Addition of red dragon fruit (*Hylocereus polyrhizus*) on yogurt:
Effect on lactic acid content, pH, and the inhibition of
*Escherichia coli* growth

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Abstract: Red dragon fruit contains carbohydrates, flavonoids and other nutrients that can be used as energy sources by lactic acid bacteria (LAB). The addition of red dragon fruit in making yogurt can improve the characteristics and functional properties of the final yogurt product. The purpose of this study was to determine the effect of various concentration of red dragon fruit (*Hylocereus polyrhizus*) in yogurt to lactic acid content, pH value, and inhibition of *E. coli* growth. The parameters measured in this study were lactic acid content, pH, and inhibition zone of *E. coli*. The design used was a completely randomized design (CRD) with three treatments and five replications. The process of making yogurt was as follow: distilled water mixed with skim milk 10% (w/v), red dragon fruit 3%, 5% and 7% (w/v) and sugar 3% (v/v). The mixture was heated at 105°C for 5 minutes, cooled, then inoculated with lactic acid bacteria starter as much as 3% (v/v) and incubated at 42°C for 12 hours. This study showed that the concentration of 7% red fruit dragon produced higher lactic acid content, a lower pH value, and higher inhibition to *E. coli* growth compared to the concentration of 3% and 5%. The result indicated that the use of 7% red dragon fruit would produce good quality yogurt.

1. Introduction

Yogurt is one of the fermented drinks which utilizes lactic acid bacteria (LAB) in its processing. Yogurt has a sour taste. The results of previous research showed that yogurt has good functional properties for human health [3]. One method for developing yogurt quality is through the diversification of flavors and the improvement of functional properties. Common diversification of flavor is done by addition of natural flavors, including red dragon fruit (*Hylocereus polyrhizus*). Beside natural flavors, red dragon fruit can also contribute to the functional properties of yogurt. Dragon fruit contains 8.42–12.97 g/100 g carbohydrates [1,2]. This nutrient used as an energy source for LAB in yogurt and further influence the metabolic activity of LAB during fermentation which produce acidity in yogurt product.

Red dragon fruit also contains anthocyanin, which is polyphenol. This compound acts as antioxidant, antibacterial, and contains a pigment which produces natural red color [4]. Anthocyanin level in red dragon fruit is 8.8 mg/100 g [5]. Dragon fruit also contains quercetin, kaempferol, isorhamnetin [6], and betacyanin flavonoids [7]. The antibacterial components of yogurt in the study were expected to inhibit the growth of *Escherichia coli* bacteria.
2. Materials and methods

2.1. Preparation of red fruit dragon (Hylocereus polyrhizus)
The pulp was separated from the peel and put into a clean container. Then pulp then mashed by pressing using a spoon. The mashed red dragon fruit pulp then used for yogurt preparation.

2.2. Starter preparation
As much as 0.15% agar was added into distilled water (w/v) then heated. After heating, the ingredients was added according to treatment group: 10% (w/v) skim milk, 3%, 5%, and 7% (w/v) dragon fruit. The mixture then placed in a sample bottle and heated at 105ºC for 5 minutes by autoclaving. Media then inoculated with commercial product culture of 3% (v/v). The mixture homogenized and incubated at 42ºC for 12 hours. Activation was carried out two times before used as a starter for yogurt. The starter culture was stored in frozen temperature.

2.3. Yogurt preparation
As much as 0.15% agar was added into distilled water (w/v) then heated. Further, 10% (w/v) skim milk, 3%, 5%, 7% (w/v) dragon fruit, and 3% sugar (v/v) were added. The mixture then heated at 105ºC for 5 minutes by autoclaving. The prepared starter then inoculated and incubated at 42ºC for 12 hours.

2.4. Escherichia coli propagation
Escherichia coli was cultured on Tryptone Soy Broth media. The bacteria culture was incubated at 37 ºC for 12 hours. Activation was carried out two times before used [8].

2.5. Parameters measurement
The parameter measured in this study was the antibacterial activity of red dragon fruit. The pH value and lactic acid content were measured by titration method [9]. The inhibition of E. coli growth was carried out by modified well diffusion agar method [8,10]

3. Results and discussion

3.1. Lactic acid content
Lactic acid is the final metabotile of the fermentation process which produced by lactic acid bacteria (LAB). The lactic acid produced is characteristic of yogurt. The lactic acid in yogurt with the addition of red dragon fruit (H. polyrhizus) presented in figure 1. Results showed that a higher concentration of red dragon fruit produced higher lactic acid. Red dragon fruit with a concentration of 7% produced higher lactic acid compared to the concentration of 3% and 5% with a highly significant difference (P<0.01). The acid lactic bacteria used red fruit dragon as an energy source for their metabolism process. The increase in lactic acid content was caused by LAB activity which ferments lactose and other sugars into lactic acid [11].

The activity of LAB will affect the acidity of yogurt by producing metabolites in the form of lactic acid [12]. Yogurt with the addition of red dragon fruit at a concentration of 7% was the best medium for the growth of LAB. This was indicated by the increasing number of primary metabolites, lactic acid. The ability of LAB to produce acids varies depending on species, inoculum level, condition, media and composition of growth media [9,13]. Lactic acid produced in this study were ranging from 0.45 to 1.18%. This value is in accordance with the provisions of Indonesian National Standard No. 2981-2009, which is ranging from 0.5 to 2.0%.
3.2. pH value
Yogurt is a beverage made from fermented milk by LAB. Lactic acid bacteria will degrade the constituent components of milk and produce the final metabolites, including lactic acid. An increase in lactic acid will affect pH value. The pH value of yogurt with the addition of red dragon fruit (H. polyrhizus) showed a highly significant difference (P<0.01) in the concentration of 7% to 3% and 5% (figure 2). The addition of red dragon fruit with a concentration of 7% is the best medium for the growth of microorganisms in yogurt. Factors that influence the activity of microorganisms in yogurt are bacterial strains, nutrient availability, inoculation level, incubation temperature, fermentation time, storage conditions, sugar concentration, milk content, and storage temperature [11,14].

Figure 1. The lactic acid content in red dragon fruit yogurt.

Figure 2. The pH value of red dragon fruit yogurt.
The decrease in pH of yogurt is caused by the activity of lactic acid bacteria which converts lactose in milk to lactic acid [15]. During fermentation, the LAB will degrade milk lactose to lactic acid and reduce the pH levels [9,10,16,17]. An increase in the lactic acid content is followed by an increase in the concentration of hydrogen ions (H\(^+\)), the solution becomes acidic and the pH will gradually decrease. The fermentation process by LAB was indicated by the increase in LAB growth in yogurt and caused the accumulation of organic acids which decreased the pH value. Longer fermentation time will lower the pH value of the yogurt [16,18]. The addition of red dragon fruit in this study showed pH value ranging from 5.21 to 4.28. Indonesian National Standard No. 2981-2009 stated standard pH value for yogurt was 5.5 to 4.5.

### 3.3. Inhibition zone of Escherichia coli growth

Yogurt contains a number of lactic acid bacteria which can act as an antibacterial which have antagonistic properties with other bacteria, especially those that are pathogenic. The antibacterial activity of yogurt increases with the decreasing in pH value. Non-dissociated lactic acid penetrates cell membranes and enters the cytoplasm [13]. The antibacterial mechanism can go through several ways such as disrupting the formation of cell walls, reacting with cell membranes, inhibiting enzyme activity which causes disruption of cell metabolism, and bioactive components which interfere the formation of nucleic acids, damage the genetic material and disrupt the cell cleavage process [19].

![Figure 3. The inhibition zone of E. coli growth.](image)

The ability of antimicrobial compounds to inhibit the microbial growth in the food system can be influenced by several factors including temperature, pH, acidity, and oxygen availability, as well as the interaction between several of these factors [14]. The addition of red dragon fruit (H. polyrhizus) in yogurt showed a highly significant difference (P<0.01) to the inhibition of E. coli growth (figure 3). Duncan’s test showed that each concentration of red dragon fruit produced different inhibition of E. coli growth. Increased inhibition of E. coli growth along with the increased concentration of red dragon fruit and followed by an increase in flavonoids content in yogurt. Flavanoids had antibacterial activity. Betacyanin is flavonoids compound in red dragon fruit, which gives color to this fruit [7].

The addition of red dragon fruit in yogurt showed the inhibition zone of E.coli growth ranged from 6.06 to 15.29 mm, which categorized as moderate to high inhibitory value. The inhibition zone less than 5 mm was categorized as low inhibitory value, 5–10 mm categorized as medium inhibitory value, 10–19
mm categorized as high inhibitory value, and more than 20 mm categorized as very high inhibitory value [10,20,21].

4. Conclusion
Carbohydrate in the red dragon fruit (H. polyrhizus) can be used as an energy source by LAB to form lactic acid. The addition of a higher concentration of red dragon fruit in yogurt reduced pH value, increased the acidity of yogurt, and increased the inhibition zone of E. coli growth.

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