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Enhancing Sourdough Starter Using Ambon Banana Fermentation: A Comparative Study of Type I and Type III Starters Focusing on Lactic Acid Bacteria and Quality

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Abstract

There are several main indicators that can be used to assess the quality of sourdough starter, namely pH, dough volume, and the presence and diversity of Lactic Acid Bacteria (LAB). Meanwhile, the addition of natural ingredients such as fermented Ambon bananas is considered to be able to improve the quality of sourdough starters, by increasing the number and diversity of LAB. This study is a comparative exploratory study that compares the quality of sourdough starters made with fermented Ambon bananas (Type III) and those made without Ambon bananas (Type I), and identifies the diversity of LAB in both. This study showed that all parameters are better in type III than in type I, where the pH is lower, the dough develops faster and has a larger volume, and the number of LAB is higher. Meanwhile, the LAB contained in Type III is more diverse (*Lactobacillus pentosus*, *Lactobacillus plantarum*, and *Lactobacillus paraplantarum*) compared to Type I, which only contains one isolate (*Lactobacillus pentosus*). Based on the results found, sourdough with the addition of Ambon banana fermentation can produce better starter quality and contains more diverse LAB, namely heterofermentative and homofermentative.

Keywords: sourdough starter, fermented ambon banana fruit, lactic acid bacteria.

A. Introduction

The global sourdough market reached USD 2.45 billion in 2024 and is projected to increase to USD 3.30 billion by 2029 (Islam & Islam, 2024). Meanwhile, the results of a survey in Indonesia conducted by various producers in the bread, cake, and chocolate industries also indicate a

significant increase in interest in sourdough bread (Fuzawati et al., 2024). The growth of the sourdough bread industry globally and in Indonesia is driven by increased awareness of the importance of consuming healthy, natural, and preservation-free products (Santos et al., 2024). In other words, several benefits associated with sourdough consumption—such as slowing starch digestion, reducing glycemic response, increasing protein digestibility, enhancing mineral absorption, and supporting postbiotic and antioxidant activity, as well as lowering FODMAPs (Fermentable Oligosaccharides, Disaccharides, Monosaccharides, and Polyols)—have become key factors in increasing consumer interest (El Fechtali et al., 2023; Islam & Islam, 2024; Rachmawati et al., 2023).

On the other hand, optimal sourdough quality is determined by monitoring important parameters such as dough volume, pH value, and the number of Lactic Acid Bacteria (LAB) microorganisms (Calvert et al., 2021). Munch-Andersen et al. (2024)explained that the ideal sourdough has a pH range between 3.5 and 4.0. It was further explained that this condition indicates that the LAB community has achieved stability in number and effectiveness of activity. This is also indicated by the volume of dough that can expand to more than twice the initial volume (Rachmawati et al., 2023).

Meanwhile, LAB is the main bacteria in sourdough starter (De Vuyst et al., 2023). The optimal amount of LAB in sourdough ranges from 10^6 to 10^9 CFU per gram (Hernández-Figueroa et al., 2024). In this amount, the starter culture is in a stable condition and leads to an effective fermentation process (Sevgili et al., 2021). Another advantage of the presence of LAB is that pathogenic microbes that cause spoilage are inhibited or cannot grow (Petkova et al., 2021).

Based on the previous explanation, it can be said that the most important sourdough starter parameter is the presence of diverse LAB and the optimal amount. Meanwhile, one of the easiest and safest methods offered so far and has been reported is adding fruit juice (Calvert et al., 2021). Older studies such as those reported by Zhang et al. (2017) also stated that the addition of fruit juice can accelerate the dominance of lactic acid bacteria so that it can accelerate the decrease in the pH of the sourdough environment. Strengthened by recent research that among various fruits, fermentation of Ambon bananas for seven days is known to have the highest potential to create a sourdough environment dominated by LAB (Fuzawati et al., 2024).

Considering the primary components of Ambon bananas (Musa acuminata), Wulandari et al., (2018) stated that in 100 grams there are 24.72 g of carbohydrates, 72.28 g of water, and 1.02 g of protein. The high carbohydrate composition can support the growth of LAB (Finanda et al., 2021). However, empirically, research related to the use of Ambon banana fermentation for sourdough starters still focuses on pH parameters and the amount of LAB (Fuzawati et al., 2024), while the identification of LAB species is still limited.

In other words, previous studies have also not compared the performance of starter fermentation as a whole. Thus, further research is needed that not only assesses physical and microbiological parameters, but also reveals the diversity of LAB species that play a role in sourdough fermentation. Thus, this study was conducted to fill this gap.

This study aims to evaluate the quality and types of LAB in sourdough starter with the addition of Ambon banana fermentation (Type III), and compare it with sourdough starter without the addition (Type I). This research is expected to provide practical contributions in the development of functional starters based on fermented local fruits, and scientifically can be an additional reference that strengthens the explanation of natural fermentation in flour-based food products.

B. Literature Review

1. Lactic Acid Bacteria (LAB)

LAB are Gram-positive bacteria, and in general, these bacterial colonies have a characteristic circular shape (Nasution et al., 2020). LAB ferment carbohydrates (glucose, maltose, and fructose) available in flour into lactic acid and acetic acid. These acidic compounds cause a gradual decrease in pH, creating an increasingly acidic environment that is conducive to the growth of acidity-tolerant microbes, such as *Lactobacillus* and *Pediococcus* (Hernández-Figueroa et al., 2024; Lafuente et al., 2023). Based on the type of fermentation, LAB can be categorized into two types, namely heterofermentative and homofermentative. Heterofermentative LAB can produce more than one by-product during fermentation, namely lactic acid, acetic acid, ethanol, and CO_2. Meanwhile, homofermentative LAB only produce one by-product, namely lactic acid. Heterofermentative metabolism contributes more to sourdough volume compared to homofermentative LAB species. Optimal sourdough starter is dominated by heterofermentative LAB (Calvert et al., 2021).

2. Sourdough Types

Sourdough can be classified into three types, namely Type I, Type II, and Type III. Type I sourdough refers to traditional sourdough that requires continuous multiplication (backslopping). This is done by refreshing it using fresh flour and water periodically (Lau et al., 2021). Type I avoids the addition or inclusion of LAB in sourdough fermentation (Islam & Islam, 2024). (Islam & Islam, 2024). Type II sourdough inoculates the culture as a dough acidifier. Type II is a fermentation process that starts from an acid-tolerant starter culture and is then accelerated at a controlled temperature of 30–37°C. This method can increase acidification and/or target other sourdough bacteria. This process takes one to three days and often reaches a pH value lower than 4.0 (De Vuyst et al., 2023). Finally, the Type III sourdough production process is a combination of the Type I and Type II sourdough production processes. Fermentation is initiated from a starter culture of a mixture of flour and water (as in Type II), followed by daily replenishment (as in Type I) (De Vuyst et al., 2023).. Type III sourdough is usually dried for easier storage and utilization (Lau et al., 2021).

C. Methodology

1. Research Design

This study is a comparative exploratory study to evaluate the quality of sourdough starter, including pH value, volume, total plate count (TPC) of lactic acid bacteria (LAB), and identification of the types of LAB formed. Comparisons were made between two types of sourdough, namely type I (without the addition of Ambon banana fermentation) and type III (with the addition of Ambon banana fermentation). This study did not use inferential statistical analysis but focused on descriptive comparisons to provide an initial understanding of the effect of the addition of Ambon banana fermentation on the quality of sourdough starter and microbiological characteristics.

2. Materials

The tools used in this study were: autoclave, Bunsen burner, scissors, tweezers, glass jars, measuring cylinders, loop needles, Erlenmeyer flasks, analytical scales, Petri dishes, hot plates, incubators, object glasses and cover glasses, test tubes, test tube racks, microscopes, Laminar Air Flow (LAF), refrigerators, micropipettes, blue tips, yellow tips, magnetic stirrers, digital pH meter, Styrofoam boxes, and vortex mixers. The materials used were as follows: Ambon banana, high-protein wheat flour, mineral water, granulated sugar, 13.64 g of MRS Agar media (De Man, Rogosa, and Sharpe Agar), 5.51 g of MRS Broth media (De Man, Rogosa, and Sharpe Broth), Ringer's solution, $CaCO_3$ (calcium carbonate), 3% H_2O_2 (hydrogen peroxide), Gram crystal violet dye, iodine, safranin, distilled water, 70% alcohol, aluminum foil, plastic wrap, cotton, plastic, rubber, tissue, and gauze.

3. Procedures and Data Collection

The work procedures carried out in this study consisted of several stages, namely: (1) fermentation of Ambon bananas for 7 days; (2) sourdough fermentation for 15 days; (3) The measurement of the volume of the sourdough starter sample was carried out by referring to the procedure adapted from Sevgili et al. (2021). The sourdough sample was placed in an 800 mL jar with a diameter of 9 cm, then incubated for four hours at a temperature of 27-28°C. After incubation, the volume of the expanded dough was measured using the cylinder volume formula, namely V= πxr^2xt), pH, and LAB TPC, conducted in duplicate; and (4) LAB identification, which was carried out by observing bacterial colonies macroscopically and microscopically (Gram staining). Biochemical tests were also conducted, namely catalase tests and fermentation type tests (gas production from glucose). In addition, molecular observations were performed using the MALDI-TOF method, referring to Landis et al. (2021).

Identification of LAB species was performed using VITEK® MS (bioMérieux, France) mass spectrometry based on Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF). One pure colony was transferred and spotted directly onto the VITEK® MS target plate. After drying, 1 µL of α-cyano-4-hydroxycinnamic acid (CHCA) matrix dissolved in a mixture of formic acid and acetonitrile was added according to the manufacturer's protocol, then dried again at room temperature. The plate was then inserted into the VITEK® MS instrument for ionization and mass spectrum analysis. The resulting protein spectrum was compared with the VITEK® MS IVD (in vitro diagnostic database) database for species identification.

4. Data Analysis

Data related to sourdough quality (volume, pH, and TPC LAB) were analyzed descriptively and quantitatively. Data related to the identification of LAB in sourdough (macroscopic, microscopic, biochemical, and molecular observations) were analyzed descriptively and qualitatively.

D. Findings and Discussion

1. Findings

a. Sourdough Starter Quality

Sourdough with the addition of Ambon banana fermentation (Type III) has been proven to improve the quality of the resulting sourdough. The improvements include an increase in sourdough volume, a higher total number of LAB detected through TPC, and a lower pH value compared to sourdough without the addition of Ambon banana fermentation water (Type I). Type III showed a faster increase in volume, from the 13th day (209.83 cm^3) to the 15th day (412.3 cm^3). Meanwhile, Type I sourdough experienced a slower increase in volume, from the 14th day (209.83 cm^3) to the 15th day (241.63 cm^3) (Figure 1).

Sourdough with the addition of Ambon banana fermented water (Type III) produced a higher number of lactic acid bacteria colonies, reaching 7.75×10^9 CFU/g, compared to sourdough without the addition of banana fermentation (Type I), which only reached 6.55×10^8 CFU/g. In addition, the addition of Ambon banana fermented water (Type III) also caused a more significant decrease in pH value, from pH 5.73 on the first day to pH 3.69 on the 15th day. In contrast, in sourdough without the addition of Ambon banana fermented water (Type I), the pH value decreased from 6.56 to 4.00 on the 15th day (Figure 2).

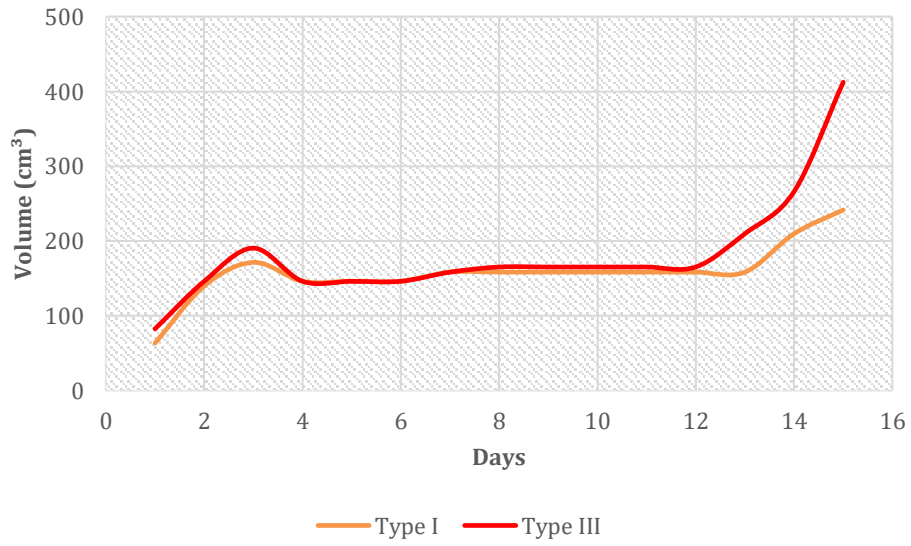

Figure 1. Comparison of Volume Changes in Type I and Type III Sourdough Starters During 15 Days of Fermentation

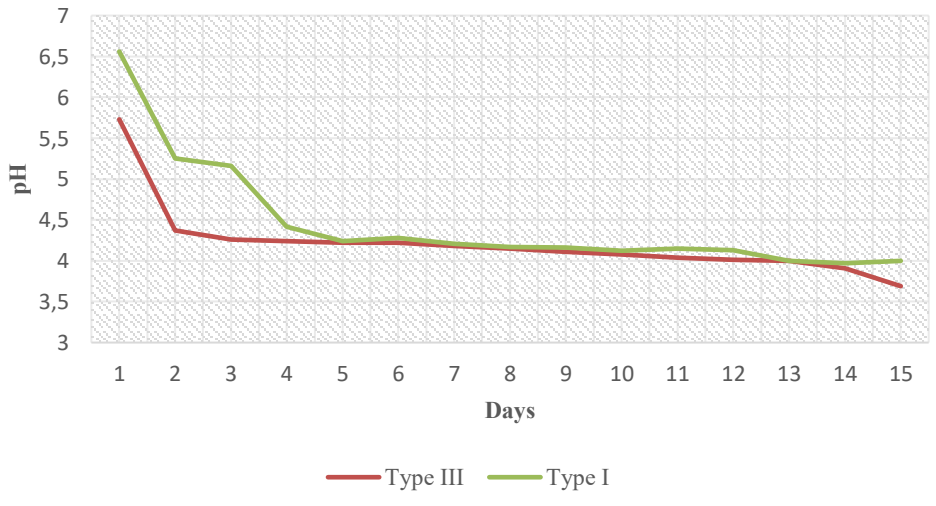

Figure 2. Comparison of pH Changes in Type I and Type III Sourdough Starters During 15 Days of Fermentation

b. Identification of Lactic Acid Bacteria

Macroscopically, the LAB observed showed a round morphology, milky white color, with a diameter ranging from 1–1.2 mm and flat colony edges. Microscopic identification using the Gram staining technique showed that all LAB colonies were Gram-positive and had rod-shaped cell morphology (bacilli) (Figure 3). The results of biochemical tests on catalase enzyme activity showed that all isolates did not produce bubbles, indicating that the isolates were catalase-negative, namely SD-1 and SD-2 from Type I, and SDP-1, SDP-2, and SDP-3 from Type III.

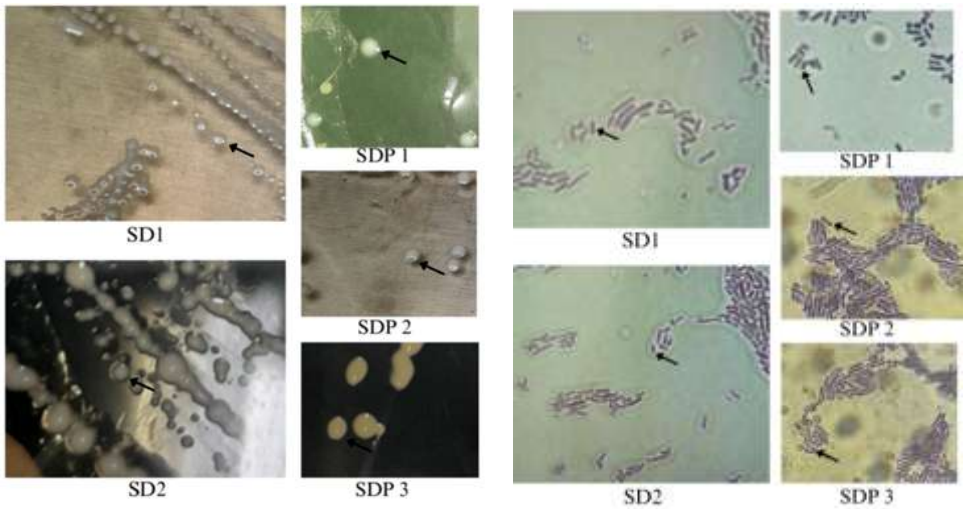

Figure 3. Macroscopic and Microscopic Characteristics of Lactic Acid Bacteria (LAB) Colonies Isolated from Sourdough Starter Types I and III

Based on the fermentation type test, isolates SD-1, SD-2, and SDP-2 were identified as heterofermentative, while isolates SDP-1 and SDP-3 belonged to the homofermentative group (Table 1). Molecular identification using the Matrix-Assisted Laser Desorption/Ionization Time-of-Flight (MALDI-TOF) method revealed three LAB species identified from the sourdough microbiota: *Lactobacillus pentosus*, *Lactobacillus plantarum*, and *Lactobacillus paraplantarum*.

Table 1. Biochemical Test Results of Lactic Acid Bacteria (LAB) Isolated from Sourdough

Isolate code	Catalase	Fermentation Type	LAB Species
SD 1	Negative	Heterofermentative	*Lactobacillus pentosus*
SD 2	Negative	Heterofermentative	*Lactobacillus pentosus*
SDP 1	Negative	Homofermentative	*Lactobacillus plantarum*
SDP 2	Negative	Heterofermentative	*Lactobacillus pentosus*
SDP 3	Negative	Homofermentative	*Lactobacillus paraplantarum*

2. Discussion

The main objective of this study was not only to compare the quality of the sourdough starter but also to identify differences in LAB types between two different types of sourdough. The first finding in this study was that the volume of sourdough with the addition of Ambon banana fermented water (Type III) increased to twice its original volume, while sourdough without the addition of Ambon banana fermented water (Type I) had not reached twice its volume by the 15th day. The addition of Ambon banana fermented water, which contains natural sugars such as glucose and fructose, acts as a source of nutrients for LAB in the sourdough dough (Martiningsih et al., 2016). The availability of fructose-based sugars, such as sucrose and fructan, can support fermentation activity, thereby producing CO_2 gas, which affects the volume development of sourdough (Islam & Islam, 2024).

The two-fold increase in the height of both types of sourdough on the first and second days occurred due to the initial fermentation activity by microorganisms originating from flour and water—*Enterococcus*, *Lactococcus*, and *Leuconostoc*—which metabolize simple sugars from the flour to produce large amounts of CO_2 gas, causing the dough to rise rapidly (McKenney et al., 2023). These microorganisms grow rapidly in the early stages of fermentation because the environmental conditions are still neutral and there is no dominance by other microbes. However, over time, and with the decrease in pH due to LAB activity, they are displaced by microbes that are more resistant to acidic conditions (Landis et al., 2021).

Furthermore, on the fourth day, after the backslopping process (removing part of the dough and adding new flour and water), there was a decrease in dough height due to the dominance of LAB such as *Lactobacillus* and *Pediococcus*, which produce organic acids. The increasingly acidic environment reduces the activity of gas-producing microbes and inhibits dough development (McKenney et al., 2023). Then, on the 13th to 15th day, an increase in dough volume occurs as fermentation enters the stabilization phase, with *Lactobacillus* dominance. The LAB population reaches an optimal point, dominated by strains most adaptive to acidic conditions. The pH tends to stabilize in the range of 3.5–4.0, indicating that the microbial ecosystem has reached equilibrium (Munch-Andersen et al., 2024). This change is part of the natural microbial succession process toward a more stable and distinctive climax community in the sourdough starter (McKenney et al., 2023).

The second finding is that the decrease in pH in sourdough occurs alongside microbial development during the fermentation process. On the first to second day, fermentation enters the initiation phase, where microorganisms from the flour and the environment begin to grow. At this stage, the pH is still relatively high (around 6.5–5.5), so organic acid production is still limited (Munch-Andersen et al., 2024). Entering the third to fourth day, fermentation becomes more active with an increase in the LAB population. Increased LAB activity produces lactic acid and acetic acid, causing a decrease in pH and creating a more acidic environment. From the fifth to the 15th day, fermentation enters the stabilization phase with *Lactobacillus* dominance. The dominant LAB indicates that this type is the most able to adapt to acidic environments. Munch-Andersen et al. (2024) explained that pH in the range of 3.5–4.0 is the optimal condition for LAB to achieve a balanced condition.

The third finding, increasing LAB is related to decreasing pH. As shown in the literature review, Ambon bananas have high carbohydrates and LAB utilize them as a carbon source. The result of metabolism is high acid which results in a fairly low pH decrease (Fuzawati et al., 2024). Meanwhile, the addition of Ambon banana fermentation water plays a role in providing LAB as well as additional nutrients so that fermentation conditions run more ideally and effectively. The findings indicate a fairly strong relationship between the three parameters observed. The addition of fermentation water increases the number and type of LAB, and ultimately more lactic acid is produced. Higher lactic acid can also have an impact on decreasing pH. A fairly low and optimal pH supports the gas formation process during fermentation so that the volume of the dough increases. These comprehensive results are supported by previous studies which stated that an increase in LAB TPC is an indication of high fermentation activity which then has an impact on improving the quality of the bread produced (Hernández-Figueroa et al., 2024)

The fourth major finding is that three species of lactic acid bacteria (LAB) identified from the sourdough microbiota were *Lactobacillus pentosus*, *Lactobacillus plantarum*, and *Lactobacillus paraplantarum*, consistent with several previous research reports (Arora et al., 2021; De Vuyst et al., 2023; Nataresa & Kuntariati, 2023). The confidence value of identification is calculated automatically by the system, with a threshold of ≥90% as the standard for the validity of species-level identification. In this study, the three identified LAB species showed a confidence value of 99.9%, indicating a very high level of match to the reference and can be confirmed as a valid species identification.

In general, sourdough is inhabited by fewer than three different LAB species, consisting of both homofermentative and heterofermentative *Lactobacillus* (De Vuyst et al., 2023). Sourdough without the addition of Ambon banana fermentation water (Type I) produced a heterofermentative LAB, namely *Lactobacillus pentosus*. Meanwhile, sourdough with the addition of Ambon banana fermented water (Type III) produced LAB with a more diverse fermentation type, consisting of *Lactobacillus pentosus* (heterofermentative), and *Lactobacillus plantarum* and *Lactobacillus paraplantarum* (both homofermentative). Sourdough is usually inhabited by less than three dominant LAB species (De Vuyst et al., 2023). The finding of three species in type III is still within the upper range of the common range, namely one to three dominant species in a stable sourdough ecosystem. The increase in the number of LAB species in type III is due to the presence of additional microbial inoculum from Ambon banana fermentation water, which can enrich the initial microbial community and encourage species diversification.

Although the addition of Ambon banana fermented water increased the types of LAB that developed in the sourdough, this study also produced slightly different results from previous findings. Earlier studies reported the presence of other LAB species considered dominant representatives of the sourdough microbiota, namely *Fructilactobacillus sanfranciscensis* and *Lactobacillus brevis* (Corsetti & Settanni, 2007). The absence of *Fructilactobacillus sanfranciscensis* and *Lactobacillus brevis* in all sourdough samples isolated in this study may be

due to their metabolic properties, they can be eliminated due to losing competition with Lactobacillus pentosus (Urshev et al., 2024).

E. Conclusion

The addition of fermented ambon banana water has been proven to improve the quality of sourdough starter (type III) compared to no addition (type I). This can be seen from several important parameters, namely the total amount of BAL (TPC) which is greater in type III. Sourdough quality is also indicated by chemical parameters such as lower pH and larger and faster dough volume. Other parameters, sourdough starter type III contains more diverse types of Lactobacillus, namely Lactobacillus pentosus (heterofermentative), Lactobacillus plantarum, and Lactobacillus paraplantarum (homofermentative), while sourdough starter (type I), only one species was found, namely Lactobacillus pentosus.

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