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The Change in pH on *Lactobacillus acidophillus* Medium Containing D-fructose

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Abstract

This study aims to analyse the pH value on *Lactobacillus acidophilus* medium containing 3% (w/v) D-fructose. *L. acidophilus* was incubated at 37°C using MRS agar medium. Changes in pH values were measured for 48 hours. The non-sugar addition was also used as a comparison. Based on this research it could be seen that D-fructose slightly decreased pH in the medium at 48 hour incubation. This research might be useful to provide information on the potential use of D-fructose as a medium to maintain the reduction in pH.

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Introduction

Member of *Lactobacillus* that is believed to have probiotic characteristics is *Lactobacillus acidophilus* and it contributes to provide health benefits for probiotics as well as provide the function as antimicrobial agents to avoid gastrointestinal infections, improvement in lactose metabolism, anti-mutagenic properties, anti-carcinogenic properties, reduction in serum cholesterol, anti-diarrheal properties and immune system stimulation (Shah, 2007). Based on research, fructose has been shown to double the growth of lactobacilli (Nutter *et al.*, 2017) due to its energy for growing. D-fructose was known as commercial sugar because of its unique physiological properties (Zhang *et al.*, 2016). This type of sugar may be affected the pH in the medium containing this lactic acid bacteria (LAB) (Moriya *et al.,* 2017).

This study aims to analyze the pH value on *L. acidophilus* medium containing D-fructose. The benefit of this study was to obtain information about changes in pH value after the addition of D-fructose.

Materials and methods

Strains of *L. acidophilus* ATCC 4356 was obtained from Gadjah Mada University, Yogyakarta. D-fructose was obtained from Kagawa Rare Sugar Research Center, Kagawa University, Japan and other ingredients were reagent grade.

Medium for Bacterial Growth

L. acidophilus was inoculated in the MRS medium and this method was adopted from Moriya *et al.* (2017) with some modifications. One-time dilution using MRS Broth was applied in *L. acidophilus* and followed by incubation for 24 hours at 37°C. This step was done for two times.

Production of D-fructose and D-allulose

Production of medium containing D-fructose was carried out using the methods of Yoshihara *et al.* (2016) with some modifications. D-fructose at 3% (w/w) was dissolved in 0,88% NaCl. This solution was filtered using 0.2 μm syringe filter. Obtained *L. acidophilus* was cultured in the MRS broth at ratio 1:9. This procedure was conducted two times in serial. The incubation time was 24 h in 37˚C. The resulted culture was then applied in the medium containing D-fructose using similar previously ratio. The medium was incubated for 48 hours at 37°C and immediately prepared for pH analysis.

Analysis of pH in Medium

Analysis of pH was carried out using the methods of Yoshihara *et al*. (2016) with the following procedure. 5 ml of D-fructose contained medium was tested for the pH using pH meter.

Data Analysis

The data was calculated in the percentage of pH change using initial and final data.

Results and Discussion

The result of pH measurement revealed that D-fructose decreased ±3% pH of medium if compare to the those of medium with no D-fructose. The decrease in pH might explained *L. acidophilus* activity in producing organic acids during fermentation (Zubaidah *et al*., 2012) and the production depended on temperature, medium, presence of salt, presence of preservatives, and growth inhibitors (Soliman *et al*., 2015; Probst *et al*., 2013; Vinderola *et al*., 2002). Three percent of differences might be categorized as slight change because of the similarity of metabolism between D-fructose and other carbohydrates (Zhang *et al*., 2016).

Glucose is known as supporting agent for the growth of lactic acid bacteria therefore, this study used MRS. As well known, MRS contains 3% glucose which was considered the same D-fructose treatment in this study. The reduction in pH of medium was a common phenomenon that occurs in lactic acid bacteria fermentation (Rhee *et al*., 2011), however this decrease was lower than the decrease MRS medium only. This may be explained because of no competitive substrate used by *L. acidophillus* for growth (Khay *et al*., 2014).

Conclusion

During the 48 hour incubation process, the decrease in pH in *L. acidophilus* medium containing D-fructose could be determined as ±3% reduction.

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