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# Optimization of Probiotic Drinks Fermentation in Bidara (*Ziziphus mauritiana*) Fruit Juice with *Lactobacillus plantarum* InaCC B616 using Response Surface Methodology

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## ABSTRACT

Bidara fruit (*Ziziphus mauritiana*) is known to contain various bioactive phytochemicals, including phenolic acid, ascorbic acid, saponins, terpenoids, flavonoids, and tannins, making it highly promising for development into functional beverage products. Several studies state that fermentation with probiotic bacteria can improve the functional properties of products. This research aims to optimize the fermentation process of bidara fruit juice with *Lactobacillus plantarum* InaCC B616 using Response Surface Methodology and determine the effect of fermentation time on the content of bioactive compounds such as total phenols, total flavonoids, antioxidant capacity, and inhibition of the α-glucosidase enzyme. Optimization of fermentation was carried out using a Central composite design with 13 experimental samples and test data using Design Expert® 13 software, the optimization of fermentation time was conducted using a Completely Randomized Design (CRD) with four treatment groups and three replications per group, including a negative control treatment: no fermentation (F0), fermentation for 12 hours (F1), fermentation for 24 hours (F2), and fermentation for 48 hours (F3). The research results showed that 18.61% bidara fruit juice and 0.34% skim milk was the optimum formula for fermenting bidara fruit juice. The best fermentation duration was in the F3 group (fermentation for 48 hours), in this group the bacterial growth reached $3.1x10^9$ cfu/mL with a total phenolic compound content of 81.80 mg GAE/mL, total flavonoids 2.81 mg QE/g, capacity antioxidant 99.28 µg AAE/mL, and inhibition of the α-glucosidase enzyme 87.42%. The fermentation of bidara fruit juice with *Lactobacillus plantarum* InaCC B616 has the potential to enhance total phenol content, total flavonoids, antioxidant capacity, and α-glucosidase enzyme inhibition, making it a promising candidate for probiotic beverages. This drink could be developed as an alternative treatment for type 2 diabetes mellitus due to its inhibitory activity against the α-glucosidase enzyme.

**Keywords**: Fermentation; *Ziziphus mauritiana*; *Lactobacillus plantarum* InaCCB616; Response Surface Methodology; Probiotic drinks

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## INTRODUCTION

Fermentation has been traditionally carried out for generations. It offers various advantages, such as enhancing the functional properties of food and beverages and improving the flavour of the products. To produce better quality fermented products, it is necessary to select a quality starter culture and handle the fermentation process. Fermentation can be carried out by utilizing lactic acid bacteria on plant-based foods, such as fruits[7]. One fruit that has many benefits but is not widely used at the moment is bidara fruit (*Ziziphus mauritiana*). This fruit is known to contain various bioactive phytochemicals, including phenolic acids, ascorbic acid, saponins, terpenoids, flavonoids, and tannins. Bidara fruit is also abundant in vitamin A, vitamin B complex, and vitamin C, with vitamin C content ranging from 65.8 to 76.0 g/100 g of fresh fruit[2,25]. Bidara fruit also contains total phenolic compounds of 207.6 mg Gallic Acid Equivalent (GAE)/100 g, while the total flavonoids are 102.9 mg Quercetin Equivalent (QE)/100 g. The carbohydrate content in bidara fruit is 82.43% and contains 0.61% fiber. soluble food and 2.03% insoluble dietary fiber, bidara fruit also has antioxidant activity and high nutritional value[1,24]. Based on Liquid Chromatography-Mass Spectrometry (LC-MS) analysis, bidara fruit contains 42 identified compounds and is divided into five compound groups, namely 12 alkaloid compounds, 10 flavonoid glycosides, 12 terpenoids, 4 glycosides, and 4 phenols[26]. Several studies report the pharmacological activity of various parts of the bidara plant such as fruit, leaves and stems as antioxidant, cytotoxic, anti-microbial, anti-diarrheal, antidepressant, immunomodulatory, anti-inflammatory and hepatoprotective[22]. Another study revealed that bidara fruit has the potential to be a source of vitamin C which plays a very important role in improving the immune system and warding off free radicals[2,25]. Bidara fruit can be mixed with salt to treat digestive and gallbladder disorders[3].

Fermenting bidara fruit juice with *Lactobacillus plantarum* and formulating fermented products into functional drinks need to be a big concern for developing health products. *Lactobacillus plantarum* is able to renovate complex compounds into simpler compounds with the final result being lactic acid. *Lactobacillus plantarum* produces lactic acid at the end of its metabolic process and can increase acidity by 1.5 to 2% in the substrate[27]. Several studies report that food products fermented with lactic acid bacteria will increase antioxidant activity and inhibit the enzyme α-glucosidase[4]. The inhibition of the α-glucosidase enzyme occurs due to the presence of secondary metabolites, specifically exopolysaccharide compounds. Exopolysaccharides produced by lactic acid bacteria are beneficial for health, especially in controlling type 2 diabetes mellitus[5,8,9].

The useful characteristics of various parts of the bidara fruit can be further enhanced by fermentation. Bidara fruit fermented with *Lactobacillus plantarum* InaCC B616 has the potential to be used as a probiotic drink which is beneficial for health, especially controlling type 2 diabetes mellitus. Fermentation can also improve the taste of bidara fruit. However, before fermentation is carried out, it is necessary to consider the formula optimization process. Optimization techniques can be carried out using an experimental design approach. This is done to determine the best formula using evaluation data from the formulation created. A widely used optimization technique is the Response Surface Methodology (RSM), which is a set of statistical and mathematical methods designed to model and analyze multiple variables[10].

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This research aims to optimize the fermentation process of the probiotic drink bidara fruit juice with *Lactobacillus plantarum* InaCC B616 using Response Surface Methodology and determine the effect of fermentation duration on the content of bioactive compounds such as total phenols, total flavonoids, antioxidant capacity, and inhibition of the α-glucosidase enzyme.

## METHODS

### Tools and materials

The tools used in this research were a juicer (Philips HR1811), Erlenmeyer 250 mL, measuring cup, laminar air flow, hose needle, Bunsen lamp, aluminum foil, autoclave, incubator, temperature controller, filter paper, vacuum filter, sugar refractometer (Adblue®), pH meter (Schott lab 850), burette, scales, pipette, petri dish, spectrophotometer, microplate reader, test tube, and Design Expert® 13 software. The materials used are bidara fruit (*Ziziphus mauritiana*), pure bacterial culture *Lactobacillus plantarum* InaCC B616, Skim milk powder (NZMP™), Nutrient broth (Merck), Nutrient agar (Merck), Aquades, Glucose, NaOH 0.1 N, phenolphthalein 1%, NaCl 0.9%, Folin-Ciocalteu Reagent, Methanol , Ethanol, Gallic acid, Ascorbic acid, AlCl3, 0.1 M phosphate buffer (pH 7.0), Potassium acetate, DPPH solution, α-glucosidase enzyme, $Na_2CO_3$, and p-nitrophenyl α-D-glucopyranoside.

### Formula optimization design and fermentation duration

Optimization of fermentation formula bidara fruit juice with *Lactobacillus plantarum* InaCC B616 was conducted using the Response Surface Methodology. The design used was the Central Composite Design with 13 experiments. The effect of fermentation duration was carried out using a completely randomized designwith 4 groups, namely: F1 (12 hour fermentation), F2 (24 hour fermentation), F3 (48 hour fermentation), and F0 (no fermentation) as control.

### Bacterial strains

The pure culture of *Lactobacillus plantarum* InaCC B616 bacteria was obtained from the InaCC (Indonesian Culture Collection)-BRIN (National Research and Innovation Agency) Laboratory.

### Inoculum preparation

The working procedure for growing *Lactobacillus plantarum* InaCC B616 bacteria is carried out by taking bacteria from pure culture stock with a sterile tube needle, suspending them in 25 mL of sterilized nutrient broth and 1% glucose medium, then incubating in an incubator at 37 $^{O}$C for 48 hours. Bacteria that have successfully grown can be seen from the color of the medium turning cloudy.

### Making bidara fruit juice

Bidara fruit *is* freshly obtained from Medang Island, Sumbawa, West Nusa Tenggara – Indonesia. Only ripe bidara fruit is used which is characterized by a reddish-yellow color and soft flesh. The bidara fruit was washed with running water and then drained and the juice was extracted using a Philips HR1811 slow juicer, then the juice was stored at -18 $^{O}$C before use.

### Fermentation procedure

Bidara fruit juice and skim milk powder (NZMP™) were put into a 250 mL Erlenmeyer with various predetermined concentrations (Table 1). then distilled water was added until the mixture was 100 mL, then sterilization was carried out at 121 $^{O}$C for 15 minutes. After cooling, inoculation was carried out with 1 mL of the grown *Lactobacillus plantarum* InaCC B616 suspension, then incubated in an incubator at 37 $^{O}$C for 48 hours. The effect of fermentation duration was carried out in the same way, only the incubation time was different between groups, namely 12 hours, 24 hours and 48 hours with the optimum concentration of bidara fruit juice and skimmed milk powder (result of

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formula optimization determination). The fermented bidara fruit juice is then filtered and ready for analysis.

**Analysis of pH, brix, and total acid**

Analysis of the degree of acidity (pH) was measured with a pH meter (Lab 850; SCHOTT, Germany). pH is determined by placing an electrode into the test sample until the pH meter displays a stable reading, after which the scale reading or numbers shown on the pH meter display are recorded[6]. Sugar content is determined by dropping the sample on a Brix refractometer, then holding it to light. From this tool, you can see the results of measuring the percentage of sugar content in % Brix for each sample tested. Brix is defined as the percentage of total dissolved solids in a solution, expressed as grams of solute per 100 grams of solution[29]. Total lactic acid was analyzed using the volumetric method. 10 mL sample solution was placed into an Erlenmeyer flask containing 100 mL of distilled water, and 3 drops of phenolphthalein indicator were added. The solution was then titrated with 0.1 N NaOH until a stable pink color was observed. Next, the volume from the NaOH titration results is entered into the acid content calculation formula[18]. The titration formula is as follows:

$$\text{Total acid (\%)} = \frac{V_1 \times N \times B}{V_2 \times 1000}$$

Note:
V1: Volume of NaOH (mL)
V2: Sample Volume (mL)
N: Normality NaOH (0.1 N)
B: Molecular Weight of Lactic Acid (90)

**Analysis of bacterial growth**

Analysis of the growth of lactic acid bacteria was carried out using the TPC (total plate count) method. A total of 1 mL of fermentation liquid was added to 9 mL of 0.9% NaCl to obtain a dilution of $10^{-1}$. Dilution was carried out to obtain a dilution of $10^{-8}$. Next, 1 mL of the diluted sample was placed into a petri dish containing agar media, which was then incubated at 37°C for 48 hours. After the incubation period, the number of colonies that had developed was counted[18].

**Total phenol analysis**

The total phenolic content in the fermentation of bidara fruit juice was measured using the Folin-Ciocalteu reagent. The fermented bidara fruit juice was diluted 1:10 with distilled water, and 0.5 mL of the solution was mixed with 5 mL of Folin-Ciocalteu reagent (diluted 1:10 with distilled water) and 4 mL of $Na_2CO_3$ solution (1 M). The mixture was allowed to stand for 15 minutes. Total phenolic content was determined by colorimetry at a wavelength of 765 nm. A standard curve was created using gallic acid in a methanol/water mixture (50:50, v/v), and measurements were performed in triplicate for each sample. The total phenolic content was expressed as Gallic Acid Equivalent (mg GAE/mL)[17,20].

**Total flavonoid analysis**

The total flavonoid content was determined using the colorimetric method with AlCl3 reagent. A 10 µL sample of fermented bidara fruit juice was combined with 60 µL of ethanol, 10 µL of 10% AlCl3, 10 µL of 1 M potassium acetate, and 120 µL of distilled water, then placed in a plate. The mixture was then homogenized and incubated for about 30 minutes. The absorbance of the solution was measured at a wavelength of 415 nm. Total flavonoid content was calculated using a quercetin calibration curve ($y = 0.001x - 0.006$, $R^2 = 0.998$), and the results were expressed as Quercetin Equivalent (mg QE/g). Measurements were carried out in triplicate for each sample[21].

**Antioxidant capacity analysis**

The antioxidant capacity was evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. 40 µL sample of fermented bidara fruit juice was combined with 250 µL of 125 µM DPPH solution (dissolved in ethanol) and placed into a microplate. The mixture was then

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incubated for 30 minutes in the dark at room temperature. The absorbance was measured at a wavelength of 515 nm. Ascorbic acid was used as the standard, and ethanol served as the blank. The antioxidant capacity was expressed as micrograms of Ascorbic Acid Equivalent per milliliter (µg AAE/mL)[12].

**Inhibition analysis of α-glucosidase enzyme**

The analysis of α-glucosidase enzyme inhibition was conducted in the following steps: The reaction mixture for the sample consisted of 10 µL of the sample, 50 µL of 0.1 M phosphate buffer (pH 7.0), 25 µL of 0.5 mM PNPG substrate (4-nitrophenyl α-D-glucopyranoside), and 25 µL of 0.04 U/mL α-glucosidase enzyme solution, all placed in a microplate. The blank reaction mixture contained 50 µL of 0.1 M phosphate buffer (pH 7.0), 25 µL of 0.5 mM PNPG substrate, and 25 µL of α-glucosidase enzyme solution. The negative control did not include the α-glucosidase enzyme. The positive control used was acarbose, with 10 µg/mL acarbose solution added to the reaction mixture, similar to the sample. The reaction mixture was then incubated at 37°C for 30 minutes. The reaction was halted by adding 100 µL of 0.2 M sodium carbonate, and absorbance was measured at a wavelength of 410 nm using a spectrophotometer. The percentage of α-glucosidase enzyme inhibition was calculated using the following formula[5,16]:

$$\frac{K-(A1-A0)}{K}\times 100\%$$

K : Blank absorbance minus negative control
A0: Absorbance of positive control
A1: Sample absorbance

## RESULTS AND DISCUSSION

**Optimization of fermentation formula**

A total of 13 experimental samples, each treated with varying concentrations of bidara fruit juice and skim milk, were tested, and the responses are shown in Table 1. These responses help identify the model that can be used to determine the optimal concentrations of bidara fruit juice and skim milk for producing fermented bidara fruit juice. which has pH, total lactic acid, and sugar content according to the desired criteria.

**Table 1.** Response to treatment with bidara fruit juice concentration and skim milk concentration

| Sample Code | A: Bidara fruit juice (%) | B: Skim Milk (%) | pH | Total lactic acid (%) | Sugar Content (%Brix) |
|---|---|---|---|---|---|
| **1** | 29.14 | 6.00 | 4.73 | 0.41 | 8.4 |
| **2** | 15.00 | 0.34 | 3.97 | 0.60 | 2,4 |
| **3** | 15.00 | 6.00 | 5.68 | 0.43 | 7.0 |
| **4** | 15.00 | 6.00 | 5.99 | 0.34 | 6,8 |
| **5** | 15.00 | 6.00 | 5.62 | 0.37 | 7.0 |
| **6** | 0.86 | 6.00 | 6.20 | 0.25 | 5.4 |
| **7** | 5.00 | 10.00 | 6,10 | 0.34 | 7.6 |
| **8** | 15.00 | 6.00 | 5.79 | 0.38 | 6.6 |
| **9** | 15.00 | 6.00 | 6.07 | 0.33 | 7.4 |
| **10** | 25.00 | 2.00 | 4.21 | 0.46 | 5.2 |
| **11** | 25.00 | 10.00 | 5.80 | 0.38 | 10.8 |
| **12** | 5.00 | 2.00 | 6.12 | 0.33 | 2,2 |
| **13** | 15.00 | 11.66 | 6.15 | 0.31 | 11.2 |

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Table 1 shows actual data on pH response, total lactic acid and sugar content during the fermentation process with different combinations of bidara fruit juice concentration and skim milk concentration. The highest pH value, namely 6.20, was obtained in experimental sample number 6 with a bidara fruit juice concentration of 0.86% and skim milk concentration of 6%, while the lowest pH value, namely 3.97, was obtained in experimental sample number 2 with a bidara fruit juice concentration of 15%. and skim milk concentration of 0.34%. The highest total lactic acid, namely 0.60%, was obtained in experimental sample number 2 with a bidara fruit juice concentration of 15% and a skim milk concentration of 0.34%, while the lowest total lactic acid, namely 0.25%, was obtained in experimental sample number 6 with a juice concentration. bidara fruit 0.86% and skim milk concentration 6%. The highest sugar content, namely 11.2%, was obtained in experimental sample number 13 with a bidara fruit juice concentration of 15% and skim milk concentration of 11.66%, while the lowest sugar content, namely 2.2%, was obtained in experimental sample number 12 with a fruit juice concentration. bidara 5% and skim milk concentration 2%. The relationship between the independent variables (bidara fruit juice concentration and skim milk concentration) and the dependent or response variable (pH, total lactic acid, and sugar content) can be described using a quadratic or linear model which can be seen in Table 2. The alignment between the data distribution and the model can be demonstrated through the results of the ANOVA test, which includes values for model significance, lack of fit, and the coefficient of determination (predicted R-squared, adjusted R-squared) using the Design Expert® 13 software.

In Table 2, the values of the Analysis of Variance (ANOVA) response model for pH, total lactic acid and sugar content. The F value in the table shows the significance value of the resulting model, the F value of the three responses shows that the model is significant because the calculated F is greater than the F table (4.75), meaning that simultaneously the independent variables (bidara fruit juice concentration and skim milk concentration) has a significant influence on the dependent variables (pH, total lactic acid, and sugar content). The pH response, total lactic acid, and sugar content form a model that can describe the data well at a significance level of 5%. This can be seen from the p-value <0.05.

The lack of fit values for the pH response, total lactic acid, and sugar content showed insignificant results (P>0.05), with respective values of 0.1042; 0.1169 and 0.1866.

**Table 2.** Mathematical model analysis of pH response, total lactic acid, and sugar content

| Response | Mathematical Model | F-value | p-value | Lack off Fit | $R^2$ | Adjusted $R^2$ Model | Predicted $R^2$ Model | Adequate Precision |
|---|---|---|---|---|---|---|---|---|
| pH | Quadratic | 14.51 | 0.0014 | 0.1042 | 0.912 | 0.8491 | 0.4947 | 12.3329 |
| Total lactic acid (%) | Linear | 6.08 | 0.0187 | 0.1169 | 0.5487 | 0.4584 | 0.0813 | 7.2251 |
| Sugar Content (%Brix) | Linear | 238.3 | <0.0001 | 0.1866 | 0.9794 | 0.9753 | 0.9612 | 42.4295 |

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This provides a clear indication of how well the model fits the pH, total lactic acid, and sugar content responses. The R2 (R-squared) values suggest that the model explains 91.20% of the variation in the pH response, 54.87% in the total lactic acid response, and 97.94% in the sugar content response. The model for sugar content response was considered to have good agreement, as it met the criterion of having a difference between Adjusted R2 and Predicted R2 of less than 0.2. However, the models for pH and total lactic acid responses did not meet this criterion, as the difference between Adjusted R2 and Predicted R2 exceeded 0.2. The Adequate Precision value for all responses is greater than 4, indicating that the models generated by Design Expert® 13 to predict water content, water activity, and ash content are acceptable and can be applied within the design space.

To demonstrate the impact of the independent variables on each response, response surface graphs (Figure 1) are utilized, which display the interaction between two independent variables and their effect on each response (pH, total lactic acid and sugar content).

In Figure 1, the values of pH response, total lactic acid, and sugar content in the fermentation of bidara fruit juice with different concentrations of bidara fruit juice and skim milk are shown. It can be seen that there is a decrease in pH as the concentration of bidara fruit juice increases. On the other hand, pH increases as the concentration of skim milk added to the medium during fermentation of bidara fruit juice increases. The decrease and increase in pH indicate that both the concentration of bidara fruit juice and the concentration of skim milk significantly affect pH values with $p<0.05$. The total lactic acid increases with the addition of bidara fruit juice concentration, although the increase is not significant $p>0.05$. Conversely, the total lactic acid decreases with the addition of higher concentrations of skim milk in the medium during fermentation, indicating that an excess of skim milk inhibits microbial growth. The decrease in total lactic acid occurs significantly with $p<0.05$. Meanwhile, the sugar content increases with the addition of bidara fruit juice concentration, as well as with the addition of skim milk concentration, showing an increase in sugar content. This suggests that both the concentration of bidara fruit juice and the concentration of skim milk significantly affect sugar content with $p<0.05$.

Table 3 it outlines the target values, upper and lower limits, and the importance of each optimization variable. The concentration of bidara fruit juice is set to range from 0.86% to 29.14%, while the concentration of skim milk is set between 0.34% and 11.66%.

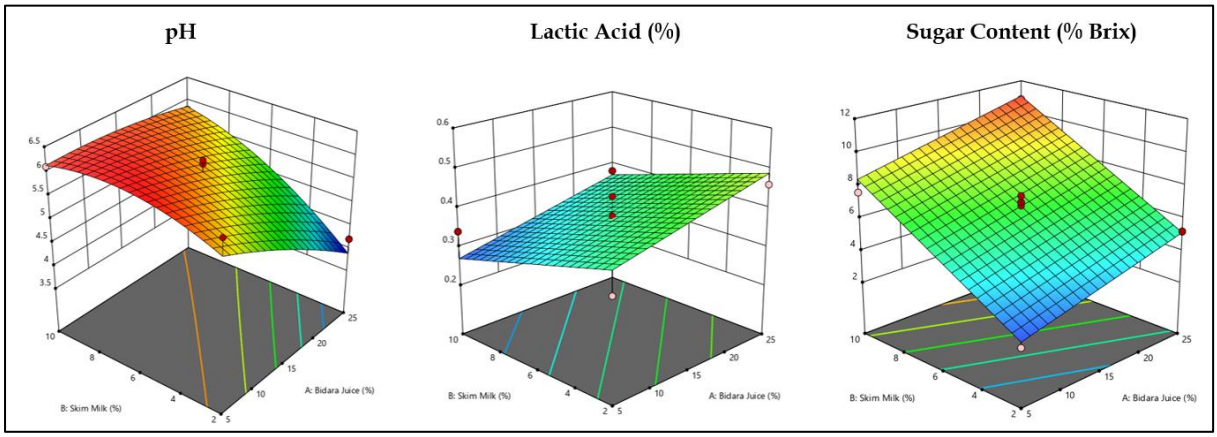


**Figure 1.** 3D Response Surface

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**Table 3.** Criteria for determining the optimum combination of bidara fruit juice and skim milk

| Variable | Target | Lower limit | Upper limit | Interest |
|---|---|---|---|---|
| Bidara fruit juice | Within range | 0.86 | 29.14 | 3 (+++) |
| Skim milk | Within range | 0.34 | 11.66 | 3 (+++) |
| pH | Within range | 3.00 | 4.00 | 3 (+++) |
| Total lactic acid | Maximum | 0.25 | 0.6 | 5 (+++++) |
| Sugar level | Minimal | 2,2 | 11.2 | 3 (+++) |

**Table 4.** Optimum combination solution for bidara fruit juice and skim milk

| Number | Bidara Juice (%) | Skim Milk (%) | pH | Total acid | Sugar level | Desirability | |
|---|---|---|---|---|---|---|---|
| 1 | 18.61 | 0.34 | 3.97 | 0.48 | 3.09 | 0.744 | Selected |
| 2 | 18.56 | 0.34 | 3.98 | 0.48 | 3.09 | 0.743 | |
| 3 | 18.54 | 0.34 | 3.98 | 0.48 | 3.09 | 0.743 | |
| 4 | 18.42 | 0.34 | 3.99 | 0.48 | 3.07 | 0.743 | |
| 5 | 22.60 | 1.58 | 4.00 | 0.48 | 4.53 | 0.694 | |

**Table 5**. Response to verification model for the combination of bidara fruit juice and skim milk

| Response | Prediction | Verification | 95% PI low | 95% PI high | 95% CI low | 95% CI high |
|---|---|---|---|---|---|---|
| pH | 3.97 | 3.89 | 2.13 | 5.81 | 3.39 | 4.55 |
| Total lactic acid (%) | 0.48 | 0.55 | 0.16 | 0.81 | 0.40 | 0.56 |
| Sugar Content (%Brix) | 3.09 | 2.80 | 0.95 | 5.24 | 2.55 | 3.64 |

The upper and lower limits have been determined as expected. The pH response is targeted to fall within the range of 3 to 4, as this pH range is anticipated to support optimal bacterial growth during the fermentation process. Meanwhile, total lactic acid is targeted to reach the maximum value, this is because lactic acid is the main metabolic product of the *Lactobacillus plantarum* bacteria, so high levels of lactic acid are a sign that the microbe is growing well. The sugar content is set at a minimum value, because based on experimental results it shows that sugar that is too high will inhibit microbial growth as indicated by the low level of acid produced.

Based on the criteria for achieving the expected targets as described in Table 3, a combination of bidara fruit juice and skim milk was selected in Table 4. The selected combination of bidara fruit juice and skim milk for verification testing was one that had a desirability value close to one. or the highest, namely bidara fruit juice with a concentration of 18.61% and skim milk 0.34%. A desirability value close to one represents the most optimal outcome, as it indicates higher accuracy in the optimization process. The predicted response values for the selected combination are pH 3.97, total lactic acid 0.48%, and sugar content 3.09%.

Based on the predicted values in Table 4 and the laboratory test process for pH response parameters, total lactic acid and sugar content, verification values were obtained. Model verification is needed to

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confirm the predicted data for optimum conditions with actual data from retesting using a combination of bidara fruit juice with a concentration of 18.61% and skim milk with a concentration of 0.34%. The response data from the predictions and verification of pH, total lactic acid, and sugar content are shown in %Table 5.

Table 5 shows the verification results of the combination of bidara fruit juice with a concentration of 18.61% and skim milk with a concentration of 0.34%. produces actual response values, namely pH 3.89, total lactic acid 0.55%, and sugar content 2.80%. This value shows that the response value is in the range of 95% Confident Interval (CI) and 95% Prediction Interval (PI). The actual test results are within the predicted range and show that the model can be used to predict the three responses well.

**The effect of fermentation duration**

The effect of the fermentation duration of bidara fruit juice with *Lactobacillus plantarum* InaCC B616 in this study was analyzed. The analysis included total phenolic compounds, total flavonoids, antioxidant capacity, and α-glucosidase enzyme inhibition. Based on the optimization results, the formulation consisted of 18.61% bidara fruit juice and 0.34% skim milk. The average values and standard deviations of the results are presented in Table 6.

The fermentation duration of bidara fruit juice with *Lactobacillus plantarum* InaCC B616 was shown to impact the levels of total phenolic compounds, total flavonoids, antioxidant capacity, and the inhibitory activity of the α-glucosidase enzyme. The best fermentation duration was in the F3 group (fermentation for 48 hours), in this group the bacterial growth reached $3.1x10^9$ cfu/mL with a total phenolic compound content of 81.80 mg GAE/mL, a total of flavonoids of 2.81 mg QE/g, capacity antioxidant 99.28 µg AAE/mL, and α-glucosidase enzyme inhibitory activity 87.42%.

The increase in phenolic compounds in fermented bidara fruit juice is closely related to the metabolic activity of microbes during fermentation which are able to modify bioactive components such as polyphenol groups and flavonoids. This can occur because lactic acid bacteria can produce hydrolytic enzymes that hydrolyze complex phytochemicals into simpler ones. The increase in the amount of simple phenolic acids, such as gallic acid, is

**Table 6.** Effect of fermentation duration of bidara fruit juice with *Lactobacillus plantarum* InaCC B616 on the content of total phenolic compounds, total flavonoids, antioxidant capacity, and inhibition of the α-glucosidase enzyme

| Group | Total Phenol (mg GAE/mL) | Total Flavonoids (mg QE/g) | Antioxidant Capacity (µg AAE/mL) | Inhibition of the α-Glucosidase Enzyme (%) |
|---|---|---|---|---|
| **F0** | $68.13 \pm 3.12^{a}$ | $1.94 \pm 0.04^{a}$ | $96.35 \pm 0.11^{a}$ | $80.92 \pm 0.80^{a}$ |
| **F1** | $73.56 \pm 2.49^{ab}$ | $1.96 \pm 0.04^{a}$ | $97.19 \pm 1.24^{ab}$ | $81.59 \pm 0.31^{a}$ |
| **F2** | $77.39 \pm 1.71^{bc}$ | $2.52 \pm 0.15^{b}$ | $98.73 \pm 0.19^{bc}$ | $86.61 \pm 0.46^{b}$ |
| **F3** | $81.80 \pm 4.20^{c}$ | $2.81 \pm 0.04^{c}$ | $99.28 \pm 0.58^{c}$ | $87.42 \pm 0.42^{b}$ |

Note:
Data are presented as mean ± SD (Standard Deviation), n=3, Values with different letter symbols in the same column indicate significant differences ($p < 0.05$) between groups based on the Tukey HSD test, F0: Not fermented, F1: Fermented for 12 hours, F2: Fermentation for 24 hours, F3: Fermentation for 48 hours

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related to the conversion of complex phenolics into free forms and the depolymerization of phenolic components by phenol oxidase enzymes produced by lactic acid bacteria[15,23]. The increase in antioxidant activity in bidara fruit juice fermented with *Lactobacillus plantarum* InaCC B616 is related to the increase in bioactive compounds such as phenols and flavonoids, flavonoids have been shown to enhance antioxidant activity[28]. while the increase in inhibition of the α-glucosidase enzyme occurs due to the presence of exopolysaccharides produced by microbes during the fermentation process. Exopolysaccharides produced by lactic acid bacteria play a role in inhibiting the α-glucosidase enzyme and have antioxidant activity so they play a role in controlling type 2[5,8,9] diabetes mellitus. Inhibition of the α-glucosidase enzyme is considered a powerful strategy for managing hyperglycemia[13]. In addition to exopolysaccharides, the components that have antidiabetic activity are phenolic compounds and flavonoids. This occurs because flavonoids are secondary metabolites that can inhibit the enzyme alpha-glucosidase[14,19].

The increase in the levels of total phenolic compounds, total flavonoids, antioxidant capacity, and α-glucosidase enzyme inhibition in bidara fruit juice fermented with *Lactobacillus plantarum* InaCC B616 are directly proportional to bacterial growth during the fermentation process. The growth of *Lactobacillus plantarum* InaCC B616 bacteria is shown by Number of bacterial colonies (cfu/mL) in Figure 2.

In Figure 2, the growth in the number of bacterial colonies *Lactobacillus plantarum* InaCC B616 during the incubation process of fermentation of bidara fruit juice.

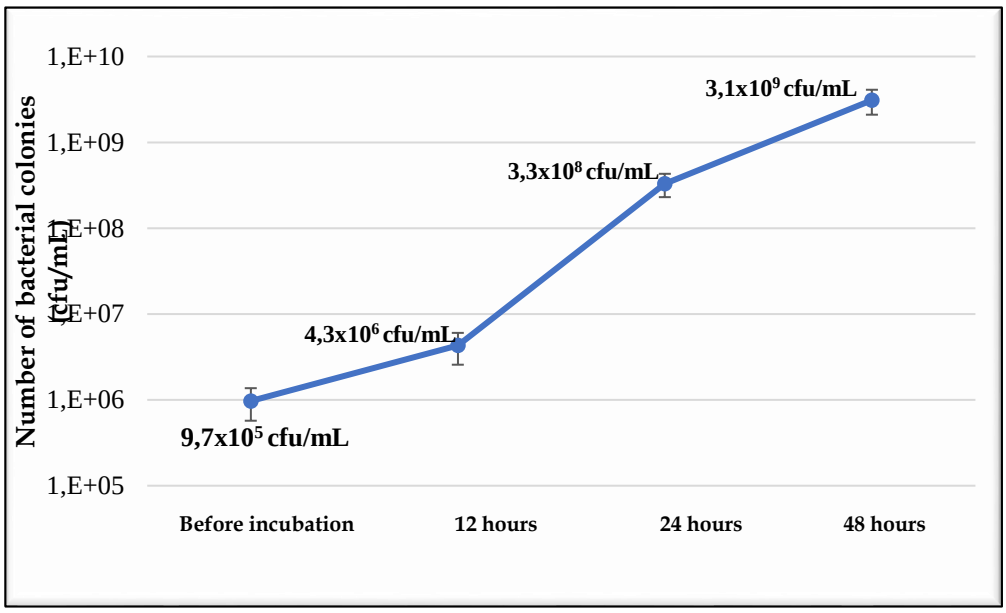


**Figure 2.** Growth of *Lactobacillus plantarum* InaCC B616 in fermented bidara fruit juice

The number of bacterial colonies before incubation is the average number of *Lactobacillus plantarum* InaCC B616 bacterial colonies that were inoculated in bidara fruit juice immediately before the incubation process or the initial number of bacterial colonies before the fermentation process started. At the 12th hour after incubation, *Lactobacillus plantarum* InaCC B616 began to show Although the number did not differ much from the initial inoculation, bacterial growth began to increase significantly at 24 hours of incubation and continued to increase in the number of colonies at 48 hours of incubation.

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## CONCLUSION

The optimum formula for fermenting the probiotic drink bidara (*Ziziphus mauritiana*) fruit juice is a combination of 18.61% bidara fruit juice and 0.34% skim milk. The best fermentation duration was 48 hours, with bacterial growth reaching 3.1x109 cfu/mL, total phenolic compound content of 81.80 mg GAE/mL, total flavonoids of 2.81 mg QE/g, antioxidant capacity of 99.28 µg AAE/mL, and the inhibitory activity of the α-glucosidase enzyme was 87.42%. Optimum formula probiotic drink resulting from the fermentation of bidara fruit juice with Lactobacillus plantarum InaCC B616 has the potential to be further developed as a probiotic drink that is beneficial for health.

### Conflicts of Interest

The authors declare no conflict of interest.

### Author's Declaration

The authors hereby declare that the work presented in this article is original and that any liability for a claim relating to the content of this article will be borne by them.

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