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Fermentation of Gembili Juice: Innovation in Functional Beverages Based on *Lactobacillus plantarum* B1765

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Abstract: *Gembili* tubers (*Dioscorea esculenta*) are abundant in Indonesia but remain underutilised despite their potential as a functional food. One effort that has been made is to produce a symbiotic fermented beverage, as it is known to contain prebiotic inulin that can be fermented using probiotics. *Lactobacillus plantarum* B1765 is charactherized by its probiotic potential. This research evaluated the impact of fermentation duration using L. plantarum B1765 starter culture on the quality of gembili juice products, including Total Lactic Acid Bacteria (LAB), pH, Total Titratable Acids (TTA), and organoleptic quality. *Gembili* juice was made by fermenting *gembili* juice at 37°C for 0, 6, 12, 18, 24, and 36 hours. The total LAB count was enumerated using the total plate count (TPC) method with MRS-Agar medium containing CaCO3. pH determination was performed using a pH meter. TTA was calculated by acid-base titration, and organoleptic quality was assessed by untrained panellists, who rated the level of liking using a hedonic scale. The total LAB and pH data were analyzed using the Kruskal–Wallis test, while the TTA data were analyzed using a one-way ANOVA to determine the significant differences among the fermentation durations. The fermentation time of *gembili* fermented beverages had an effect ($p < 0.05$) on the total number of LAB, pH, and TTA. LAB growth increased and reached an optimum of 7.55×10^8 CFU/mL at 18 hours. However, the pH continued to decrease to 3.84, and the TTA continued to increase to 0.32% until the end of fermentation. Sensory evaluation (hedonic test) showed that fermentation time had no effect ($p>0.05$) on color and aroma. Still, it had an impact ($p < 0.05$) on taste, with the highest preference for the 18-hour fermentation. This product complies with SNI standards and is suitable for development as a fermented functional beverage. The inulin and phenolic compounds present in gembili may contribute to its antioxidant activity and α-glucosidase inhibitory effects.

Keywords: *Gembili*; Inulin; *Lactobacillus plantarum* B1765; Product Quality; Symbiotic Fermented Beverage.

Introduction

Gembili (*Dioscorea esculenta*) is a tuber crop from the Dioscorea genus. That has long been cultivated in several regions of Indonesia, including Java, Maluku, and Papua. Traditionally, it is consumed by boiling, roasting, or cooking in vegetable dishes. Gembili serves as an alternative carbohydrate source and has potential for both medicinal and industrial applications, such as starch production [1,2]. Despite its availability and nutritional value, *gembili* remains underutilized, especially in the development of functional food products. One promising direction is its use in symbiotic fermented beverages, as *gembili* is a natural source of inulin, a prebiotic that supports the growth of probiotic microorganisms.

Research on *gembili* fermentation has been relatively limited and fragmented. Previous studies have examined *gembili* tuber flour as a source of fructooligosaccharides (FOS) [3], fermented *gembili* juice with yeast to determine ethanol production [4], and *gembili* milk fermentation using mixed starter cultures (*Lactobacillus acidophilus*, *Streptococcus thermophilus*, and *L. plantarum*), which demonstrated a 43.59% reduction in blood glucose levels in vivo [5]. While these studies suggest potential health benefits, most have focused on flour-based fermentation or ethanol generation, leaving a gap in exploring *gembili* juice as a probiotic-rich, functional beverage.

Therefore, this study conducted *gembili* fermentation in the form of fermented *gembili* juice using the LAB *L. plantarum* B1765 starter culture, and its product quality was evaluated as the novelty of this research. The nutritional composition of *gembili* strongly supports lactic acid fermentation. Its starch and inulin contents provide fermentable substrates for lactic acid bacteria (LAB), which can metabolize them into organic acids, reducing pH and shaping sensory characteristics such as flavor, aroma, and color [6]. *Gembili* contains approximately 14.77% inulin [7], which can be hydrolyzed by inulinase into FOS, glucose, and fructose. These compounds are subsequently metabolized into short-chain fatty acids (SCFAs), contributing to a lower pH and potential health benefits [8–11]. *Lactobacillus plantarum* B1765, in particular, has been shown to exhibit inulinase activity (0.047 U/mL), with peak activity occurring during the logarithmic growth phase at approximately 12 hours [12,13].

Based on these considerations, this study investigates the effect of fermentation duration using *L. plantarum* B1765 on the quality of *gembili* juice. Parameters assessed include LAB count, pH, total titratable acidity (TTA), and sensory properties. By focusing on *gembili* juice fermentation, this work introduces a novel symbiotic beverage concept and contributes to the broader utilization of underexplored tuber crops in the functional food sector. To date, no scientific reports have been published on the fermentation of gembili

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juice using Lactobacillus plantarum B1765 as a starter culture, making this study a pioneering investigation in the development of locally sourced functional fermented beverages.

Research Methods

Preparation of Starter Culture

Starter culture of *L. plantarum* B1765 was prepared from a bekasam isolate grown in sterile MRS Broth (autoclaved at 121°C, 15 Psi, 15 min). An initial inoculum of 1 mL of culture was transferred into 9 mL of sterile MRS Broth and incubated at 37°C for 20 hours. Subsequently, 1 mL of this culture was inoculated into 9 mL of fresh MRS Broth and incubated for 24 h at 37°C. Cells were harvested by centrifugation (3500 rpm, 5 min), washed twice with 0,85% NaCl solution, and resuspended in 10 mL of 0,85% NaCl to prepare the starter culture [14].

Preparation of Fermented Gembili Juice

Fresh *gembili* tubers were peeled, washed, and diced. Approximately ±350 g was blended with distilled water in a 1:5 ratio, filtered through a cloth to obtain juice. The juice was pasteurized at 80 °C for 15 min, cooled, and inoculated with 5% (v/v) *L. plantarum* B1765 starter culture. Fermentation was conducted at 37 °C for 0, 6, 12, 18, 24, and 36 h [9].

Enumeration of Total LAB

Total LAB were enumerated using the Total Plate Count (TPC) method with MRS agar supplemented with 1% $CaCO_3$ media. Serial dilutions (10^{-1}-10^{-8}) were prepared in 0.85% NaCl, then incubated at 37°C for 48 hours. Colonies characterized by a clear zone were quantified, and the data were reported as CFU/mL[15].

Measurement of pH and TTA (Total Titratable Acids)

The pH of approximately 20 mL of the sample was measured using a calibrated pH meter. TTA was determined by the acid-base titration method. For titration, 10 mL of the sample was diluted to 100 mL, then 20 mL aliquots were titrated with 0.1 N NaOH using phenolphthalein as an indicator until a persistent pink color appeared [16].

Sensory Evaluation

Organoleptic tests (aroma, taste, and color) were evaluated using a hedonic test; untrained panellists rated each attribute on a 5-point scale, where 1 = dislike very much to 5 = like very much.

Results and Discussion

Growth of Total LAB, pH, and TTA

Statistical analysis using the Kruskal-Wallis test revealed that fermentation time had a significant effect on total LAB counts ($p < 0.05$). Post Hoc Mann-Whitney analysis confirmed a significant difference ($p<0.05$) between 0 and 18 hours of fermentation time, while no further differences were observed in LAB at 24 and 36 hours. Figure 1 presents the total LAB, pH, and TTA of fermented gembili juice.

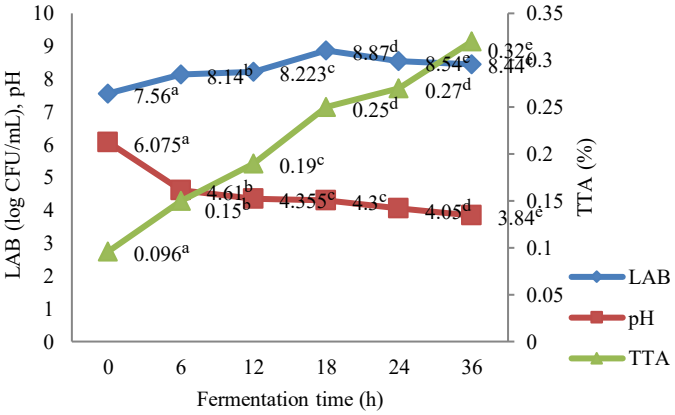

Figure 1. Graphic of total LAB, pH, dan TTA

Optimal LAB growth occurred at 18 hours, reaching the log phase with a 1 log cycle increase from the start of fermentation and achieving 7.55 × 10^8 log CFU/mL. This was followed by a stationary phase at 24-36 hours, with counts of 3.47 x 10^8 log CFU/mL and 2.8 x 10^8 log CFU/mL, respectively. The log phase was supported by nutrient availability in the *gembili* juice medium, which contains carbohydrates and inulin, which is hydrolyzed by the inulinase into glucose and fructose, serving as an energy source for *L. plantarum* B1765. Similar findings were reported in jicama fermentation, where *L. plantarum* B1765 exhibited growth in inulin-rich substrates [14], [17].

Changes in microbial population were closely related to physicochemical parameters. Increasing fermentation time resulted in a decline in pH and an increase in Total Titratable Acidity (TTA). Figure 1 shows that increasing the fermentation time can increase LAB, decrease the pH value, and increase the TTA value. The highest pH (6.07 ± 0.11) was observed at 0 hours and decreased to 3.84 ± 0.05 at 36 hours, while TTA increased from 0.096 ± 0.002 to 0.32 ± 0.009.

Statistical analysis using the Kruskal-Wallis test confirmed that fermentation time had a significant effect on pH ($p < 0.05$), with Post-Hoc Mann-Whitney tests showing a difference ($p < 0.05$) within the 0-12 hour period. Similarly, the One-Way ANOVA results indicated that fermentation time had a significant effect ($p < 0.05$), with Post-Hoc Bonferroni analysis confirming consistent increases across fermentation times. TTA increased from 0.15% ± 0.02 to 0.29% ± 0.03.

The results of this study align with previous study on jicama beverages, which also showed decreasing pH and increasing TTA during fermentation [14], [17], [18]. The observed trends are attributed to the metabolic activity of *L. plantarum* B1765 a facultative heterofermentative bacterium that produces lactic acid and other organic acids.

Organoleptic Characteristics Testing

Organoleptic evaluation provided complementary insights into consumer acceptability. Sensory testing of color, taste, and aroma by 20 panellists indicated that no significant difference ($p>0.05$) in colour and aroma occurred along the fermentation time. The colour parameter at each

fermentation period showed no significant difference, with all samples appearing milky white (Figure 2).

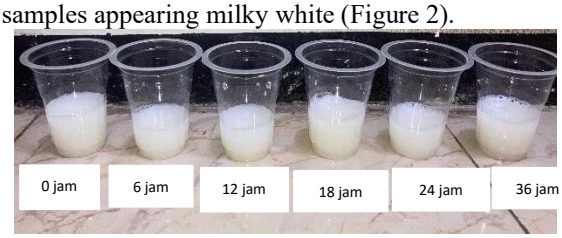

Figure 2. The color of fermented gembili juice at each fermentation period

However, taste was significantly affected ($p<0.05$), with the highest preference at 18 hours of fermentation (Figure 3). Beyond this point, preference declined due to the intensifying sourness associated with further decreases in pH and increases in TTA. Thus, although fermentation for 24–36 hours produced acceptable microbiological and physicochemical profiles, prolonged acid accumulation reduced sensory appeal. This pattern is consistent with findings in other lactic acid–fermented beverages, where excessive acidification negatively impacts consumer acceptance despite compliance with quality standards

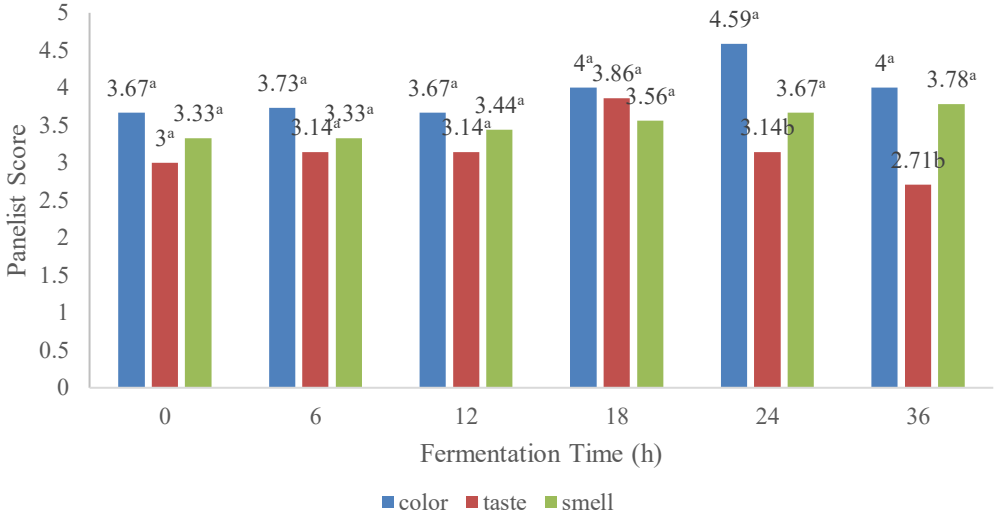

Figure 3. Graphic of organoleptic testing of gembili extract beverages

Based on LAB counts, pH, TTA, and sensory attributes, fermented *gembili* juice at 18 hours of fermentation meets the minimum requirements for fermented products according to SNI 7552:2009 ($\geq 10^6$ CFU/mL live LAB, pH $\leq$ 4.6, and TTA $\geq$ 0.2%) while maintaining optimal consumer acceptability [19-20].

Conclusion

The study showed that the fermentation of gembili juice increased the number of lactic acid bacteria from 10^7 to 10^8 CFU/mL, with optimal growth achieved at 18 hours of fermentation. The pH value decreased to 3.84, while total titratable acidity (TTA) increased to 0.29% at the end of the 36-hour fermentation period. The organoleptic evaluation revealed no significant difference in aroma and color, but the highest preference for taste was observed at 18 hours of fermentation. This product complies with SNI standards and is suitable for development as a fermented functional beverage. The presence of phenolic compounds contributes to increased antioxidant capacity and α-glucosidase inhibition, offering potential health benefits, including protection against oxidative stress and antidiabetic effects. Accordingly, this product holds promise for advancing functional food innovation and promoting the utilization of Indonesia's local carbohydrate-rich resources.

Author's Contribution

Rohilatul Bidayah Amaliyah: responsible for conducting research, collecting data, analysing results, and drafting manuscripts. Prima Retno Wikandari: acted as corresponding author, reviewing and revising article drafts.

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