

*The Effect of Fermentation Time on the Quality of Probiotic Products from Jackfruit Seed Extract with *Lactobacillus plantarum* B1765 as The Starter Culture Bacteria*

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Abstract— The purpose of this study was to determine the effect of fermentation time on total lactic acid bacteria (LAB), pH, total titrated acid (TTA), and organoleptic qualities including colour, taste, and aroma in probiotic drink jackfruit seed extract with starter culture of *Lactobacillus plantarum* B1765. *Lactobacillus plantarum* B1765 had been studied has probiotic bacteria characteristics. Fermentation was carried out for 0, 24, 48, and 72 hours. Total LAB was measured by the Total Plate Count (TPC) technique. Meanwhile, pH and TTA were measured with a pH meter and acid-base titration and the organoleptic quality using hedonic test. The results showed that the length of fermentation affected the total LAB, pH, TTA, taste, aroma, and colour. Total LAB increased by 1 log cycle from 1.81×10^8 CFU/mL to 2.18×10^9 CFU/mL at 24 hours of fermentation and then reduced to a death phase up to 4.03×10^6 CFU/mL. pH decreased from 5.48 to 3.25 while TTA increased from 0.25% to 1.16%. The average value of the panelists preference for colour, taste, and aroma, respectively, is 2.85; 2.60; 2.78 which shows a tendency to like. The best fermentation time is 24 hours. Based on the number of total LAB, pH and TTA, this product had fulfilled the Indonesian National Standart (INS) for the probiotic drinks, so it could be developed potentially as an alternative of probiotic beverage products.

Keywords— Probiotic drink, Jackfruit seed extract, *Lactobacillus plantarum* B1765, Product qualities

I. INTRODUCTION

The natural wealth of local food sources in Indonesia is abundant, one of them is jackfruit. Jackfruit is a potential local food source, but only the fruit is used, while the other plant components are used as waste. The production of jackfruit in Indonesia increased from 2018 to 2020 [1] causing high jackfruit seed waste. Whereas jackfruit seed has maximum nutritional content such as protein, carbohydrates, organic acids, minerals, vitamins B and vitamin C as well as antioxidant activity that has the potential to develop functional food [2]. The functional food is not only capable of being energy but also can reduce the risk of colon cancer which is characterized by the presence of resistant starch, the level of resistant starch in jackfruit seed starch is around 29.7% [3], [4].

The data shows that jackfruit seed has the potential to be used as processed products, namely jackfruit seed juice drinks. Beside to improve the functional properties, the nutritional content of jackfruit seeds juice drink could be a good substrate for LAB in the fermentation process. One of the fermented products that spread in the market is a probiotic fermented beverage product. Probiotics are very beneficial for the health of the body, especially the digestive system because they can maintain normal microbiota in the intestines, so they can be used to treat or prevent problems in the digestive system and can improve the immune system [5]. The most commonly used lactic acid bacteria as probiotic agents are the *Lactobacillus* genus which can overcome lactose intolerance, Balance the number of bacteria in the digestive tract, and can inhibit the growth of pathogenic bacteria in the intestine [6].

Lactobacillus plantarum B1765 is a lactic acid bacterium which has been studied to have probiotic characteristics, including resistance to the pH conditions of the digestive tract and bile salts, resistance to antibiotics, and antagonist against pathogenic bacteria so that it can be used as a probiotic agent that has been proven capable of use as a probiotic culture [7]. Based on the research, in this study *Lactobacillus plantarum* B1765 will be used for the fermentation of jackfruit seeds as a probiotic starter culture.

Based on [8] the fermented jackfruit seed flour using another strain of *Lactobacillus plantarum* 10% w/v bacteria at 37°C with fermentation time of 8, 16, 24, 32 hours showed the growth of a total LAB for 2 log cycle from 3.63×10^6 - 9.70×10^8 CFU/mL and a reduced pH of 5.48-5.16. Fermented jackfruit seed drink fermented with *L. casei subsp. casei* R-68 5% at 37°C for 24 hours with variations concentration of skim milk produced a fermented drink of jackfruit seed extract with a total LAB reach of 8.59×10^7 CFU/mL, TTA 1.04, and pH 5.63 at the optimal skimmed milk concentration of 10% [9].

This research has examined the effect of fermentation time of jack fruit seed extract fermented with starter culture of *Lactobacillus plantarum* B1765 on total LAB, pH, TTA, and organoleptic qualities including colour, taste, and aroma. The results of this study are expected could be used as an alternative to the development of functional food with the presence of probiotic beverage products from jackfruit seed extract in addition to reduce waste.

II. RESEARCH METHODS

2.1. Tools and Materials

The equipment used in this experiment were a knife, basin, pan, electric stove (Maspion), spoon, glass bottle, plastic wrap, thermometer, measuring cup, autoclave (*Hirayama HVE-50*), laminar airflow (*Thermo Fisher Scientific 1300 Series A2*), vortex mixer (LAB-NET), centrifuge (*Eppendorf*), magnetic stirrer, stirrer (DLAB), beaker, centrifuge tube (GP), blue type (*Eppendorf*), incubator (Memmert), stative and clamps, micropipette (D -LAB), digital pH meter, erlenmeyer, blender (Maspion), petri dish and analytical balance (*Denver Instrument*).

The materials used in this study included jackfruit seeds, granulated sugar, skim milk (*Petit Eric*), *Lactobacillus plantarum* B1765, MRS broth (*Merck*), white plain agar powder (*Satelite*), NaCl (*PUDAK Scientific*), aquademineral, CaCO₃ (*SAP Chemicals*), NaOH (*Merck*), and phenolphthalein indicator (*Merck*).

2.2. Experimental Procedure

Preparation of Starter Culture *Lactobacillus plantarum* B1765

A total of 1000 µL isolates of *Lactobacillus plantarum* B1765 were inoculated into 9 mL of the liquid medium made from 0.52 grams of MRS broth dissolved in 10 mL of aqua demineral and incubated at 37°C for 24 hours. The grown cultures were centrifuged at 3500 rpm for ± 5 minutes. After being centrifuged, the filtrate was decanted and the residue was suspended in 10 mL of sterile 0.85% NaCl solution and then centrifuged again at 3500 rpm for ± 5 minutes. The residue was resuspended into 10 mL of a sterile 0.85% NaCl solution and vortexed on a medium scale for ± 1 minute to be used as a starter culture [10].

Preparation of Probiotic Drink of Jackfruit Seed Juice

Preparation of jackfruit juice were done base on [9], [11] with some modification. Jackfruit seeds are soaked for 12 hours then washed with running water. Next, the jackfruit seeds were blanched at a temperature of 65°C for 10 minutes and the skin was removed. Jackfruit seeds were cut into the smaller size, add warm water (± 80°C) in a ratio of 1:4 and crushed using a blender to produced jackfruit seed pulp, then filtered through a 400 mesh sieve and heated by adding 2% (b/v) skim milk and 6% sugar (b/v), pasteurized at a temperature of 85°C for 15 minutes while stirring until homogeneous. Pasteurized samples were put into bottles and waited until decrease to room temperature. Then, 5% (v/v) of starter culture *Lactobacillus plantarum* B1765 was added. Subsequently, they were incubated for 0, 24, 48, and 72 hours at 37°C.

pH and TTA

The pH value was measured using a digital pH meter. The total value of the titrated acid was measured using the acid-base titration method. A total of 10 mL of the sample was put into a 100 mL volumetric flask and aquademineral was added to the

mark. Then 20 mL was taken using a volume pipette and put into an Erlenmeyer. After that, 3 drops of phenolphthalein indicator were added and then titrated with NaOH. The titration is stopped if there is a permanent pink colour change [12].

Total LAB

Total LAB test was measured using the TPC method. A total of 1 mL of sample was taken and put into 9 mL of 0.85% NaCl solution. Then, the dilution was carried out to 10^{-9} . After that, the MRS agar was inoculated from MRS broth + 1.5% (b/v) white plain + 1% (b/v) CaCO_3 . Inoculation was carried out using the triple pour plate method. Then, it was incubated at 37°C for 48 hours with the petri dish inverted. The growing colonies were indicated by the formation of a clear circular zone and the total bacterial yield was expressed in CFU/mL logs [13].

Organoleptic Quality Test

Organoleptic test were carried out by 30 untrained panelists using hedonic quality test including preference for colour, aroma, and taste with numerical scale as follows:

1 = Really dislike

2 = Dislike

3 = Like

4 = Really like

2.3. Data Analysis

This research data was processed using the IBM Statistics SPSS 25 program. The data obtained from the TTA and total LAB tests were processed using the *One Way ANOVA* test followed by the *LSD Post Hoc Test*. The data obtained from the pH test was processed using the *Kruskall Wallis* test followed by the *Post Hoc Mann Whitney Test*. While the data obtained from the organoleptic test were processed using the *Kruskall Wallis test*.

III. RESULT AND DISCUSSION

Total LAB, pH and TTA

Microbiological quality testing was measured by the total number of LAB tested using the TPC method. Meanwhile, chemical quality was measured by pH test and TTA test. The results of the total LAB, TTA, and pH test of the jackfruit seed probiotic drink is shown in Table I.

TABLE I. THE RESULT OF THE TOTAL LAB, PH, AND TTA TEST RESULT FOR JACKFRUIT SEED EXTRACT PROBIOTIC DRINK

Fermentation Time	Average Total LAB (CFU/mL)	pH	TTA(%)
0 hour	$1,81 \times 10^{8a}$	5,48 ^a	0,25 ^a
24 hour	$2,18 \times 10^{9b}$	3,39 ^b	0,74 ^b
48 hour	$5,96 \times 10^{8c}$	3,29 ^c	1,05 ^c
72 hour	$4,03 \times 10^{6d}$	3,25 ^d	1,16 ^d

Note: The letters a, b, c, d in the column show a significant difference at the 5% level with the LSD (*Least Significant Difference*) test

Based on the results of statistical tests, the total LAB and TTA data were normally distributed and homogeneous so that they could meet the requirements for the *One Way ANOVA* test and then tested with the *Post Hoc LSD* test with the aim of to find out the differences in each treatment. Statistical results with the *One Way ANOVA* test yielded a significance value ($p < 0.05$)

which indicated that there was an effect of fermentation time on LAB growth. While the results of the *post hoc* LSD further test showed a significant difference ($p < 0.05$) in each treatment of fermentation time on total LAB and TTA of jackfruit seed probiotic drink.

The statistical test of pH data is not normally distributed and homogeneous and can be done with the *Kruskal Wallis* test. The results of the *Kruskal Wallis* test showed a significant value ($p < 0.05$) which indicated that there was an effect of fermentation time on the pH value. The *Post Hoc Mann Whitney* test showed that there was a significant difference ($p < 0.05$) for each treatment with the length of fermentation time with the pH of the probiotic drink of jackfruit seed extract. Changes in the total LAB, pH, and TTA of the probiotic drink jackfruit seed extract with fermentation time are shown in Figure 1.

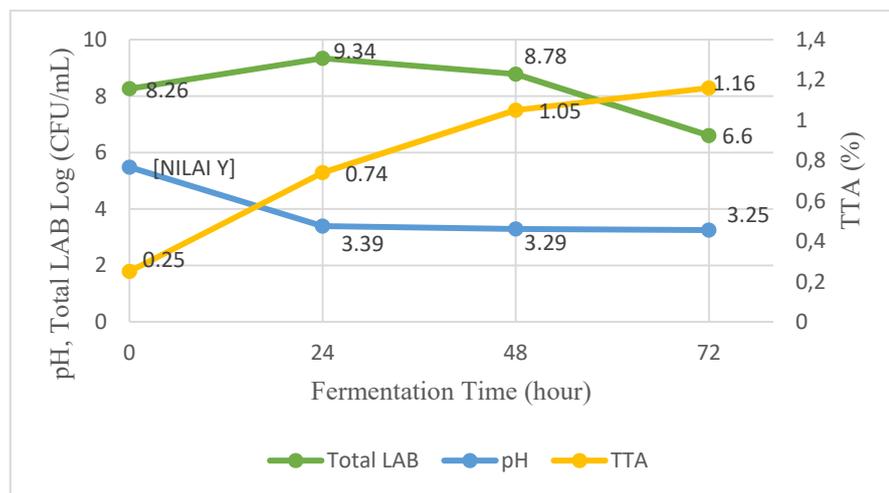


Fig 1. Graph of growth of LAB, pH, and TTA on probiotic drink jackfruit seed extract

Figure 1 shows that the duration length of fermentation affects the total LAB, TTA, and pH. The highest LAB growth was found during the 24-hour of fermentation with the number a total LAB of 2.18×10^9 CFU/mL, an increase of 1 log cycle from the initial condition of inoculation, in the logarithmic phase of *Lactobacillus plantarum* B1765. The log phase occurs because in these conditions bacterial cells utilize the nutrients in the media to carry out cell division. During the 48 to 72 hours of fermentation, the total LAB decreased, which was called the death phase, because the substrate contained in the bacteria was reduced and the total LAB reduced to 4.03×10^6 CFU/mL.

Research conducted by [14] stated that the longer the fermentation, the more the number of microbes will increase so that the ability to break down existing substrates into lactic acid and alcohol is greater. Bacteria will grow to the maximum number without the provision of nutrients in the media. Based on [15] that in the death phase, cells die more than cells that grow, which is because the nutrients contained in the media are reduced and environmental conditions are less supportive for bacteria to survive as a result of the results of microbial metabolism. The results of this study fulfil the minimum standard for the number of probiotic cultures that must be present in food. A product can be said to be a probiotic drink if the product contains $10^6 - 10^8$ CFU/mL of lactic acid bacteria that are still alive at the time of consumption [16],[17].

The results of this study are also supported by previous studies which stated that the length of fermentation time could increase the amount of LAB to the log phase. In a study [8] fresh jackfruit seed flour fermented for up to 32 hours experienced an increase in total LAB with *L. plantarum* culture. Optimum growth occurred at 24 hours of fermentation with a total LAB reaching of 8.85×10^8 CFU/mL. In a study [18] the total LAB *Lactobacillus plantarum* B1765 increased to 3.25×10^8 CFU/mL in Yacon tubers fermented for up to 48 hours. While in probiotic drink jackfruit seed extract, the total LAB *Lactobacillus plantarum* B1765 increased until the 24-hour fermentation time reached 2.18×10^9 . When compared with these studies, the results of this study can increase the total LAB up to 2.18×10^9 CFU/mL at 24 hours of fermentation with the same culture. Based on these results the seed extract Jackfruit has the potential to be used as a growth medium for *Lactobacillus plantarum* B1765 bacteria.

In this study, the pH value decreased with increasing fermentation time due to the metabolic process of the bacterium *Lactobacillus plantarum* B1765. *Lactobacillus plantarum* is part of the heterofermentative because is able to can produce other

than lactic acid, including acetic acid, ethanol, and CO₂. The higher the lactic acid produced, the higher the H⁺ ions released, because during the fermentation process lactic acid will be broken down into H⁺ ions and CH₃CHOHCOO⁻ ions so that the longer the fermentation time, the more H⁺ ions will cause the pH to decrease [19]. The decrease in pH is caused by the activity of LAB which produces lactic acid and short-chain fatty acids commonly referred to as Short Chain Fatty Acids (SCFA) such as acetic acid, butyric acid, and propionic acid [20].

Figure 1 shows the highest pH in the unfermented jackfruit seed probiotic drink with a pH value of 5.48. While the lowest pH was found in the probiotic drink of jackfruit seed extract which was fermented for 72 hours with a pH value of 3.25. The standard pH value for safe food is less than 4.6 so the pH value of this drink has meet the standard as a safe food in only 24 hours. The results of this study are supported by previous studies which stated that there was a decrease in pH from 4.34 to 3.62 in cocofiers using kefir with a fermentation time of up to 48 hours [14]. Another study also stated that there was a decrease in pH from 4.53 to 3.20 with a fermentation time of up to 10 days in red galangal drink fermented with kombucha starter [21]. When compared with these studies, the results of this study can reduce faster to 3.29 with a duration of up to 72 hours. This is because the bacteria used are different, so it can be said that the *L. plantarum* B1765 strain has the potential to lower pH faster than kefir and kombucha starters.

Figure 1 shows that increasing fermentation time can increase TTA in the probiotic drink of jackfruit seed extract even though the total LAB under goes a death phase. The highest TTA was found in the probiotic drink of jackfruit seeds fermented for 72 hours with a TTA value of 1.16%. Meanwhile, the lowest total titrated acid was found in the unfermented jackfruit seed probiotic drink with a TTA value of 0.25%. The total acid value increased due to the breakdown of glucose into lactic acid. Research conducted by [8] which states that the total acid value tends to increase in jackfruit seed flour followed by a decrease in pH due to the breakdown of glucose into lactic acid so that the organic acid content increases and glucose in the starch is used as nutrition. The results of this study are also supported by previous research which states that increasing the length of fermentation can increase the total value of titrated acid in jackfruit seed flour fermented for 24 hours with *L. plantarum* culture with a value of 0.2157% [8]. When compared with this study, the results of this study at a 24-hour fermentation period the TTA value increased 0.74% greater with bacterial culture and the same fermentation time, so it can be said that the *L. plantarum* B1765 strain has the potential to increase TTA faster in the same substrate.

The standard used to assess the total titrated acid of probiotic drinks is (SNI 7552:2009) concerning flavored fermented milk drinks with the allowable total value of titrated acid from 0.2 to 0.9%. Based on the results of this study, the TTA value that fullfil the standard was at 24 hours of fermentation with a value of 0.74%.

Organoleptic Quality Test

The organoleptic test results of jackfruit seed probiotic drink can be shown in Table II.

TABLE II. THE RESULT OF ORGANOLEPTIC TESTS OF COLOR, TASTE, AND AROMA ON PROBIOTIC DRINK JACKFRUIT SEED EXTRACT

Fermentation time	Colour	Aroma	Taste
0 hour	2,43 ^b	2,43 ^a	2,57 ^a
24 hour	3,26 ^a	2,90 ^a	3,07 ^a
48 hour	2,70 ^a	2,70 ^a	2,97 ^a
72 hour	3,03 ^a	2,36 ^a	2,50 ^a

Note: 1= really dislike 2= dislike 3= like 4= really like. The letters a, b in the column show a significant difference at the 5% level

The results of the *Kruskal Wallis* test for organoleptic test showed that the length of fermentation had not significant effect ($p > 0.05$) on the level of taste and aroma preference, but had a significant effect ($p < 0.05$) on the level of colour preference. Then proceed with the *Mann Withney* test on colour parameters. The results of the *Mann Withney* test showed a significant difference (p

< 0.05) in the fermentation time 0 with 24 hours, 0 with 48 hours, and 0 with 72 hours. While the fermentation time of 24 to 48 hours, 24 to 72 hours, and 48 to 72 hours there was no significant difference ($p > 0.05$).

Table II shows that there is an increase in the length of fermentation time that affects the level of preference for taste, colour, and aroma. The highest average value at the level of preference for the colour preferred by the panelists is 3.26 (likes) where the preferred colour is whiter than other fermentation times. The white colour is caused by degradation of the yellow pigment during fermentation [8]. Jackfruit seeds contain carotene pigment which is the main component of the yellow colour in jackfruit seed extract, pH stability affects the color produced by carotenoids so there is a change in colour with longer fermentation due to a decrease in pH [22], [23]. The results of physical observations on the probiotic drink jackfruit seed extract are shown in Figure 2.



Fig 2. Jackfruit seed probiotic drink with different fermentation time

Based on the picture, it can be seen that there is a color change in the probiotic drink of jackfruit seed extract. The unfermented beverage is yellowish white in color, the 24-hour and 48-hour fermented drink is white, while for 72 hours the drink is yellowish white in color. The white colour is caused by degradation of the yellow pigment during fermentation [8].

The preference for taste and aroma, the highest average value favored by the panelists was at 24 hours of fermentation with a value of 3.07 (like) and 2.90 with a sweet-sour taste and sour aroma. the 48 hours of fermentation, it shows a sour taste and aroma with a liking category level. While the lowest average value is 72 hours of fermentation with a value of 2.50 and 2.36 on taste and aroma because the taste and aroma of the drink is too sour. This happens because LAB utilize the sugar content of probiotic drinks from jackfruit seeds through their metabolic system as a source of energy and carbon which then produces lactic acid as a metabolic product [24]. There is a decrease in the level of preference in the 48 and 72 hour of fermentation because the taste and aroma are getting more sour, because the pH value produced in this study is decreasing.

Based on the overall organoleptic assessment, the best fermentation time for the probiotic drink of jackfruit seed extract was found in 24 hours of fermentation with a sweet-sour taste and white colour. Apart from the organoleptic assessment of the best fermentation time in probiotic drinks, jackfruit seed extract can also be viewed from its microbiological and chemical quality with the best fermentation time of 24 hours.

IV. CONCLUSION

Based on the research that has been done, it can be concluded that the longer the fermentation time will affect the total LAB, pH, TTA, and colour, but does not affect taste and aroma. The results showed that the best fermentation time for the probiotic drink jackfruit seed extract was 24 hours with a total LAB of 2.18×10^9 CFU/mL, pH of 3.39, TTA of 0.74%, and the average value. colour preference level 3.26; taste 2.9; and aroma 3.07. Based on total LAB, pH and TAT, the qualities of this product fulfilled the Indonesian National Standart (INS) for probiotic drink, so it could be used as the beverage probiotic agent.

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