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Development of probiotic fermented smoothie made from "Loka Pere" endemic banana of West Sulawesi

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ABSTRACT

Functional food products play a crucial role in maintaining human health. One such functional food product that benefits digestive health is probiotic products. Banana "Loka Pere" can be the primary raw material for probiotic products. The monosaccharide and fiber content in the banana "Loka Pere" can serve as substrates for probiotic bacteria. A probiotic fermented smoothie made from banana "Loka Pere" is one such example of a probiotic product. Smoothies were chosen for their superior fiber composition, which remains intact due to the absence of fruit fiber filtration. This study examines and analyzes the growth ability of probiotics through the fermentation process in smoothies made from "Loka Pere" bananas, focusing on the parameters of pH, total acid, and the number of probiotic bacteria. The variables used included variations in probiotic powder (Lacto B and L-Bio) and variations in fermentation time (0 hours, 24 hours, and 48 hours) with three replications. The analysis results showed significant differences in pH, total acid, and probiotic count. The results showed that the use of L-Bio probiotic powder and 48 hours of fermentation time provided the best results, with a pH value of 3.81, a total acid content of 0.69%, and several probiotic bacteria at a log of 7.65 CFU.mL^{-1}.

Keywords:
Banana, Fermented smoothie, Loka Pere, Probiotic

1. Introduction

Societal awareness of the importance of health and functional foods has increased in recent years. One crucial strategy for maintaining digestive health is the consumption of probiotic products. Probiotics are live microorganisms that confer beneficial effects on the host, particularly by maintaining gut microbial balance, when consumed in adequate amounts. The most commonly used probiotics belong to the genus *Lactobacillus*, particularly in dairy products [1]. Probiotic beverages are among the most accessible and widely accepted functional foods owing to their ease of consumption and associated health benefits. Regular consumption of probiotic beverages can support digestive health, enhance immune function, and help prevent or manage several chronic diseases [2,3].

However, most probiotic beverages available in the market are derived from dairy-based ingredients. This poses a limitation for individuals with lactose intolerance, which are unable to digest lactose properly and may experience discomfort such as bloating or diarrhea. Lactose intolerance is a prevalent gastrointestinal condition resulting from insufficient lactase activity, which impairs the digestion of lactose found in dairy products [4]. Common manifestations include abdominal discomfort, bloating, gas, and diarrhea after the intake of lactose-containing foods [5,6]. In response, plant-based alternatives are being explored to diversify probiotic sources and expand accessibility. These alternatives can effectively deliver probiotics and provide additional nutritional and health benefits to the consumer.

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Several studies have reported the successful fermentation of fruits, such as pineapple [7], apple [8], and snake fruit [9], into probiotic beverages. The use of local fruits as raw materials to produce probiotic drinks needs to be developed to increase the diversification of local products. In this study, the fruit used was a local banana cultivar known as Loka Pere (*Musa paradisiaca*), originating from Majene Regency, West Sulawesi. This banana is a local variety found exclusively in the villages of Adolang and Adolang Dhua in the Pamboang District. Nutritional analysis of the banana "Loka Pere" indicates the presence of various minerals, vitamin B6, and macronutrients such as protein, fat, carbohydrates, ash, and dietary fiber. Owing to its carbohydrate and dietary fiber contents of 31.15% and 2.5%, respectively, at full maturity, the banana "Loka Pere" holds potential as a suitable growth substrate for probiotic development. Banana "Loka Pere" is rich in carbohydrates (31.15%) and dietary fiber (2.5%) at full ripeness, making it a potential medium for the growth of Lactic Acid Bacteria (LAB) [10,11]. The banana was prepared as a smoothie and mixed with probiotic microorganisms. Compared to juices, smoothies typically offer greater nutritional benefits due to their higher fiber content, a wider range of phytochemicals, and a more filling texture. They are prepared using the entire fruit, including the pulp or puree, which helps retain dietary fiber and contributes to the overall nutritional value of the beverage [12]. The probiotic strains used in this study comprised *Lactobacillus* spp. sourced from a commercially available powdered probiotic supplement. These bacteria utilize simple sugars, such as glucose and fructose, for energy and benefit from dietary fibers, such as inulin, which act as prebiotics to stimulate their proliferation [13].

Despite its nutritional potential, the banana "Loka Pere" has not yet been utilized as a base ingredient in probiotic beverages. Therefore, this study aimed to evaluate the growth ability of probiotics through the fermentation process in smoothies made from "Loka Pere" bananas based on product characteristics, including pH value, total acid, and number of probiotic bacteria. These findings are expected to support the development of innovative plant-based functional beverages and promote local food diversification using underutilized regional commodities.

2. Methods

2.1. Materials

The materials used in this experiment include ripe banana "Loka Pere", mineral water, and probiotic powder Lacto B and L-Bio (Commercial probiotic supplements). The materials used for analysis included MRS broth media, bacteriological agar No. 1. Calcium Carbonate (CaCO3), Sodium Chloride (NaCl), Aquades, Sodium Hydroxide (NaOH), Standard Oxalic Acid, and Phenolphthalein (PP) indicator.

2.2. Banana "Loka Pere" Smoothie Sample Preparation

To make a Banana "Loka Pere" smoothie, the main ingredients are first prepared, namely ripe bananas and mineral water. The process of making the sample includes peeling the banana skin, cutting it into small pieces, mashing it with a ratio of 1:2 (banana flesh : mineral water) using a blender, packaging it in glass bottles with a volume of 100 mL per bottle, pasteurization at a temperature of $\pm$ 90 °C for 15 minutes using boiling water [14] and cooling it in the refrigerator.

2.3. *Fermentation Process*

The previously prepared banana smoothie samples were subjected to fermentation with the addition of 1 sachet (1 g) of various probiotic powders, namely Lacto B (>Log 9 $CFU.g^{-1}$, strain: *Lactobacillus acidophilus* LA1, *Bifidobacterium longum* BG7, and *Streptococcus thermophilus* ST3) and L-Bio (>Log 8 $CFU.g^{-1}$, strain: *Bifidobacterium lactis* W51, *Bifidobacterium lactis* W52, *Lactobacillus acidophilus* W55, *Lactobacillus casei* W56, *Lactobacillus salivarius* W57, and *Lactococcus lactis* W58). The probiotic powder inoculation process was performed aseptically in a Laminar Air Flow. Subsequently, homogenization was performed using a Vortex Mixer until the sample was evenly mixed with the probiotic powder. After the sample was ready, the fermentation process was continued using an incubator at 37 °C. Fermentation was carried out for 0, 24, and 48 h. The prepared Probiotic Fermented smoothie samples were subjected to the analysis process including pH analysis, total acidity, and the number of probiotic bacteria.

2.4. *Determination of pH Sample*

The acidity of the sample was determined in pH units. The pH value was determined using an ST20 OHAUS Pen pH meter.

2.5. *Total Acid Analysis*

Acid formation during fermentation in the samples was analyzed using titration with a 0.1 N NaOH solution that was standardized using a 0.1 N oxalic acid standard solution. Three drops of 1% phenolphthalein (PP) solution were added to each sample as an indicator. The results of acid formation were expressed as a percentage of the total acid using the following formula:

$$\%\text{Total Acid} = \frac{\text{Titration Volume (mL)} \times \text{NaOH (0.1 N)} \times \text{MW Lactic Acid (90.08 g.mol}^{-1}\text{)}}{\text{Sample Volume (5 mL)} \times 1000} \times 100 \qquad (1)$$

2.6. *Probiotic Cell Count Analysis*

The number of probiotic bacteria was analyzed by growing samples on MRS agar media (mixture of MRS broth media, bacteriological agar no. 1, and Calcium Carbonate [$CaCO_3$]). The samples were diluted using a sterile sodium chloride solution. The dilution process was carried out by taking 1 mL of the sample and diluting it to 10 mL by adding 9 mL of sterile sodium chloride solution. Dilution was carried out up to six dilution series for 0 h fermentation and seven dilution series for 24 and 48 h fermentation. After the sample was diluted, 1 mL was taken from each of the last three dilution series and placed in a Petri dish, which was then poured with sufficient MRS agar media, and a duplication analysis was carried out in this process. After the media hardened, the incubation process continued at 37 °C for 48 h using an incubator. The colonies that grew after incubation were counted and expressed as Log $CFU.mL^{-1}$ [15].

2.7. *Statistical Analysis*

This experiment used a completely randomized design method with three replicates. All data will be statistically analyzed using ANOVA. If the results are significantly different (P-value <0.05), the Least Significant Difference (LSD) test will be continued.

Treatments that are statistically significantly different will be distinguished based on the letter notation.

3. Results and Discussion

3.1. pH of Samples

The pH measurements showed a decrease in pH in each sample using Lacto B probiotic powder (Figure 1A) and L-Bio probiotic powder (Figure 1B) with increasing fermentation time. In the Lacto B sample (Figure 1A), the pH value decreased from 5.20 (0 h) to 4.12 (48 h), whereas in the L-Bio sample (Figure 1B), the pH value decreased from 5.04 (0 h) to 3.81 (48 h). The results of the interaction between Lacto B and L-Bio samples (Figure 1C) showed that the L-Bio sample at a fermentation time of 48 h had the lowest pH value of 3.81. In another study that used snake fruit juice as a substrate for the probiotic bacteria strain *Lactiplantibacillus plantarum* subsp. *plantarum* Dad-13 in the fermentation process, the results showed a decrease in pH during the 24 h fermentation process from 4.13 to 3.77 [9].

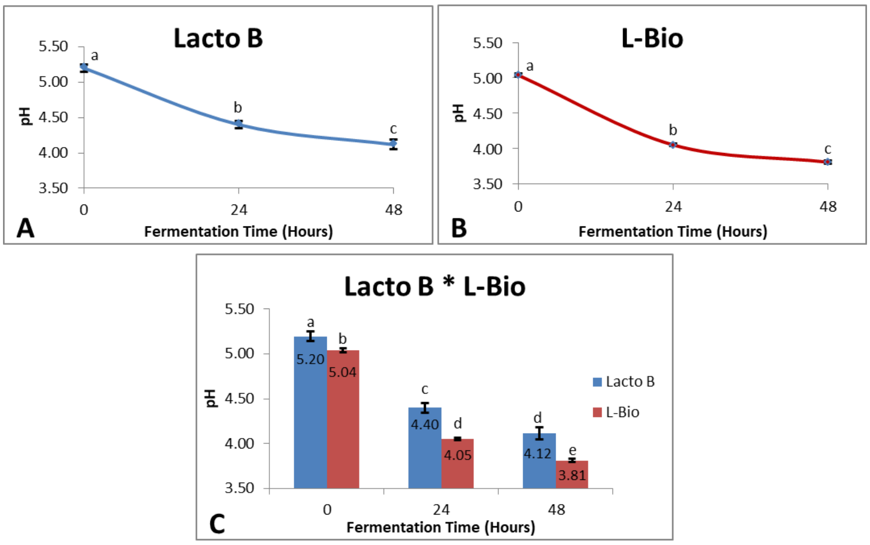

Figure 1. pH evaluation during fermentation process. (A) Lacto B probiotic powder, (B) L-Bio probiotic powder, and (C) Interaction of the two probiotic powders. Different letters showed significantly different (p-value <0.05)

In this study, the decrease in pH during fermentation was caused by the accumulation of metabolites produced by probiotic bacteria [16]. The metabolites produced can be organic acids because the bacterial content in the Lacto-B and L-Bio probiotic powders was dominated by *Lactobacillus* spp. [17]. *Lactobacillus* spp. can use monosaccharides and fiber contained in the sample as substrates during fermentation and produce organic acids as metabolites [18]. The acidic characteristics of organic acids reduce the pH of the sample during fermentation [19]. A variety of organic acids are produced depending on the microbial species, substrates, and fermentation conditions. Although lactic acid is often the main product, other acids, such as acetic [20], butyric [21], propionic [22], and caproic acids, can also be significant [23].

3.2. *Total Acid of Samples*

Acid formation during fermentation can be detected using titration. The titration results for each are expressed as the% Total Acid. During fermentation, the % Total Acid increased in each sample using Lacto B probiotic powder (Figure 2A) and L-Bio probiotic powder (Figure 2B) with increasing fermentation time. In the Lacto B sample (Figure 2A), there was an increase in the Total Acid content from 0.20% (0 h) to 0.47% (48 h), while in the L-Bio sample (Figure 2B), there was an increase in the Total Acid content from 0.18% (0 h) to 0.69% (48 h). The results of the interaction between the Lacto B and L-Bio samples (Figure 2C) showed that the L-Bio sample showed the largest acid formation, namely 0.69% at a fermentation time of 48 h, when compared to other samples. In the probiotic fermented drink from snake fruit juice, there was also an increase in total acid during the 24 h fermentation process from 0.26% to 0.33% [9].

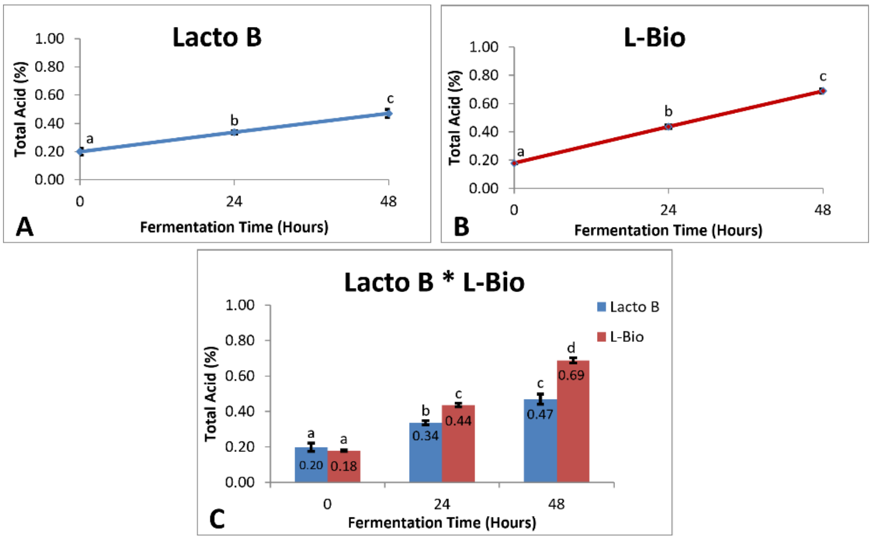

Figure 2. Total acid evaluation during fermentation process. (A) Lacto B probiotic powder, (B) L-Bio probiotic powder, and (C) Interaction of the two probiotic powders. Different letters showed significantly different (p-value <0.05)

In this case, the organic acids formed during fermentation were dominated by lactic acid because the probiotic powders used (Lacto-B and L-Bio) contained *Lactobacillus* spp. bacteria. *Lactobacillus* spp. are lactic acid bacteria that can produce organic acids, namely lactic acid, during fermentation [24]. The formation of acid in fermented products can provide a unique taste characteristic compared with other products. Acid-fermented products are appreciated for their soft sourness, freshness, and complex aromas, which can mask off-flavors and enhance consumer acceptance [25,26].

3.3. *Probiotic Cell Amount of Samples*

The number of probiotic bacteria in each sample was expressed in Log CFU.mL^{-1} units. During the fermentation process, the results showed that the sample with Lacto B probiotic powder (Figure 3A) showed an increase in the number of bacteria from 6.84 Log CFU.mL^{-1} (0 h) to 7.60 Log CFU.mL^{-1} (24 h) and then decreased to 48 h fermentation to 7.22 Log CFU.mL^{-1}. The number of bacteria in the sample with L-Bio probiotic powder (Figure 3B) showed an increase in the number of bacteria from log

5.98 CFU.mL^{-1} (0 h) to log 7.71 CFU.mL^{-1} (24 h), and then remained stable at 48 h fermentation, namely log 7.65 CFU.mL^{-1}. The interaction between the two (Figure 3C) shows that the highest number of bacteria was observed in the Lacto B 24 h (Log 7.60 CFU.mL^{-1}), L-Bio 24 h (Log 7.71 CFU.mL^{-1}), and L-Bio 48 h (Log 7.65 CFU.mL^{-1}) samples compared to other samples because the statistical notation of these three samples did not show a significant difference. Most regulatory and scientific bodies recommend a minimum of 7 CFU.g^{-1} or CFU.mL^{-1} at the point of consumption to ensure that probiotics confer health benefits. This is based on evidence that lower counts may not survive gastrointestinal transit or exert measurable effects [27].

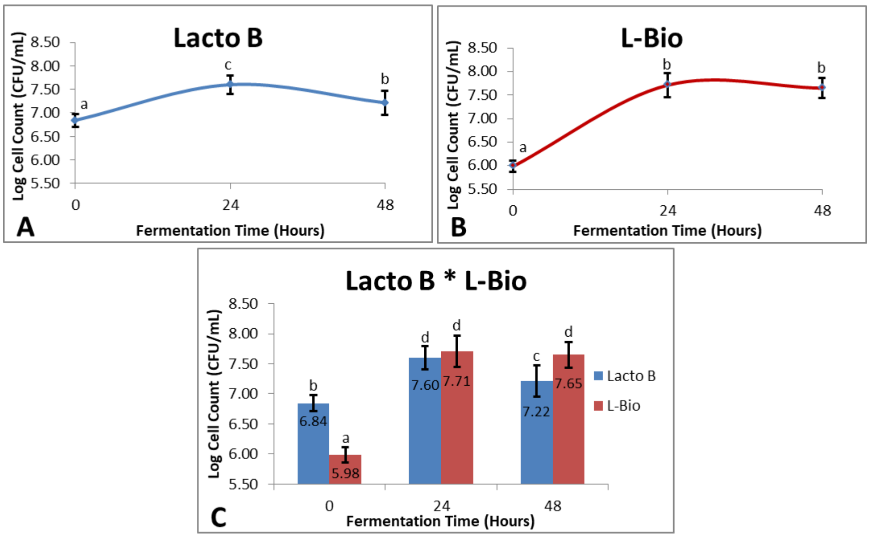

Figure 3. Probiotic cell amount evaluation during fermentation process. (A) Lacto B probiotic powder, (B) L-Bio probiotic powder, and (C) Interaction of the two probiotic powders. Different letters showed significantly different (p-value <0.05)

In the Lacto B sample, there was a decrease in the number of bacteria during the 48 h fermentation time, which could be caused by competition in utilizing the substrate in the sample, so that bacteria that do not get the substrate will die. This was caused by the initial bacterial content of the Lacto-B sample (0 h fermentation) as Log 6.84 CFU.mL^{-1}, being greater than the L-Bio sample (Log 5.98 CFU.mL^{-1}). If the number of bacteria is greater than the substrate, the fermentation process will not be optimal; conversely, if the substrate is abundant compared to the number of bacteria, bacterial growth will be good, and fermentation will run optimally [28].

The success of the fermentation process can be observed from the increasing or stable number of bacteria. This can be seen in the L-Bio sample, whose bacterial count was stable at 24 h and 48 h of fermentation due to the initial bacterial count (0 h fermentation) being lower than that of the Lacto B sample. Lactic acid bacteria, especially *Lactobacillus*, consume simple sugars, such as fructose and glucose, as substrates during fermentation [29]. The simple sugar and fiber content in the banana "Loka Pere" smoothie is used as a substrate for the growth of probiotic bacteria during the fermentation process. In another study by [30], banana powder (*Musa acuminata* Colla) from China was fermented in vitro for 24 h using fecal samples from six Chinese donors. The banana polysaccharides gradually degraded, reaching approximately 80% degradation. The production of short-chain fatty acids (SCFAs) was also measured, and the results showed that the addition of banana powder increased the concentrations of organic acids (acetate, propionate, and butyrate), with acetate being produced in higher amounts than propionate and butyrate.

4. Conclusion

The results of this study indicate that "Loka Pere" Banana Smoothie can be a substrate for probiotics to support their growth through fermentation. The banana "Loka Pere" Probiotic Fermented Smoothie experienced a decrease in pH, an increase in total acid, and an increase in the number of probiotic bacteria during the fermentation process. The sample using L-Bio probiotic powder with a fermentation time of 48 h gave the best results because it had the lowest pH value (3.81) and the highest total acid content (0.69%). The number of probiotic bacteria was also stable at Log 7.65 CFU.mL^{-1}, thus providing health benefits when the product was consumed. This study is basic research that will serve as a reference for further studies. In the future, research will be conducted on formulations with organoleptic analysis and stability testing with storage tests to ensure compliance with commercial product standards.

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