Viability of Lactic Acid Bacteria and Its Sugar Metabolism Capability in Fermentation Using Local Salak Extract

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ABSTRACT

Fruit-based fermentation is widely known as a food product resulting from the metabolism of lactic acid bacteria. Fermentation using Lactobacillus plantarum is known to have many benefits for digestive health, because the product contains high fiber, low calories, and contains many functional components. Using fermentation processes, the functional properties of salak fruit juice can be improved, and beverages with different chemical characteristics can be produced according to the fermentation times. For this reason, in this study, total lactic acid bacteria (LAB), the total sugar, reducing sugar content, and total lactic acid formed at 0,6,12,18, and 24 hours of fermentation were examined. The total LAB obtained was 2,10 x 10^8 – 4,64 x 10^8 CFU/ml, total sugar obtained was 12.48%-19.72%; reducing sugar content 15.53% – 9.82%; and total lactic acid 0.70% - 2.06%. The results showed that there were no significant differences in total LAB, while there were significant differences in decreasing total sugar, reducing sugar content, and total lactic acid.

Keywords: *salak fruit extract, fermentation, Lactobacillus plantarum*

INTRODUCTION

Fruit fermentation has been widely studied in Indonesia using various strains of lactic acid bacteria, either used as pure culture or obtained from backslope culture. Through fruit fermentation, a drink with good sensory properties is obtained.

Salak is a tropical fruit native to Indonesia. Its use as a fermented beverage is currently being investigated using yeast (1) and several strains of lactic acid bacteria that are naturally found in milk-based foodstuffs (2).

Lactobacillus plantarum is commonly found in Indonesian fermented foods. These bacteria are classified as lactic acid bacteria and heterofermentative which produce lactic acid as the main product of sugar metabolism, in addition to other organic acids as secondary metabolites.

L.plantarum has the potential to be used in fruit extract fermentation because its natural habitat comes from vegetables and fruits, and it simplifies the preparation and preparation of starters because it does not involve the addition of skim or other milk-based ingredients. At the same time, these bacteria are heterofermentative, capable of producing lactic acid and other organic acids that have important benefits in maintaining the health of our bodies.

The use of *L. plantarum* in fruit extract fermentation has been reported by several researchers but has not been used as a sole starter in the fermentation of salak fruit juice. So it is necessary to research to see the ability of *L. plantarum* to ferment substrates made from salak fruit, as a preliminary study to explore its potential to produce other secondary metabolites.

The purpose of this study was to determine the total lactic acid bacteria, total sugar, reducing sugar content, and total lactic acid during the fermentation of salak fruit juice. The fermentation time used was 0, 6, 12, 18, and 24 hours.

MATERIAL AND METHODS

The experimental design used was a one-factor Completely Randomized Design (CRD), with fermentation time treatments 0, 6, 12, 18, and 24 hours. Repeated 4 times. The hypothesis used in this study is as follows, H0: there is no significant effect of fermentation time on total lactic acid bacteria, total sugar, reducing sugar content, and total lactic acid in salak fruit juice. H1 : there is a significant effect of fermentation time on total lactic acid bacteria, total sugar, reducing sugar content, and total lactic acid of salak fruit juice.

Salak fruit extract is prepared in the following steps: 900g of salak is sorted with the same maturity level, peeled the skin, removed the seeds, are cut into small pieces, and washed thoroughly with running water. Then it's drained and added water with a ratio of water and salak 4:1. Then it's mashed with a blender until it becomes juice, and filtered to get the extract of the salak fruit. Salak fruit extract was poured and then sterilized using an autoclave at a temperature of 121 C at a pressure of 1 atm for 15 minutes. Salak fruit extract was poured into a sterile 100 ml Erlenmeyer, according to the treatment and number of replications. Each was added 5% (v/v) starter *L. plantarum* which had been activated in MRS Broth medium. Then it was incubated at 37°C with variations in fermentation time for 0,6,12,18,24 hours.

Salak fruit extract was analyzed for total lactic acid bacteria, total sugar, reducing sugar content, and total lactic acid (3,4). The data obtained were analyzed statistically using variance (ANOVA). If there is a difference between treatments, which means that there is an effect of the length of fermentation on the observations at a significance level of 5%, then proceed with Duncan's Multiple Area Test to determine the level of difference between treatments.

RESULT

3.1. Total LAB of Fermented Salak Fruit Extract during Fermentation

Total LAB represents the growth of *L.plantarum* during 24 hours of fermentation and is equipped with parameters such as pH, acidity, sugar, and reducing sugar content. As a heterofermentative bacteria, it uses a carbon source other than lactose and converts it become lactic acid as the main metabolite through the Embden-Meyerhof-Parnas metabolic pathway in anaerobic condition (5). Other compounds such as aldehyde, ketone, and other organic acids are also produced aside from lactic acid as secondary metabolites (6).

Table 1 and Figure 1 has described the total LAB during fermentation time. As a treatment, fermentation time did not significantly affect the total LAB. The population obtained in this research has met the minimum requirement of probiotics in fermented food (10^6 -10^8 CFU/ml) according to FAO/WHO (2006) (7). During fermentation time, the bacterial population tends to utilize sugar as an energy source for growth although it obtained a constant population result. Buruleanu *et.al*. (2010) stated that the initial amount of sugars is important in the sense to be enough for lactic acid bacteria growth and multiplication (8).

During fermentation, the bacterial population didn't change significantly, suspected that there was a growth restriction due to accumulation of metabolite. This is in line with Passos *et al.* (1994) that there was a reduction specific growth of *L.plantarum* rate due to product inhibition apparently (9). Leroy and Vuyst (2001) and Othman *et al.* (2017) had explained the mechanism of lactic acid to inhibit the cell growth. This was related

with the damage of membrane plasma permeability (10,11). Chui *et al* (2016) added his statement about the osmotic pressure due to continual accumulation of various substances and one of them is lactic acid had its effect to cell growth inhibition. LAB growth restriction in this research is in line with Priadi *et al.* (2020), that LAB population viability had observed to be decreased during 24 hours of fermentation due to acidification effect to the cell membrane damage and intracellular components as well. LAB growth restriction due to the accumulation of lactic acid are reported by several researchers in fermented fruit juice or extract as describe in the following table. However viability of LAB and its functionality during fermentation need to be given more attention in research related with the benefits obtained as an oral probiotic drink can improved as optimum as the number of population is reached. The fast growing characteristic, its stability, and its metabolic activity are the key of LAB benefits and application

Table 1. Total Lactic Acid Bacteria during 24 hr of Fermentation

Fermentation Time (hr)	Total LAB (CFU/ml)
0	2,10 x 10^{8a}
6	6,06 x 10^{8a}
12	2, 22 x 10^{8a}
18	5,45 x 10^{8a}
24	4,64 x 10^{8a}

*Values in a column with the same letters are not significantly (P>0,05) different.

3.2. *Total Sugar of Fermented Salak Fruit Extract during Fermentation*

The total sugar obtained in the 0th hour of fermentation was 19.7288 %. Salak fruit as a fermentation material already contains a sugar component. This is by what was stated by Lee, *et al.* (2013) that salak fruit contains glucose and fructose < 5g/100 ml of its juice, and sucrose 10-15 g/100 ml of its juice. At the end of fermentation total sugar, it was 12,48830% (12).

Table 2 and Figure 1 has described the total sugar during fermentation time. As a treatment, fermentation time significantly affects the total sugar. Total sugar decreased significantly during 24 hr of fermentation (P<0.05). This decrease was due to the metabolic activity of *L. plantarum* using glucose as its growth nutrient, which then produced lactic acid and other metabolites.

Utami (2018) that there was a decrease in total sugar during 10 hours of fermentation of salak fruit extract (2). Widyantara (2020) obtained similar results using a pineapple fruit substrate, which was a 10% reduction in total sugar for up to 18 hours of fermentation (13). The decreasing in total sugar is tend to sugar utilization by LAB to convert into lactic acid and other metabolites.

Table 2. Total Sugar during 24 hr of Fermentation

Fermentation (hr)	Total Sugar (%)
0	19,7288 [a]
6	16,7940 [b]
12	15,4030 [c]
18	15,2793 [d]
24	12,4830 [e]

*Values in a column with the same letters are not significantly (P>0,05) different.

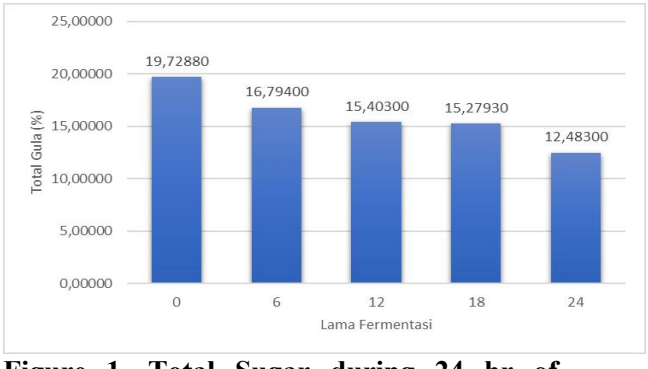

Figure 1. Total Sugar during 24 hr of Fermentation

3.3. *Reduction of Sugar of Fermented Salak Fruit Extract during Fermentation*

The reduction sugar obtained in the 0th hour of fermentation was 15,5343 %. Salak fruit as a fermentation material already contains a reduced sugar component. This showed that salak fruit contains glucose and fructose and supported by Lee, et al. (2013) that, salak fruit contains glucose and fructose < 5g/100 ml of its juice, and sucrose 10-15 g/100 ml of its juice (13). At the end of fermentation reduction sugar, it was 9,8290 %.

Table 3 and Figure 2 has described the reduction of sugar during fermentation time. As a treatment, fermentation time significantly affects the reduction of sugar. Reduction sugar decreased significantly during 24 hr of fermentation ($P<0.05$).

L. plantarum is heterofermentative, utilized glucose and fructose in the glycolysis through the Embden-Meyerhoff Parnas pathway (Moat and Foster, 1998), and were converted to lactic acid as a primary metabolite. The decreasing in reduction sugar as stated by Winarno (1992) is related with the increasing of invertase activity that caused the hydrolysis of sucrose into reducing sugar, and subsequently converts it into organic acids and alcohol (13). The Pearson correlation coefficients will ascertain that reducing sugar is decomposed into lactic acid, as follow: the value (r) is -0.96159.

Table 3. Reducing Sugar during 24 hr of Fermentation

Fermentation (hr)	Reducing Sugar (%)
0	15,5343 [a]
6	13,2238 [b]
12	12,1283 [c]
18	12,0308 [d]
24	9,82900 [e]

*Values in a column with the same letters are not significantly ($P>0,05$) different.

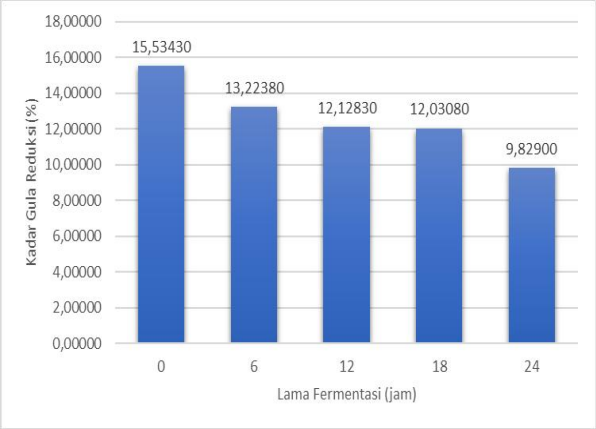

Figure 2. Reducing Sugar during 24 of hr Fermentation

3.4. *Acidity of Fermented Salak Fruit Extract during Fermentation*

At the beginning of the fermentation, acidity was 0,7% indicating that spontaneous fermentation of fresh salak fruit due to the activity of microorganisms naturally present in it, or as a contaminant due to salak post-harvest handling. It was reported by Lee *et al* (2013) that fresh salak fruit does not contain lactic acid. Acidity became 2,06% at the end of the fermentation.

Table 4 and Figure 3 has described the effect of fermentation time on acidity. The treatments significantly affect acidity. Total lactic acid increased significantly during fermentation. *L.plantarum* has aldolase and phosphoketolase to ferment hexose into lactic acid. Lactic acid will be secreted out of cells and accumulated in the substrate thereby increasing the acidity of the product.

Table 4. The Acidity during 24 hr of Fermentation

Fermentation Time (hrs)	Total Lactic Acid (%)
0	0,70300 [e]
6	1,39100 [d]
12	1,52750 [c]
18	1,82975 [d]
24	2,06075 [a]

*Values in a column with the same letters are not significantly ($P>0,05$) different.

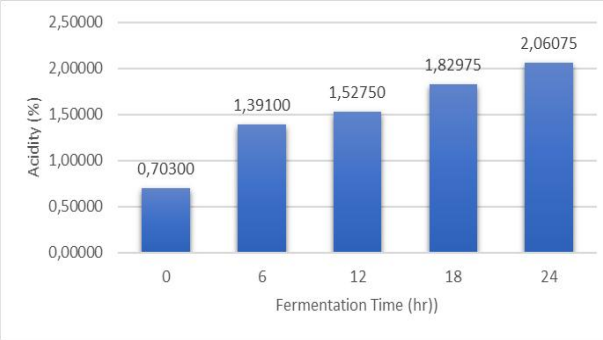

Figure 3. The Acidity during 24 hr of Fermentation

CONCLUSION

In this study, it can be concluded that *L.plantarum* was viable while there was a stability in its population, and able to utilize the sugar contained in salak fruit in its metabolism into lactic acid. The total LAB obtained was 2,10 x 10^8 – 4,64 x 10^8 CFU/ml, total sugar obtained was 12.48%-19.72%; reducing sugar content 15.53% – 9.82%; and total lactic acid 0.70% - 2.06%. There was no significant difference in total LAB, while there were significant differences in the total sugar, reducing sugar content, and the total lactic acid.

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