



Antibacterial Activity of Synbiotic Yoghurt Peel Extract of Red Dragon Fruit (*Hylocereus polyrhizus*) Evaporation with Honey

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Synbiotic yogurt contains both probiotics and prebiotics. The peel of red dragon fruit contains 10.79% pectin, which can be used as a prebiotic. Red dragon fruit peel synbiotic yoghurt has an unpleasant aftertaste. Honey can enhance the functional properties of dragon fruit peel synbiotic yogurt. Yogurt with the addition of prebiotics can increase the functional properties of probiotic yogurt and increase the viability of bacteria in the digestive tract. The purpose of this study was to determine the antibacterial activity of synbiotic yogurt with evaporated red dragon fruit peel extract and honey. This research was conducted at the Laboratory of Animal Products Technology, Faculty of Animal Husbandry, Universitas Brawijaya. The research material is 3-month-old male 15 white rats (*Rattus norvegicus*) weighing between 150-180 g and divided into 3 groups, probiotic yogurt,

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synbiotic yoghurt with addition of red dragon fruit peel extract (*Hylocereus polyrhizus*) 20% made from 10% skim milk and yoghurt starter containing *Lactobacillus bulgaricus* and *Streptococcus thermophilus* (1:1), and the addition of 2% honey to synbiotic. Animals were given probiotic yogurt (P0), 20% red dragon fruit peel extract synbiotic yogurt (P1), and 20% red dragon fruit peel extract synbiotic yogurt with 2% honey sweetener (P2). The duration of experimenting is 30 days. Afterward, white rats will be dissected and the faeces in the ileum will be taken for testing. The data obtained were analyzed using descriptive qualitative. The results showed that P0 did decrease coliform and *Enterococcus spp* but increase total bacteria, mold and yeast, and *Escherichia coli*. The addition of evaporated red dragon fruit peel extract in synbiotic yogurt (P1) did decrease total bacteria, coliform, and *Enterococcus spp* but increase in mold and yeast and *Escherichia coli*. The addition of honey (P2) did decrease total decrease, mold and yeast, and *Enterococcus spp* but increase *Escherichia coli*. The addition of honey to the synbiotic yogurt of red dragon fruit peel extract can affect the total number of bacteria, mold and yeast, coliform, *Escherichia coli*, and *Enterococcus spp*.

Keywords: Synbiotic yoghurt; red dragon fruit peel; honey; antibacterial activity; *Escherichia coli*; *Enterococcus spp*.

1. INTRODUCTION

Yoghurt is a product obtained from fermented milk using *Lactobacillus bulgaricus* and *Streptococcus thermophilus* bacteria and/or other suitable lactic acid bacteria, with/without the addition of other food ingredients and permitted food additives. Probiotics used in fermented milk such as yogurt are *Lactobacillus bulgaricus* and *Streptococcus thermophilus* because they have a synbiotic relationship [1]. Yoghurt with the addition of prebiotics is expected to increase bacterial survival and store food for microbes in the digestive tract [2]. The addition of prebiotics to probiotic yogurt is called synbiotic yogurt [3]. One of the ingredients that can be a prebiotic ingredient is red dragon fruit peel (*Hylocereus polyrhizus*).

Red dragon fruit peel becomes waste even though the content contained in red dragon fruit peel can be utilized as prebiotics and increase the functional value of yogurt. The content of red dragon fruit peel (*Hylocereus polyrhizus*) contains higher fiber and betasianin when compared to white dragon fruit peel (*Hylocereus undatus*). Red dragon fruit peel has 46.7% fiber, 10.79% pectin, 150.46/100g betasianin, alkaloid compounds, steroids, saponins, and tannins [4]. The addition of red dragon fruit peel extract also affects the flavor of the yogurt. Making synbiotic yogurt with red dragon fruit peel extract produces an aftertaste that consumers do not like [5].

The addition of natural sweeteners such as honey is an attempt to increase the sweetness of yogurt. The addition of honey to yoghurt can improve the quality and nutritional value of yoghurt and improves organoleptic properties [6].

The content contained in honey is 41% fructose, 35% glucose, and 1.9% sucrose [7]. Honey is one of the energy sources for the growth of Lactic Acid Bacteria (LAB) in yogurt making because it is one of the oligosaccharides that can be used as a source of prebiotics [8]. The addition of honey up to 3% can improve the quality of yoghurt without having a detrimental effect on Lactic Acid Bacteria (LAB) characteristics and yoghurt characteristics [9].

Yogurt as a functional food has many benefits because the content of probiotics and prebiotics becomes synbiotic such as preventing the growth of pathogenic bacteria in digestion. *Enterococcus spp* and *Escherichia coli* bacteria can cause digestive disorders such as poisoning and diarrhea [10]. An increase in coliform microbial growth and a decrease in the number of non-pathogenic bacteria attached to the intestinal villi will result in the formation of mucin compounds resulting in damage to epithelial cells and intestinal villi [11]. Synbiotic products are expected to increase the viability of counteract pathogenic bacteria that cause digestive disorders.

Yoghurt with the addition of dietary fiber from red dragon fruit peel extract (*Hylocereus polyrhizus*) and natural sweetener honey can be studied to test its antimicrobial properties. This research needs to be followed up on natural sweeteners used in synbiotic yogurt in terms of antibacterial activity against experimental animals.

2. MATERIALS AND METHODS

The material in this study was experimental animals, namely 3-month-old male 15 white rats

(*Rattus norvegicus*) weighing between 150-180 g and divided into 3 groups. These animals were healthy and did not carry disease. The animals were first adapted to the environment for 7 days and given basal feed. First week, one rat in each group was dissected for pre-treatment variables. Afterward the animals were given probiotic yogurt (P0), synbiotic yogurt with 20% red dragon fruit peel extract (P1), and synbiotic yogurt with 20% red dragon fruit peel extract sweetened with 2% honey (P2) for 30 days. Afterward, white rats will be dissected and the faeces in the ileum will be taken for testing.

3. RESULTS AND DISCUSSION

3.1 Treatment Effect on the Average Total Number of Excreta Bacteria in Animals Given Synbiotic Yoghurt

Based on Table 1, the average total bacteria indicate the presence of antibacterial activity by synbiotic yogurt with honey-sweetened dragon fruit peel extract. In Table 1, the results of these observations show that the P0 treatment has increased and P1 and P2 have decreased. The results of the study showed that the synbiotic yogurt of dragon fruit peel extract with honey sweetener has antibacterial activity. The difference in the total number of bacteria is due to the addition of food ingredients to the yogurt. Addition of foodstuffs can affect the interaction between microbes in products [12].

The P0 treatment increased because the lactose in milk is utilized by lactic acid bacteria so as to increase the growth and activity of lactic acid bacteria. Lactic acid bacteria in yogurt digest protein and lactose to build new cells and affect the growth rate of lactic acid bacteria [13] *Lactobacillus bulgaricus* and *Streptococcus thermophilus* are a combination of bacteria that coexist and benefit living things in the intestines. Probiotic yogurt can increase and improve the balance of microflora in the intestinal tract [14].

The P1 treatment decreased. The decrease occurred because adding red dragon fruit peel extract can increase the growth and activity of lactic acid bacteria. This increase in activity

exceeds yoghurt without addition because red dragon fruit peel extract has dietary fiber which is a prebiotic. The food fiber contained in red dragon fruit peel is around 46.7% [4]. This prebiotic can increase the activity and growth of lactic acid bacteria. In addition, red dragon fruit peel extract also contains antibacterial flavonoids that can reduce total bacteria. Flavonoids can damage the cell walls of bacteria resulting in bacterial death. Anthocyanins and betasianins in dragon fruit peel extract that provide color also have antibacterial properties. Betasianin provides a purplish-red color in red dragon fruit peels and has antimicrobial and antifungal characteristics [15]. The hydrophilic nature of betasianin can inhibit the growth of bacteria by damaging the cell membrane or inhibiting protein synthesis [16]. Damage to the cell membrane causes a loss of internal and external pH of the cell so that it is unbalanced and causes death [17].

P2 treatment also decreased more when compared to control and P1. The addition of honey can increase the activity and growth of lactic acid bacteria. Lactic acid bacteria can convert carbohydrates such as the lactose in milk and fructose in honey sweeteners into lactic acid. The content contained in honey is 41% fructose, 35% glucose, and 1.9% sucrose (Yurliasni, et al., 2019). This is match with Zakaria's research [18], the addition of sugar up to 15% tends to increase lactic acid levels in synbiotic yogurt through a fermentation process carried out by lactic acid bacteria against carbohydrates, lactose, and fructose. Fructose in large quantities can inhibit the growth of bacteria. This is in accordance with Watanabe's [19] which shows fructose has higher antibacterial properties than glucose and sucrose. Other ingredients such as flavonoids in honey also have antibacterial properties. Phenol compounds and phenolic compounds derivatives can also cause the denaturation of proteins contained in the cell wall so that they can damage the composition and change the permeability mechanism of microsomes, lysosomes, and cell walls [12]. Flavonoids also cause lysis resulting in aggregation of the bacterial membrane which results in a reduction in nutrient uptake [20].

Table 1. Average total number of excreta bacteria in experimental animals given synbiotic yogurt (Log CFU/g)

Ileum	P0		P1		P2	
	Before	After	Before	After	Before	After
TPC	10,18	10,47	10,89	10,60	10,80	10,18

3.2 Effect of Treatment on Average Total Mold and Excreta Yeast in Experimental Animals Given Synbiotic Yogurt

Based on Table 2, the average total mold and yeast showed that the P0 and P1 treatments had increased. This can happen in the process of feeding experimental animals. According to Auchtung [21], the formation of mold and yeast colonization in the intestines and feces is due to the transition from food consumed and the oral cavity to the digestive system of experimental animals. Molds and yeasts can grow in the ileum due to additional nutrients from the food consumed [22]. Some foods that cannot be digested in the upper digestive tract such as the mouth and stomach will be channeled to the lower digestive tract such as the small intestine and will be digested by mold and yeast and existing microbes [23]. The addition of red dragon fruit peel extract can also be an indicator of the increase in mold and yeast. Pectin contained in the red dragon fruit peel extract can be fermented by mold and yeast to increase the population in the ileum [23].

The treatment in P2 decreased the average total value of the amount of mold and excreta yeast. According to Van Riel [24], several factors that can be antifungals in honey are the rich composition of fructose has caused dehydration of existing microorganisms and makes microorganisms die. Glucose and fructose in honey can cause molds and yeasts to release fluids in their bodies, resulting in death [25]. Flavonoid compounds in honey can directly inhibit growth in fungi by denaturing protein bonds in cell membranes, and lysis occurs causing mold and yeast not to grow back [25].

3.3 Effect of Treatment on the Average Total *coliform* Excreta Bacteria in Experimental Animals Given Synbiotic Yogurt

By Table. 3, the average total coliform shows the P0, P1, and P2 treatments have decreased. This shows that yogurt, synbiotic yogurt, and synbiotic yogurt with honey sweetener influence the number of coliforms. The ability of Lactic Acid Bacteria (LAB) to produce antimicrobial activity because LAB can produce bacteriocin can inhibit coliform growth [26]. Bacteriocin secreted in the ileum will attach to the cells of pathogenic microorganisms, damage the cell membrane, and prevent the re-formation of the cell

membrane [27]. Lactic acid bacteria colonies in the ileum will compete with pathogenic bacteria in nutrition. Lactic acid bacteria in the intestine compete with pathogenic bacteria by attaching to receptors in the ileum and secreting antibacterials to gain access to nutrients in the ileum [28].

In the P1 and P2 treatments, it decreased due to the addition of red dragon fruit peel extract (P1) and honey sweetener (P2). Pectin and honey as prebiotics can increase the growth and activity of lactic acid bacteria to produce lactic acid. The greater the amount of LAB in yogurt, the higher the lactic acid content [29]. Increased lactic acid levels can reduce the pH of the synbiotic yogurt. Coliform will not survive at low pH and the metabolism of lactic acid bacteria as antibacterial. The presence of antibacterial content in red dragon fruit peel extract and honey can also reduce the number of coliforms. This is in accordance with the research of Yong, et al. [17], betasianin can reduce the number of gram-negative bacteria by entering the pores in the cell wall and reducing permeability. Red dragon fruit peel extract contains flavonoids and phenolics which are active compounds. Flavonoid compounds can penetrate polar peptidoglycan because flavonoids are also polar, while on the other hand, phenolic compounds damage the bacterial wall by breaking peptidoglycan bonds [30]. The breaking of peptidoglycan bonds will make the cell wall disintegrate and result in the death of coliform cells. In addition, this phenol compound will damage the permeability of microsomes and lysosomes [12].

3.4 Effect of Treatment on Average Total *Escherichia coli* Excreta Bacteria in Experimental Animals Given Synbiotic Yogurt

Based on Table 4, the average total *Escherichia coli* bacteria showed antibacterial activity by synbiotic yogurt with honey-sweetened dragon fruit peel extract. In Table 4, the results of this observation show that the P0, P1, and P2 treatments have increased. This happens because the nature of *Escherichia coli* is resistant to low acids, namely pH 2 to 2.5 [31]. This causes *Escherichia coli* to pass through the stomach with a pH of 1 to 3, and into the ileum [32]. In the ileum, *Escherichia coli* bacteria will grow by utilizing nutrients, namely mono and disaccharides produced from the degradation of complex polysaccharides by anaerobic microbes in the ileum [33].

Table 2. Average value of total amount of mold and yeast in excreta of experimental animals given synbiotic yogurt (Log CFU/g)

Ileum	P0		P1		P2	
	Before	After	Before	After	Before	After
Mold and Yeast	8,66	8,92	8,57	8,94	8,48	8.18

Table 3. Average total coliform excreta in experimental animals given synbiotic yogurt (Log CFU/g)

Ileum	P0		P1		P2	
	Before	After	Before	After	Before	After
Coliform	7,69	7,64	7,68	7,22	7,93	7,40

Table 4. Average total *Escherichia coli* excreta bacteria in experimental animals given synbiotic yogurt (Log CFU/g)

Ileum	P0		P1		P2	
	Before	After	Before	After	Before	After
<i>Escherichia coli</i>	7,20	7,55	7,44	7,58	7,29	7,96

Table 5. Average total bacteria *Enterococcus spp* excreta in experimental animals given synbiotic yogurt (Log CFU/g)

Ileum	P0		P1		P2	
	Before	After	Before	After	Before	After
<i>Enterococcus spp</i>	6,24	6,21	6,93	6,74	6,76	6,47

3.5 Effect of Treatment on Average Total Bacteria *Enterococcus spp* Excreta in Experimental Animals Given Synbiotic Yogurt

Based on Table 5, in the P0 treatment, the average total bacteria *Enterococcus spp* decreased. This indicates the antibacterial activity of probiotics against *Enterococcus spp*. Lactic acid bacteria produce lactic acid which has bactericisin as an antibacterial. Bactericisin secreted in the ileum will attach to the cells of pathogenic microorganisms, damage the cell membrane and prevent the re-formation of the cell membrane [27]. Lactic acid bacteria compete with pathogenic bacteria for nutrients in the ileum. Lactic bacteria in the gut compete with pathogenic bacteria by attaching to receptors in the ileum and secreting antibacterials to gain access to nutrients in the ileum [28]. Lactic acid bacteria produce lactic acid that will diffuse into the cells of pathogenic bacteria and disrupt the nutrient transport system [34].

Based on Table 5, in the P1 treatment, the average total *Enterococcus spp* bacteria decreased. This is due to the addition of red dragon fruit peel extract in yogurt. Red dragon

fruit peel extract has antibacterial content such as betasianin and flavonoids. This is in accordance with research from Tenore, et al. [35], betacyanin content has antimicrobial properties against *Enterococcus spp*. Betacyanin has antimicrobial and antifungal characteristics [15].

Based on Table 5, in the P2 treatment, the average total *Enterococcus spp* bacteria decreased. This is due to the addition of honey to the synbiotic yogurt. Flavonoids contained in honey have antibacterial properties for *Enterococcus spp*. Antibacterial action on *Enterococcus spp*, which is gram-positive bacteria, is slightly different from gram-negative bacteria such as *Escherichia coli*. This is because *Enterococcus spp* has a cell wall that is different from *Escherichia coli* bacteria. The cell wall of gram-positive bacteria consists of very thick peptidoglycan which provides rigidity to maintain cell integrity [36]. Flavonoid compounds can penetrate polar peptidoglycan because flavonoids are also polar, while on the other hand, phenol compounds damage the bacterial wall by breaking peptidoglycan bonds [30]. The mechanism of action of these organic compounds as antibacterial substances is by

poisoning the protoplasm, damaging and penetrating the cell wall, and precipitating microbial cell proteins. Large-molecule phenolic compounds are able to inactivate essential enzymes in microbial cells, even at deficient concentrations. Phenol compounds can break peptidoglycan bonds when breaking through cell walls [37].

4. CONCLUSION

The addition of red dragon fruit peel extract and honey can affect the total microbial count, mold and yeast, coliform, *Escherichia coli*, and *Enterococcus spp.* The total number of microbes, coliforms, *Escherichia coli*, and *Enterococcus spp.* decreased due to the antibacterial content of red dragon fruit peel extract and honey. The decrease in the number of molds and yeasts in the addition of honey is because honey has antifungal properties, which can dehydrate moulds and yeasts.

ETHICAL APPROVAL

The study was approved by the Animal Care and Use Ethics Committee Brawijaya University (Reference 007-KEP-UB-2023)

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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