, probiotics are defined as living microorganisms that, when given adequate amounts, provide health benefits to their hosts. The probiotic strains must be able to survive through the upper digestive tract, multiply, colonize, and function in the intestine. Most probiotics come from humans [7]. There is an increasing popularity in the use of probiotics because of the promotion of their health effects, as well as their ability to prevent or treat various diseases, including common infectious diseases [8]. In the United States, probiotics are available as conventional foods, food supplements, medical foods, and medicines. Probiotics are often given in capsules, liquid or powder form [9]. The most commonly used microorganisms as probiotics are bacteria and yeast. There are four bacterial genera and one yeast genus used for most preparations: *Enterococcus*, *Bifidobacterium*, *Escherichia*, *Lactobacillus*, and *Saccharomyces*. Probiotics act through a variety of mechanisms that affect the composition or function of intestinal microbiota and also affect host epithelial and immune responses [8]. Lactic acid bacterial strains, specifically *Lactobacillus* spp. and *Bifidobacterium* is commonly used as a probiotic in fermented milk products such as kefir, yogurt, or koumiss [10]. In kefir, for example, *Lactobacillus* spp., *Bifidobacterium*, and other bacteria, including *Enterococcus* spp., *Leuconostoc* spp. moreover, yeast such as *Saccharomyces* spp. it has also been used as a probiotic [11].

Kefir is a unique fermented milk product produced by a mixture of lactic acid bacteria, acetic acid bacteria, and yeast. The results of studies in vitro showed that milk kefir has potential and activity varies according to the type and time of fermentation. In addition to using fresh milk raw materials, kefir can also be obtained by adding 20% starter kefir grain into colostrum, the first liquid released by mammals 24-72 hours postpartum [12]. Therefore, this research investigated the psychochemistry and activity of colostrum kefir on eight types of foodborne bacteria.

2. Materials and methods

2.1 Colostrum kefir preparation

Two types of colostrum were collected from a private farm in Manglayang mountain, Bandung. Colostrum obtained 24 hours (K) and 48 hours (P) postpartum was used in this experiment, while kefir grain was obtained from the Indonesian kefir community. A total of 200 g of viable kefir grains was inoculated in 1000 mL colostrum (20% w/v) and cultured at 25°C for 24, 48, or 72 h. At the end of the the fermentation process, the grains, and fermented colostrum were separated using a sterilized plastic filter (2-mm pore size).

2.2 Psychochemical analysis of colostrum kefir

Psychochemical analysis was conducted to determine viscosity, pH, and titratable acidity, while proximate analysis was performed according to AOAC [13] procedures to determine ash, proteins, moisture, fat, and carbohydrates contents in colostrum kefir.

2.3 Bacterial strains

A total of eight strains of frequently reported as foodborne pathogens were included: *Bacillus cereus*, *Bacillus pumilus*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, *Salmonella* Typhimurium, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* were used in antimicrobial activity tests. Each strain was streaked onto Mueller Hinton Agar (Difco) and they are incubated for 24 h at 37°C for antimicrobial activity tests.

2.4 Antimicrobial test with colostrum kefir

For antimicrobial activity tests, 3.256 g colostrum kefir was centrifuged at 10,000 rpm for 2 × 5 min, and the supernatant was sterilized by filtration using a 0.45-µm pore-size syringe filter (Millipore Co., USA). A free-cell colostrum kefir extract stored at 4°C before used. Chlorhexidine 1% used as a

positive control antimicrobial. Colostrum kefir extract was tested for antimicrobial activity using the disk diffusion method as described by CLSI [14]. Bacteria species with a concentration in range 10^6 – 10^8 CFU/mL were spread on Mueller Hinton agar (MHA) with a sterile cotton swab. Sterile filter paper discs with 6 mm diameter were placed on top of the culture, and ten μ L of free-cell colostrum kefir extract was loaded on the paper discs. 0.1% of commercial chlorhexidine (CHX) was used as a positive control, whereas 10% DMSO as a negative control. The plates were incubated at 37 °C for 24 hours. Evidence of clear zone indicates bacterial growth inhibition, and the diameter was measured in mm.

3. Result and discussion

The pH, titrable acidity, alcohol, and specific gravity values of the colostrum kefir during the fermentation process are shown in Table 1. pH values in both types of colostrum kefir decreased with increasing fermentation time. While the levels of titrable acid, alcohol, and specific gravity have increased.

Table 1. Psychochemical data of colostrum kefir.

Parameter	Type of Colostrum Kefir ^a			
	K1	K2	P1	P2
pH	3.65	3.40	3.61	3.40
Titrable acidity (%)	1.96	2.55	1.98	2.52
Alcohol (%)	0.39	0.55	0.38	0.57
Specific gravity (g/mL)	1.030	1.046	1.038	1.043

^a K1= colostrum kefir P1, 24 h; K2= colostrum kefir P1, 48 h; P1= colostrum kefir P2, 24 h; P2= colostrum kefir P2, 48 h.

Antimicrobial activity of the colostrum kefir against the food pathogen bacteria is also presented in Table 2. Antimicrobial activity generally increased along with fermentation time in all types of kefir. This effect may be related to the decreased pH and increase in titrable acidity observed during the fermentation process. Our findings in accordance with previous reports [15]. Colostrum kefir obtained following the CODEX standard for kefir [16].

Table 2. Inhibition zone of the tested colostrum kefir against some foodborne bacteria.

Strain	Inhibition zone (mm)					
	K1	K2	P1	P2	CHX	DMSO
Gram (-)						
<i>L. monocytogenes</i>	8.7	8.7	9.3	8.7	25.3	n.a
<i>B. cereus</i>	7.7	7.3	7.3	7.7	18.7	n.a
<i>E. coli</i>	8.0	8.3	9.7	9.0	20.7	n.a
<i>K. pneumoniae</i>	8.3	8.3	8.3	9.0	22.0	n.a
Gram (+)						
<i>Salmonella</i> Typhi	7.7	8.0	6.3	6.5	16.7	n.a
<i>B. pumilus</i>	7.3	8.0	8.7	10.0	20.7	n.a
<i>S. aureus</i>	8.3	9.3	9.3	10.0	20.3	n.a
<i>P. aeruginosa</i>	8.3	8.3	8.3	9.0	19.7	n.a

K1: colostrum kefir day-1(fermentation time 48 h); K2: colostrum kefir day-1(fermentation time 72 h); P1: colostrum kefir day-2(fermentation time 48 h); P2: colostrum kefir day-2 (fermentation time 72 h); negative control: DMSO 10%; positive control, CHX: chlorhexidine 1%; n.a: no activity; diameter of inhibition zone in mm (including disc).

4. Conclusion

In this research, colostrum kefir has been successfully obtained by adding 20% kefir grain into colostrum. Psychochemical characteristics and activity varied according to colostrum kefir type and fermentation time. Colostrum kefir has antibacterial properties against both groups of gram-positive and gram-negative foodborne bacteria. For the type of colostrum kefir K1, K2, P1, and P2 after fermentation for 48 h, it shows that antimicrobial activity increase along with the increasing of titrable acidity and decrease in pH value.

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