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The Use of Lady Finger Banana Fruit (*Musa acuminata Colla*) As Probiotic Ice Cream With The Addition Of Starter Cultures Of *Lactobacillus plantarum* B1765

Kurrotu Aini[1], Prima Retno Wikandari[2*]
[1,2*]*Department of Chemistry, Faculty Mathematics and Natural Sciences, Universitas Negeri Surabaya, Indonesia*
Corresponding Author: primaretno@unesa.ac.id

Abstract
This study aims to determine the effect of fermentation time on microbiological quality of total Lactic Acid Bacteria (LAB), chemical qualities of pH and Total Titratable Acid (TTA), and organoleptic qualities of flavor, taste, and texture on probiotic ice cream of Lady Finger Banana fruit (Musa acuminata Colla) with the addition of starter culture Lactobacillus plantarum B1765. L.plantarum B1765 has been studied has probiotic characteristics. Fermentation is carried out for 0, 4, 6, and 8 hours. The total of Lactic Acid Bacteria (LAB) was enumerated using the TPC method. pH and TTA are determined using a pH meter and acid-base titration respectively. Whereas, organoleptic was tested using the hedonic test. The results showed that fermentation time affects the total LAB, pH, TTA, and texture, but does not affects the flavor and taste. There was an increase in the total LAB of 2 logs cycle at 8 hours fermentation reached 7.40 x 10^9 CFU/mL. The pH decreased from 6.17 to 5.10 and TTA increased from 0.40% to 1.84%. The average value of panelist level of preference of flavor, taste, and texture were 3.03; 3.21; 3.17 respectively and showed a tendency in the like catagories. The best fermentation time was 8 hours. Based on total LAB, pH and TTA, Lady Finger bananas ice cream could be used as probiotics agent.

Keywords: *probiotic ice cream, Lady Finger banana fruit, L.plantarum B1765, product quality*

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I. INTRODUCTION

Ice cream is a processed food that is much loved by various circles of society. At this time, innovation is needed to improve the quality of ice cream that is not only delicious but also good for consumer health, namely probiotic ice cream using a probiotic as a starter culture.

Probiotics are bacteria that have a good effect on health when consumed in sufficient quantities [1]. *Lactobacillus plantarum* B1765 is one type of lactic acid bacteria that is able to show characteristics as probiotics, such as the resistant to gastrointestinal pH in the pH range of 1-8, resistant to bile salts in the range of 0.1-1% with survival above 90%, and shows antagonistic properties in pathogenic bacteria such as *S. aureus* and *E. coli* [2].

Banana is one of the fruits that can be used as a probiotic growth medium because it contains high enough carbohydrates, low cholesterol, vitamin B6, vitamin C, and minerals. The starch and inulin components found in bananas can be converted into glucose and fructose when bananas ripen [3]. One of the varieties of bananas that have the potential to be processed probiotic foods is Lady Finger banana. Besides its contents, this fruit is also economically valuable, and easy to obtain, and so far its use is only eaten directly. Another advantage is the antioxidants substance contained in this fruit. According to [4] bananas contain antioxidant compounds, namely phenols, flavonoids, tannins, and glycosides.

Previous research showed that probiotic ice cream banana of banana peel flour with the addition of starter culture *Lactobacillus casei* as much as 4% produce a total LAB of 5.43 x 10^{10} CFU/mL, total acid of 0.05%, and pH 4.15 [1]. There is also a symbiotic ice cream made from Jicama and Red Dragon Fruit by utilizing *Lactobacillus plantarum* 1 producing ice cream with total LAB of 9.02 log CFU/mL, pH of 4.41, total solids 27.58%, overrun 29.61%, melting time 11.32 minutes, as well as organoleptic ice cream is pink purplish, the resulting flavor and taste sweet slightly sour, and soft texture [5].

Potential of *L.plantarum* B1765 as a probiotic has never been reported in the manufacture of Lady Finger banana fruit probiotic ice cream. Therefore, this study will examine the effect of fermentation time on Lady Finger banana fruit ice cream product inoculated with *L.plantarum* B1765 probiotics. The qualities of product which were examined including microbiological quality (total LAB), chemical qualities (pH and TTA),

and organoleptic qualities (preference level of flavor, taste, and texture). The results of this study are expected to be used as an alternative to functional food development and diversification of processed food products of Lady Finger banana fruits.

II. MATERIALS AND METHODS

1. Material and Equipments

The materials used in this research is Lady Finger banana fruit, *Lactobacillus plantarum* B1765, full cream UHT milk, whipped cream, sugar, skim milk (*Petit Eric*) and stabilizer namely lecithin and CMC (*Koepoe-Koepoe*), MRS broth (*Merck*), $CaCO_3$ (*SAP Chemicals*), NaCl (*PUDAK Scientific*), NaOH 0.1 N, gelatin powder white plain (*Satelit*), phenolphthalein indicator (*Merck*), and aquademineral. The equipment used in this research is pan, electric stove, blender (*Philips*), knife, plastic wrap, stopwatch, thermometer, autoclave (*Hirayama HVE-50*), micropipette (*D-LAB*), magnetic stirrer (*D-LAB*), centrifuge tube (*GP*), centrifuge (*Eppendorf 5810*), Incubator (*Memmert*), digital pH meter (*ATC*), stands and clamps, and glassware.

2. Methods

The Preparation of Starter Culture Lactobacillus plantarum B1765

There are 1000µL stocks of *Lactobacillus plantarum* B1765 is introduced into 9 mL of MRS broth, after which it is incubated at a temperature of 37°C for 24 hours. Next, the grown culture is centrifuged at a speed of 3500 rpm for 5 minutes. The supernatant is decanted and the residue is suspended into 10 mL of 0.85% sterile NaCl solution and then centrifuged again at 3500 rpm for 5 minutes. The residue is suspended again into 10 mL of 0.85% sterile NaCl solution as a starter culture [6].

Producing Probiotic Ice Cream of Lady Finger Banana

Procedure of producing of lady finger banana based on [1] with modification. Washing Lady Finger bananas with soap, then bleaching using hot water 60°C for 10 minutes. After that, cutting Lady Finger banana. Then, mixing 100 mL of fresh milk with pieces of its banana as much as 6% (b/v) and other additives including skim milk 5% (b/v), sugar 15% (b/v), CMC 0.2% (b/v), lecithin 0.1% (b/v), and whipped cream 5% (b/v). Next, pasteurizing up to a temperature of 80±2°C for 10 seconds. The next step is homogenized using mixer for 10 minutes and adding *L.plantarum* B1765 starter culture as much as 1% and homogenized. Then, incubating the dough at 37°C for 0, 4, 6, and 8 hours. Next, the dough was aged for 14 hours at 4°C. After that, whisking and freezing 3 times using a mixer for 10 minutes. The final step is frozen in the freezer for 24 hours.

Microbiology Quality Testing

Microbiology quality testing is done by calculating the total LAB using the method of Total Plate Count (*TPC*). Sample of banana Lady Finger fruit probiotic ice cream taken 1 mL then diluted in 0.85% sterile NaCl solution at dilution 10^{-1}-10^{-9}. Planting in a petri dish is carried out at a dilution of 10^{-5}-10^{-9} a total of 1000µL. Then, adding MRS broth media of gelatin that is added by $CaCO_3$ 1%. Then, incubating upside down for 48 hours at a temperature of 37°C. The growth of the LAB colony is characterized by a pure ring zone. Total of LAB result is stated in CFU/mL [7]. Calculation of LAB is determined by the following formula:

$$\text{Total LAB (CFU/mL)} = \text{total colony x } \frac{1}{\text{dilution factor}}$$

Chemical Quality Testing (pH and TTA)

Chemical quality testing includes pH and TTA. The pH value is determined using a digital pH meter. The total titrated acid (TTA) test is measured in the form of a lactic acid percentage. 10 mL probiotic ice cream samples are taken and diluted in a 100 mL measuring flask with aquademineral, then pipetted as much as 20 mL and put in Erlenmeyer, after which added indicators of phenolphthalein a total of 3 drops, then titrated with NaOH 0.1 N until there is a permanent pink color change [8]. The value of the titrated acid was calculated by the formula:

$$\frac{\text{FP x mL NaOH titration x N NaOH x BM x 100\%}}{\text{W sample}}$$

FP = dilution factor
N = normality
BM = lactic acid molecular weight
W = sample weight (g)

Organoleptic Quality Testing

Organoleptic quality testing was carried out by means of hedonic tests which included preference for flavor, taste, and texture on probiotic ice cream of Lady Finger banana fruit. The test was conducted using 30

untrained panelists. In this assessment, panelists were asked to give an impression on the quality of flavor, taste preference, and texture quality of the probiotic ice cream of Lady Finger banana fruit product with a numerical scale as follows:
1 = Very Disliked
2 = Dislike
3 = Like
4 = Very Like

Data Analysis

The data obtained from the total LAB, pH and TTA tests were processed using the IBM Statistics SPSS 25 program. The total LAB data was processed using the *One Way ANOVA* test and to be continued with the *Post Hoc LSD* (Least Significant Difference) test. The pH and TTA data were processed using the *Kruskal-Wallis* test and the continued using the *Post Hoc Mann-Whitney* test. Organoleptic data were processed using the *Kruskal-Wallis* test and the *Post Hoc Mann-Whitney* test.

III. RESULT AND DISCUSSION

1. Total LAB, pH, and TTA

The test results of total LAB, pH, and TTA of Probiotic Ice Cream of Lady Finger Banana Fruit can be shown in Table 1.

Table 1. Results of Microbiological Quality Test (Total LAB) and Chemical Quality (pH and TTA) *Probiotic Ice Cream of Lady Finger Banana Fruit*

Fermentation Time	Total LAB (CFU/mL)	pH	TTA (%)
0 hour	$3.57 \times 10^{7\,a}$	6.17^{a}	0.40^{a}
4 hours	$5.10 \times 10^{8\,b}$	5.89^{b}	0.68^{b}
6 hours	$3.60 \times 10^{9\,c}$	5.44^{c}	1.72^{c}
8 hours	$7.40 \times 10^{9\,d}$	5.10^{d}	1.84^{d}

Note: The letters a, b, c, d in the column indicate a significant difference at the 5% level.

Statistical test results showed that the total data of LAB is normally distributed and homogeneous so that it is eligible for the *One Way ANOVA* test. Test results of *One Way ANOVA* showed that fermentation duration has a significant influence ($p < 0.05$) on total LAB. After that, continued with the *Post Hoc LSD* test to know the difference in each treatment. Test results of *Post Hoc LSD* showed a significant difference ($p < 0.05$) in each treatment of fermentation duration of total LAB of Lady Finger banana probiotic ice cream. Meanwhile, the results of the statistical test of pH and TTA data were not normal and homogeneous distributed, so that it could not qualify for the *One Way ANOVA* test. However, it qualified for the *Kruskal Wallis* test. The results of the *Kruskal Wallis* test showed that fermentation duration significantly influence ($p < 0.05$) on pH and TTA value. After that, continued with the *Post Hoc Mann Whitney* test to acquire the difference in each treatment. The results of the *Post Hoc Mann Whitney* test showed a significant difference ($p < 0.05$) in each treatment of fermentation duration of pH and TTA in Lady Finger banana probiotic ice cream. The results of LAB total, pH, and TTA test on probiotic ice cream of Lady Finger banana with fermentation duration of 0, 4, 6, and 8 hours are shown in Figure 1.

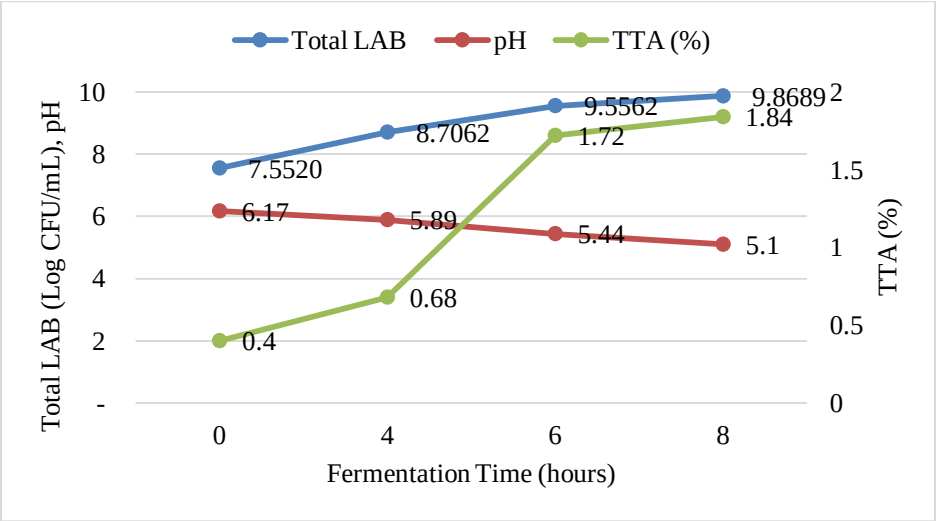

Figure 1: Graph of the correlation among total LAB, pH and TTA

Figure 1 shows that the longer the fermentation time, the increase of the total LAB and TTA, on the contrary the longer the fermentation time will cause the pH value decrease. At first, fermentation of the total LAB was 3.57 x 10^7 CFU/mL on the treatment of fermentation duration 2 hours (control), and increased into 2 log cycle reached 7.40 x 10^9 CFU/mL on a fermentation of 8 hours. The LAB total obtained in each fermentation time was qualified to be called a probiotic product because the total LAB were $\geq 10^6$ CFU/mL or CFU/g based on ISO 19344: 2015 standards [9]. According to [10] , the number of LAB will increase along with the number of medium nutrients during the fermentation process. This is because the LAB uses these nutrients to reproduce cells by converting glucose into lactic acid and utilizing carbon sources as energy to make macromolecules of cells. Thus, the more nutrients located in the medium and the duration of fermentation, the LAB total will increase. In this study, the LAB total were still increasing up to 8 hours of fermentation.

Lady Finger banana probiotic ice cream that has been tested total LAB subsequently stored in freezer for 7 days and retested its LAB total to observe the viability of *L.plantarum* B1765 in frozen storage, and it was obtained that LAB total was 5.63 x 10^8 CFU/mL, there was a decrease of 1 log cycle from the old treatment of fermentation for 8 hours. Despite there was a decrease, *L. plantarum* B1765 is still capable of growing and maintaining its viability in frozen storage. In previous research [11] stated that Symbiotic Banana and Edamame exctrats Ice Cream (60%:40%) using starter culture *Lactobacillus casei* produced total LAB of 1.70 x 10^8, decreased to 1.42 x 10^8 in frozen storage for 10 days. The viability of *Lactobacillus casei* and *Lactobacillus plantarum* on Ambon banana velva with 8 hours fermentation treatment, resulting in a total LAB of 2.02 x 10^{10} CFU/mL and after being stored for 6 weeks LAB total decreased by 2 log cycles of 2.23 x 10^8 CFU/mL [12]. This could happen because during the frozen storage period, LAB has dormant and death situation due to osmotic pressure, so that LAB could not perform metabolic activities and produce lactic acid [13]. According to [11], the difference between the storage temperature and the optimal temperature for bacteria to grow will inhibit their growth rate. Decrease of LAB total can also occur due to the formation of ice crystals that will resulting in the differential pressure between the fluid outside and inside the bacterial cell, causing death or lysis.

With the increase in LAB total, there is also an increase in total titrated acid (TTA). The correlation between LAB total and TTA is shown in Figure 1. There is an increase in TTA from 0.40% in the 2 hours fermentation treatment (control) that increase to 1.84% in the 8 hours fermentation treatment. In accordance with the research that the total titrated acid will increase as the total LAB increase. In a research of [10] stated that the increase levels of lactic acid is due to the activity of LAB in breaking down glucose through the process of glycolysis. The increasing growth of LAB will cause the lactic acid value is getting increase.

In Figure 1 is also shown that the increase in LAB total, as well as TTA followed by a decrease in pH value. In this research, the pH value amounted to 5.44 in the old treatment of fermentation 6 hours, decreased from the initial pH in the old treatment of fermentation 2 hours (control) of 6.17. In research [1] stated that banana peel flour ice cream with the addition of 4% starter of *L. casei* fermented for 6 hours produced a pH value of 4.19. Previous research also stated that fruit ghurt fruit peel Ambon banana (*Musa paradisiaca* L.) with variations in the LAB concentration as much as 7% fermented for 10 hours produced a pH value of 4.1 [14]. When compared with its research, the pH value achieved in this research is still relatively high, it is due to the lack of duration fermentation time and the number of concentrations of LAB added. This is in accordance with the statement [14], where the higher concentration given, it will cause a decrease in the pH value. Because more

LAB amount will convert glucose into lactic acid. Organic acids are formed through dissociated acids in the form of H^+ ions. The more H^+ ions indicates that the more acid will be produced.

Commonly, ice cream products do not require lactic acid levels, so the levels of lactic acid in Lady Finger banana probiotic banana ice cream refers to the standard rules of Indonesian National Standart (INS) of yogurt with lactic acid levels of 0.5-2.0%, and the pH value of 4.99-6.96 [15]. In this research, the TTA and pH value of probiotic Lady Finger banana probiotic ice cream has fulfilled the standard of SNI.

2. Organoleptic Quality

The test result of organoleptic of probiotic ice cream of Lady Finger banana fruit can be shown in Table 2.

Table 2. ***Probiotic Ice Cream of Lady Finger Banana Fruit Hedonic Test Result***

Fermentation Time	Flavor	Taste	Texture
0 hour	2,93[a]	3,13[a]	2,93[a]
4 hours	2,93[a]	3,23[a]	3,03[a]
6 hours	3,07[a]	3,27[a]	3,17[ab]
8 hours	3,20[a]	3,20[a]	3,53[bc]

Note: The letters a, b, c in the column shows a significant difference at the 5% level.

Non-parametric statistical test results of *Kruskal-Wallis* showed that the duration of fermentation did not significantly influence ($p > 0.05$) on the level of preference flavor and taste, but significantly influence ($p < 0.05$) on the level of texture preference. The average value of the preferred rate of flavor by panelists was 3.03 (like). The preference value for flavor was achieved at 8 hours of fermentation (3.20). Furthermore, the average value of taste preferences preferred by panelists was 3.21 (likes). The flavor produced by Lady Finger banana probiotic ice cream was the flavor of milk and a little of banana flavor. According to [11], lactic acid produced during the fermentation process will decrease the pH value and give a distinctive flavor character as the end result of fermentation of lactose into lactic acid and volatile components, However in this research, the pH value was still relatively high so that panelists were not able to give a difference to the taste and flavor formed due to the lack of duration fermentation so that the resulting lactic acid flavor had not been created perfectly. In addition, the flavor of ice cream was also influenced by storage conditions because foodstuffs stored at cold temperatures will have lower detection power characteristics compared to foodstuffs at normal temperature or have received heat treatment [16].

In contrast to the level of preference for flavor and taste, fermentation time actually showed an influence on the level of preference texture. The average value of the texture preferred level by panelists was 3.17 (likes). The panelists liked the texture was 8 hours of fermentation treatment with a score of 3.53 (very like). The texture produced by Lady Finger banana probiotic ice cream was thick and soft, and quickly melted and light when in the mouth. The soft texture produced by its banana was closely related to the total solids produced during fermentation. One of the factors that affect the softness of ice cream was the total of solids. The more LAB total, the more total of solids. An increase in total solids could reduce the potential for greater ice crystal formation leading to a rough ice cream texture. In addition, the texture of ice cream formed was thick, the viscosity formed in fermented milk products was due to the clumping of proteins by acids produced during the fermentation process. Besides, the total solids of ice cream aimed to increase the viscosity of the ice cream dough so as to maintain the stability of air bubbles [16].

Based on research [17] the texture of ice cream was strongly influenced by the size of ice crystals, fat globules, air bubbles, lactose crystals as well as the composition of the stabilizer, processing method, and storage conditions. [18] stated that a good ice cream texture is smooth and soft, not hard, and looks shiny while the bad one is the taste of fat lumps, felt the presence of ice flakes and sandy. Then, the expansion of the volume of ice cream dough is due to shaking and makes ice cream is lighter and not too dense and has a soft texture.

Based on the overall organoleptic assessment, the best fermentation time on Lady Finger banana probiotic ice cream is 8 hours.

IV. CONCLUSION

Based on the research that has been done, it can be concluded that the fermentation time affected the microbiological quality (total LAB), chemical qualities (pH and TTA), and texture, but have no effect on flavor and taste. The results showed that the best fermentation time was probiotic ice cream Lady Finger banana fruit which was fermented for 8 hours with a total LAB of 7.40×10^9 CFU/mL, pH of 5.10, TTA of 1.84%, and the average value level of preference for flavor 3.20; taste 3.2; and texture 3.53. The total LAB, pH and TTA of this product fulfilled the Indonesia National Standar, so the product resulting from this research can be recommended as a fermented product and could be used as probiotic agent.

V. SUGGESTION

Because in this study the pH value achieved was still high causing the characteristic taste of ice cream to be less sour, so in this study it was necessary to add a longer fermentation time. So the product wishes could has the higher preferable taste and flavour.

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