Glucose and D-Allulose contained medium to support the growth of lactic acid bacteria

A N Al-Baarri1*, A M Legowo1, Y B Pramono1, D I Sari1, W Pangestika1

1 Food Technology Department, Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang, Indonesia

Email: albari@undip.ac.id

Abstract. Monosaccharide has been known as support agent for the growth of lactic acid bacteria. However the combination among monosaccharides for supporting the living of bacteria has not been understood well. This research was done for analyzing the combination glucose and D-allulose for the growth of *Lactobacillus acidophilus* and *Streptococcus thermophilus*. The NaCl medium containing glucose and D-allulose was used to analyse the growth of bacteria. The study showed that glucose and D-allulose have been detected as supportive agent to *L. acidophilus* and *S. thermophilus* specifically. As conclusion, glucose and D-allulose supported the growth of lactic acid bacteria equally. This finding might provide the beneficial information for industry to utilize D-allulose as well as glucose.

Key word: *L. acidophilus*, *S. thermophilus*, glucose, D-allulose, growth.

1. Introduction

Recently, rare sugars have attracted a great deal of attention mainly concentrated on their applications, such as potential inhibitors of various glycosidases low- or non-calorie carbohydrate sweeteners and bulking agents in the food industry. Rare sugars are monosaccharides and their derivatives that are rare in nature [1]. D-allulose is a rare sugar, is found in nature at only small amount and very difficult to synthesize chemically. D-allulose exits in tea plants and wheat in very small quantity and can be produced in low amount from D-fructose during heat treatment of food products [2]. Biological processes such as enzymatic reactions with various ketose epimerase, oxidoreductase and aldose isomerase as well as various microbial reactions are feasible process for the synthesis of D-allulose [3].

D-glucose (Glu) is conventional sugar which occurred widely in nature. Glu has been one of sugar which can cause browning reaction with occurs in food system containing sugar and amino acid by heat treatment. Melanoidin is formed at late stage of browning reaction. The structure of melanoids are quite complex and are formed by reaction of heterogeneous polymers containing nitrogen [4]. Although the previous study from [5] stated that pentose sugars were more reactive than hexose to turns brown colour, Glu was still the common sugars that widely used in food industry [6].

Lactic acid bacteria (LAB) are non-sporulating, catalase-negative, Gram positive, strictly fermentative organisms, with lactic acid as a major metabolic end product of carbohydrate fermentation. Currently, this group comprises the following general: *Carnobacterium*, *Enterococcus*,...
Lactobacillus, Lactococcus, Leuconostoc, Streptococcus, Tetragenococcus, Vagococcus and Weissella and can be found in soil, water, animal and mammal gastrointestinal tract, as well as in food and fermented products [7]. They cause rapid acidification of the raw material through the production of organic acids, mainly lactic acid. In addition, their production of acetic acid, ethanol, aroma compound, bacteriocins, expopoly-saccharides, and several enzymes is of importance and depend on the applied saccharides. This research was done to analyse the determine the growth of lactic acid bacteria in the presence of glucose and D-allulose.

2. Materials and Methods

2.1 Materials
Starins of lactic acid bacteria (LAB) Lactobacillus acidophilus (LA) and Streptococcus thermophilus (ST) were obtained from Integrated Laboratory, Diponegoro University, Semarang, D-allulose was obtained from Kagawa Rare Sugar Research Centre Japan. Glucose (3%). De man, Rogosa and Sharpe (MRS) broth and MRS agar were used to support the growth of bacteria.

2.2 Growth condition
The provided of LAB strain and growth condition was adopted from the methods with some modification. L. acidophilus and S. thermophilus were grown anaerobically in 9 ml MRS broth at 37°C for 24h prior to use.

2.3 Microbiological analysis
The microbiological analysis was adopted the methods of [8] with some modification. Sample (1 ml) were diluted using 9 ml sterile physiological saline (0.8% NaCl) prepared in tubes and autoclaved. The mixture was thoroughly shaken to uniform distribution, and several aliquots of diluents (10⁻¹–10⁻⁶) were prepared using dilution method. For counting LAB, MRS Agar was used. Plate of MRS Agar were incubated at 37°C for 48h in the anaerobic condition. After the incubation, counts of microorganisms were determined as log CFU/mL sample.

2.4 Preparation media and inoculation
The preparation of glucose and D-allulose containing medium was done by the addition 3% of glucose and D-allulose, respectively to 0.88% NaCl. Sterile physiological saline (0.88% NaCl) was used to avoid excess electrolyte. The MRS broth was used as comparison. One millilitre of LAB strains were inserted into 14 ml solution containing MRS broth, glucose, or D-allulose.

3. Result and Discussion
The amount of LAB on glucose and D-allulose containing medium appeared non significantly different. Both of them were able to provide the population of LAB at a range 7.45–7.55 Log CFU/ml (Fig. 1). Compare to MRS containing medium, population of LAB in the sugar containing medium was lower in amount of LAB resulting the different value of 0.5-0.7 CFU/ml. It is understandable since the composition of MRS broth are more complex than sugar containing medium resulting the maximum support for the living of LAB. [10] stated that the composition of MRS broth was able to support well to the development of lactic acid bacteria. Based on these data, D-allulose might support to the growth of lactic acid bacteria as maximum as glucose. [11] stated if the medium was a simple carbohydrate, it would be more quickly hydrolysed and resulting the beneficial value for supporting the living of lactic acid bacteria.
Figure 1. The detected amount of LAB in medium containing glucose and D-allulose at concentration 3% (w/v). MRS Broth medium was used as control. Data were obtained by 12 repetitions with standard deviation.

Figure 1 showed the development of considerable amount of lactic acid bacteria in the period of 48 hours of incubation time. The number of bacteria has increased since the number of inoculated LAB was about 1 Log CFU/ml (data not shown). Based on this increase, glucose and D-allulose might equally support the living of LAB during period of 48 hours. [12] stated that the exponential growth can be observed when substrate and nutrients were present in acceptable of amount.

4. Conclusion
Based on the research it can be concluded that the glucose and D-allulose might support the living of LAB but the amount still lower than in MRS.

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