Effect of pollard supplementation on probiotic (*Lactobacillus acidophilus*) growth and acidification rate

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Abstract. This study aimed to determine the effect of the addition of pollard as a potential prebiotic on growth and acidification rate of probiotic *Lactobacillus acidophilus* (*L. acidophilus*) in fermented milk. The study was conducted by adding the pollard with concentrations 1, 3 and 5% in the fermentation medium with 15% skim milk. Observed growth parameters including the total number of probiotic bacteria, decreasing pH and increasing tetherable acidity during the fermentation process. The results showed that the addition of pollard speed up the fermentation time, accelerate the process of decline in pH, increasing the tetherable acidity and total number of probiotic bacteria. This study shows that pollard supplemented in fermented milk could enhance the growth characteristics of probiotics *L. acidophilus*.

1. Introduction

*Lactobacillus acidophilus* (*L. acidophilus*) is one strain of lactic acid bacteria that is widely used as probiotic [1]. Probiotics are used in the process of making milk-based functional foods, such as probiotic milk. However, the viability of probiotics during the fermentation process, storage and in the digestive system faces several obstacles including the low pH, H2O2, toxic substances, anaerobic conditions, bile salt, and competition with other bacteria. To increase the viability of probiotics prebiotics were added [2-3].

Prebiotics are foods that can stimulate the growth of probiotics because their specific properties are not absorbed and hydrolyzed in the upper digestive system or gastrointestinal tract, stimulate the growth of beneficial bacteria and selectively inhibit pathogenic growth [2]. One of the foods which have potential as a prebiotic is pollard. This ability is based on the content of resistant starch, pentosan (non-starch polysaccharide), oligosaccharides and some soluble dietary fibers in these ingredients. Resistant starch, pentosan, oligosaccharides and soluble dietary fiber are non-digestible food ingredients in the upper digestive tract so that they can be utilized by several probiotic bacteria [3].

This study aims to examine the potential of pollard as a prebiotic. This study expected to know the effect of pollard supplementation and on the growth of probiotics, decrease in pH and acidification rate during fermentation.
2. Material and methods

2.1. Material
The materials used in this study were Lactobacillus acidophilus (NFCC 0051), skimmed milk powder, pollard, broth MRS (Oxoid), MRS agar (Oxoid), bromocresol purple, aquadest, washing buffer, cell resuspension solution and chemicals for tetherable acidity test. The equipment used in this study included centrifuges, incubators, autoclaves, laminar air flow, microscopes, and other microbiological equipment.

2.2. Methods

2.2.1. Preparation of Probiotic Starter Culture. Preparation of starter culture L. acidophilus was using the method from Ouwehand et al. [3]. Growth measurements was carried out on MRS broth medium with pH 6.7. Preparations incubated in microaerobic conditions at 37°C for 24 hours. The bacteria were harvested by centrifuging 3000 rpm for 20 minutes. The supernatant was removed and the remaining cells were washed with a washing buffer (8.6 g NaCl; 0.025 mg MgSO$_4$.$\cdot$7H$_2$O in 1 liter of buffer phosphate pH 7). Cell resuspension was carried out by adding 10 ml of suspended solution (8.5 g NaCl; 0.3 g KH$_2$PO$_4$; 0.6 g Na$_2$HPO$_4$; 0.1 g bactopepton in 1 litre sterile buffer phosphate pH 7) then homogenized. Resuspended cell biomass was used to test carbohydrate fermentation ability and cell growth analysis.

2.2.2. Preliminary Test. Preliminary tests were conducted to determine the ability of L. acidophilus in fermenting pollard as carbohydrate sources. Tests were carried out by growing L. acidophilus in pollard solutions with 0.1% bromocresol purple indicator in microaerobic conditions for 48 hours.

2.2.3. Bacterial Growth. L. acidophilus was grown in a skim milk solution with supplementation of 1, 3 and 5% pollard. Fermentation medium added with 0.3 ml of 0.1% bromocresol purple as an indicator of the growth of lactic acid bacteria. The growth parameters observed were pH decreased, tetherable acidity increased, and the total probiotic bacteria. Measurement of pH decrease is carried out with a pH meter. Measuring the acidity using the Mann Acid Test method. The calculation of the number of probiotic bacteria is carried out using the pour plate method with M-MRS agar medium.

3. Results and discussion

3.1. Preliminary Test
Preliminary analysis was performed to determine the potential of pollard as a prebiotic. Preliminary test results show that L. acidophilus can ferment pollard as an energy source. Based on the type of fermentation, L. Acidophilus is classified as a homofermentative bacteria that uses the hexose diphosphate pathway and produces lactic acid from glucose [4].

The occurrence of the fermentation process is characterized by a change in the color of the medium from purple to yellow. Changes in color from purple to yellow are a sign of the formation of acids in the medium as a result of bacterial metabolic activity. Preliminary results provide an initial picture of pollard's potential as a prebiotic. Pollard contains crude fiber, cellulose, maltose and SDF pollard, respectively 0.65; 2.05; 0.87 and 7.56%. The crude fiber content, especially SDF, is the main characteristic component of prebiotic ingredients. The high content of SDF in pollard indicates that pollard has a high potential as a prebiotic [1-5].

3.2 Probiotic Growth
The parameters used to measure the growth rate of probiotics include pH changes, tetherable acidity, and total probiotic bacteria. Decreasing pH is one indicator of bacterial growth [6]. Lactic acid bacteria produce lactic acid in large quantities during the process of metabolism cause lactic acid concentration...
and pH medium increases [6-7]. Changes of pH values and acidity in the L. acidophilus fermentation with milk medium supplemented with pollard are presented in Figure 1.

During the incubation process, there is a decrease in the pH value of the medium. Decreasing pH values in both the control and milk medium with 1, 3 and 5% pollard supplementation showed the growth activity of L. Acidophilus bacteria. Pollard supplementation with higher levels cannot accelerate the process of decreasing pH in fermentation medium. This is because the dissolved protein content in the fermentation medium has an effect as a buffer [7-8]. The fastest pH decrease occurs in 8 to 10 hours. This is due to bacterial growth in the exponential phase. During the exponential phase the bacteria experience cell division at maximum speed. Energy requirements in this phase are high enough so a lot of lactose and other carbohydrates are fermented and produces acids, especially lactic acid.

Figure 1. Decreasing pH (left) and increasing titrable acidity (right) in the fermentation process of L. acidophilus with milk medium supplemented with 1, 3 and 5% pollard.

Increased tetherable acidity during the fermentation process is caused by the metabolic activity of L. acidophilus to produce lactic acid as the final product. The increase in acidity is in the range of 0.20 to 0.98%. Pollard supplementation accelerate the tetherable acidity cause pollard supplementation in milk increases carbohydrate and protein content. Tamimie [9] states that the high production of lactic acid is associated with high levels of lactose and is also associated with an increase in bacterial nutrient concentration. Do Espírito Santo et al. [10] and Shafiee et al. [8] states that the concentration of dry matter in fermentation is directly proportional to the speed and concentration of acids formed in the fermentation process.

During the fermentation process, there is an increase in the number of bacteria. The increase of L. acidophilus is presented in Figure 2. Pollard supplementation have a significant effect on the number of bacteria during fermentation. The average increase in cell counts occurred at 8 to 16 hours incubation. This shows that at the incubation, the cell growth occurs optimally or the bacteria are in the exponential phase. In the exponential phase bacterial growth very fast according to linear equations [11].

Pollard supplementation with a higher concentration indicates an increase in the number of bacterial cells. High concentrations of pollard in the fermentation medium is carbon and nitrogen sources needed for growth [11]. Pollard supplementation also increases the content of crude fiber, cellulose, fructose, maltose and SDF in the medium needed for the growth of probiotics [4-5]. Besides, higher levels of pollard supplementation cause pH stability in the fermentation medium so that the process of decreasing pH is slower. Salovaara [4] states that proteins and some minerals have high capacity buffer capacity. In stable pH conditions, the growth of lactic acid bacteria is more optimal because it avoids the presence of acidity shock. Acid accumulation in the fermentation medium is an inhibitor of bacterial growth [10].
Figure 2. Increasing number of L. acidophilus during milk fermentation with 1, 3 and 5% pollard supplementation.

4. Conclusion
Pollard supplementation in the milk medium did not cause a difference in pH decrease but increased acidity and the total amount of probiotic bacteria. This indicates that pollard supplemented in milk fermentation can enhance the growth characteristics of probiotics L. acidophilus.

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