EFFECT OF FERMENTATION TIME ON THE CHARACTERISTICS OF FERMENTED PINEAPPLE JUICE BY PROBIOTIC (*Lactobacillus casei*)

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ABSTRACT

Diarrhea and digestive tract irritations can be brought on by an imbalance of microorganisms in the digestive tract. Consuming probiotic-containing products can help with this. Beverages with probiotics may be a good way to maintain the proper balance of gut microbiota. Pineapple juice contains potential nutrients as a growth medium for the probiotic *Lactobacillus casei*, thereby producing probiotic drink products. The aim of this research was to determine the effect of fermentation time on the characteristics of pineapple juice probiotic drinks. This study used a Completely Randomized Design (CRD) with 3 treatments and 2 repetitions. The statistical analysis used was one-way ANOVA and Duncan for post-hoc analysis. The treatments in this study were T1 (16 hours), T2 (20 hours), and T3 (24 hours). The results showed that fermentation time significantly influenced pH, total Lactic Acid Bacteria (LAB) and sensory characteristics (taste). The most preferred treatment based on taste was T1 with a pH value of 4.12 and a total LAB of 11.22 log CFU/ml.

Keywords: fermented juice; *Lactobacillus casei*; pineapple; probiotic.

ABSTRAK

Diare dan iritasi saluran pencernaan bisa disebabkan oleh ketidakseimbangan mikroorganisme di saluran pencernaan. Konsumsi produk yang mengandung probiotik dapat membantu mengatasi hal ini. Minuman dengan probiotik dapat menjadi cara yang baik untuk menjaga keseimbangan mikrobiota usus. Sari buah nanas mengandung nutrisi yang potensial sebagai media pertumbuhan probiotik Lactobacillus casei sehingga menghasilkan produk minuman probiotik. Tujuan dari penelitian ini adalah untuk mengetahui pengaruh waktu fermentasi terhadap karakteristik minuman probiotik sari buah nanas. Penelitian ini menggunakan Rancangan Acak Lengkap (RAL) dengan 3 perlakuan dan 2 kali pengulangan. Analisis statistik yang digunakan adalah ANOVA satu arah dan Duncan untuk analisis post-hoc. Perlakuan pada penelitian ini adalah lama waktu fermentasi yaitu T1 (16 jam), T2 (20 jam), dan T3 (24 jam). Hasil penelitian menunjukkan bahwa waktu fermentasi mempengaruhi secara signifikan pada pH, total Bakteri Asam Laktat (BAL), dan karakteristik sensori rasa. Perlakuan yang paling disukai berdasarkan rasa adalah T1 dengan nilai pH 4,12 dan total BAL 11,22 log CFU/ml.

Kata kunci: sari buah fermentasi; *Lactobacillus casei*; nanas; probiotik.

Article Information

Article Type: Research Article
Journal Type: Open Access
Volume: 7 Issue 1

Manuscript ID
V7N11742-2

Received Date
27 Dec 2024

Accepted Date
26 Aug 2025

Published Date
30 Aug 2025

DOI:
10.33555/jffn.v7i1.18

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Citation:
Sahab N. R. M., Khoerunisa N., Hayati F., Ilmiyati L. & Rafika M. 2025. Effect of Fermentation Time on the Characteristics of Fermented Pineapple Juice by Probiotic (*Lactobacillus Casei*). J. Functional Food & Nutraceutical, 7(1), pp 12-19.

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INTRODUCTION

One major health issue that can be brought on by pathogenic bacteria is gastrointestinal problems such as diarrhea. According to WHO, diarrhea is the third highest cause of death in children under 5, and killed 443.832 among them. Diarrhea also killed 50.851 children aged 5-9 years (WHO, 2024). Every year, there are 1.7 billion cases of diarrhea worldwide, which results in malnutrition in children under the age of five. Consuming probiotics can help manage intestinal health issues (Maftei et al., 2024).

Probiotics are live microorganisms that can provide health benefits, especially to the digestive tract, if given in sufficient quantities i.e. 10^8-10^9 (Maftei et al., 2024; Marnpae et al., 2022; Mendonça et al., 2023). Probiotic-containing food can help avoid diarrhea and other digestive disorders by balancing the microbiota in the digestive tract (Dewi et al., 2021). According to Peivasteh-Roudsari et al., 2020, probiotic bacteria produce metabolites in the form of lactic acid, organic acids, fatty acids, hydrogen peroxide and bacteriocins which can control pathogenic bacteria.

Probiotics exist in fermented foods and beverages such as kimchi, yogurt, curd, kefir, sauerkraut, kombucha, miso, pickle, tempeh, and nato (Sahab, 2023). LAB is a probiotic that is often found in food products, especially the genus *Bifidobacterium, Lactobacillus* and *Streptococcus* (Peivasteh-Roudsari et al., 2020). Researchers have recently grown interested in fermented fruit juice because it may potentially be made into a probiotic-rich beverage (Sahab, 2023). Fruit juices like orange, longan, loquat, blueberry, mulberry, and kiwi have been studied to create fermented beverages utilizing lactic acid bacteria cultures like *L. brevis and L. plantarum, L. paracasei, L. fermentium,* dan *L. acidophilus* (Kwaw et al., 2018; Lan et al., 2023; Li et al., 2021; Meng et al., 2022; Wang et al., 2021; Xu et al., 2023).

In Indonesia, pineapple is widely cultivated. According to Pusat Data dan Sistem Informasi (2023), the average pineapple production in Indonesia in 2018-2022 reached 2,149,733 tons. The three provinces in Java that are the largest contributors are Central Java Province 252,07 thousand tons, East Java 217.60 thousand tons, and West Java 213.12 thousand tons. This is not balanced with pineapple consumption, where in 2022 pineapple consumption decreased by 53.71% to 3.82 kg/capita/year which is the lowest consumption since 2010 (Darmawan, 2023). The abundant availability of pineapple in Indonesia is the reason for the author to develop pineapple products to be more varied, increase selling value and increase pineapple consumption.

Honey pineapple is a type of sweet pineapple because it contains 23.6% glucose which can be used as a carbon source for the growth of microorganisms (Pangaribuan et al., 2022). According to Ujilestari et al., 2021, one advantage of *L. casei* over other probiotic bacterial groups is that it can use sugar as a carbon source for a longer amount of time, extending the final product's shelf life. *L. casei* also has the benefit of being easily isolated from commercial products sold under the Yakult brand. Therefore, *L. Casei* is suitable for use as a starting culture in the production of fermented pineapple juice.

The characteristics of fermented fruit juice are influenced by several factors, namely starter culture, fermentation time, temperature, pH, and substrate (Sahab, 2023). Fermentation time is one of the most crucial factors of beverage fermentation since it affects taste and amount of LAB in the final product (Pangaribuan et al., 2022; Pracheta Febricia et al., 2020). According to Pracheta Febricia et al., 2020, the best fermentation time using the starter culture *Lactobacillus* sp F213 is 22 hours, while according to Nazarend & Wikandari, 2023; Pangaribuan et al., 2022 the best fermentation time using the starter culture *Lactobacillus plantarum* B1765 and *Lactobacillus rhamnosus* SKG34 is 24 hours. Based on this, it is known that the best fermentation time in making fermented fruit juice can be different. Therefore, research is needed to determine the best fermentation time in fermented pineapple juice using *L. casei*. In this research, the parameters to be observed are pH, total LAB, and sensory characteristics.

MATERIALS AND METHOD

Materials

The tools used in this research were knives, blenders, filters, cutting boards, volume pipettes, test tubes, glass beakers, Erlenmeyer, stirring rods, analytical balances, autoclaves, laminar air flow, micropipettes, petri dishes, vortexes, incubators, colony counters, and pH meter.

The materials used in this research were pineapple, *L. casei* strain Shirota culture from Yakult drinks, skim milk, glucose, distilled water, Mann Rogose and Sharpe (MRSA) media, Mann Rogose and Sharpe Broth (MRS Broth) media, NaCl, alcohol 70%, pH 4 buffer, and pH 7 buffer.

Stock Culture

L. casei was isolated from Yakult by Agavi Laboratory Bandung. One ose of the *L. casei* isolate was taken, placed in 10 ml of MRSB, and incubated for a 24-hour at 37^0Celsius. The first culture result was taken as much as 2 ml (4% v/v) and put into 50 ml pineapple juice and incubated at 37^0C for 24 hours.

Fermented Pineapple Juice Production

Pineapple fruit is blended and filtered to obtain its juice. Pineapple juice as much as 50 ml is added with 2 ml of culture stock (4% v/v), 1.5 g of sterile glucose (3% w/v), and 2.5 g of skim milk (5% w/v). Fermentation is carried out at a temperature of 37^0C for 16 hours (T1), 20 hours (T2), and 24 hours (T3). According to (Sahab, 2023), fermentation time affects total LAB, total sugar, total acid, and pH. This also relates to panelists' preference for taste (Pangaribuan et al., 2022; Pracheta Febricia et al., 2020). Therefore, it's important to determine the optimal fermentation time to achieve the best fermented pineapple juice.

Analytical Method

The analytical method used in this study was a one factor, Completely Randomized Design (CRD) Method. Analysis of Variance (ANOVA) and Duncan for post-hoc tests are used to analyze the differences between samples in pH and total LAB parameters. There are 3 treatments i.e. T1 (16 hours), T2 (20 hours), and T3 (24 hours) with 2 replications. Repetition is used to increase the reliability of the results and the statistical power of the study. The parameters evaluated in this study included pH, total LAB and characteristic sensory.

Test Method

The pH test was conducted using a pH meter. Total LAB was tested using the TPC method using MRSA media. The sensory test used is an affective test using the hedonic method with a scale of 5, namely a score of 1 very dislike, 2 dislike, 3 normal, 4 like, and 5 very like. The panelists used were 20 untrained panelists. Hedonic data was processed using SPSS with Kruskal Wallis statistical analysis to determine whether there were differences in each treatment.

RESULTS AND DISCUSSION

Acidity (pH)

Based on Table 1, the results of the ANOVA (Analysis of Variance) demonstrated that fermentation time had a significant influence on pH

Table 1. Acidity (pH value) and Total Lactic Acid Bacteria (LAB) of Fermented Pineapple Juice

Fermentation Time	Acidity (pH Value)	Total LAB (log CFU/ml)
T0	5.2	-
T1 (16 h)	4.12 ± 0.141c	11.22 ± 0.56[a]
T2 (20 h)	3.87 ± 0.848b	12.34 ± 0.05[b]
T3 (24 h)	3.56 ± 0.707a	12.08± 0.65[a]

Note: Numbers with their Standard Deviations (SDs) followed by the different letters within the same column indicate a significant difference (α = 0.05).

This analysis was conducted to test whether there were significant differences in the mean pH values among the various fermentation time groups. The p-value obtained from the ANOVA indicated that we could reject the null hypothesis (i.e., no difference in pH among fermentation time groups), thereby confirming the effect of fermentation time. Table 1 illustrates that the longer the fermentation time, the lower the pH of the probiotic pineapple juice. The pH of pineapple juice before fermentation was 5.2 then decreased to 4.12 at the 16th hour of fermentation, 3.87 at the 20th hour and 3.56 at the 24th hour. This is in accordance with Hashemi & Jafarpour, 2023 & Reddy et al., 2015, where fermentation of mango and guava juice by *Lactiplantibacillus plantarum* can reduce the pH of the juice significantly. This may be due to the formation of lactic acid and other organic acids.

According to Kuley et al., 2020, organic acids are formed during the fermentation process which can lower the pH. The two main organic acids produced by LAB during fermentation are lactic and acetic acids (Nuryana et al., 2019). Additionally, lactic acid bacteria can break down unwanted acid molecules like malic and tartaric acid into lactic acid, which has a flavor that people find pleasing (Yang et al., 2024). This is in line with Küçükgöz et al., 2024, who reported that lactic acid and acetic acid significantly increased and malic acid significantly decreased as a result of LAB fermentation. According to Bergentall et al., 2024, bilberry juice which has a high malic acid content will be converted by LAB into lactic acid so that it has more favourable taste characteristics.

Organic acids produced by lactic acid bacteria not only function to improve the taste of the product, but also function as antimicrobials (Hu et al., 2019; Ji et al., 2023). Organic acids effectively enter microbial cells because of their simple, small molecular structure. Once inside, they disrupt the cell's internal processes, ultimately leading to cell death (Sorathiya et al., 2025). According to Ji et al., 2023, the influx of organic acid ions into bacterial cells elevates their intracellular osmotic pressure. To balance the osmotic pressure inside and outside the cells, bacteria release precursors and cofactors that they need to grow into the extracellular space, thereby hindering normal growth and metabolism. Furthermore, this internal acid ion enrichment can disrupt and block nuclear DNA synthesis, negatively impacting the metabolic processes that produce bacterial energy and leading to the blunting or denaturation of crucial intracellular enzyme

Table 2. Sensory Attributes Acceptance Scores of Fermented Pineapple Juice

Treatment	Color	Aroma	Taste
T1	3.00 ± 0.795	2.85 ± 0.988	3.50^{a} ± 0.761
T2	2.90 ± 0.447	2.75 ± 0.716	2.75^{b} ± 0.786
T3	3.40 ± 0.821	2.85 ± 0.875	2.75^{b} ± 0.786

Note: Numbers with their Standard Deviations (SDs) followed by the different letters within the same row indicate a significant difference (α = 0.05)

Total LAB

Table 1 shows that fermentation time influences the number of LAB, where the highest number of lactic acid bacteria is T2 (20 hours). At 16 hours (T1), the total LAB was 11.22 log CFU/ml, and increased to 12.34 log CFU/ml at the 20th hour, then decreased to 12.08 log CFU/ml at the 24th hour. *L. casei* may have begun to enter the death phase at the 24-hour fermentation stage, causing a little but insignificant drop in numbers. These three treatments can be categorized as probiotic drinks because they meet the requirements for the amount of probiotics based on SNI/WHO, namely a minimum of 10^{6}-10^{7} CFU/ml or 6-7 log CFU/ml (Aspri et al., 2020; Nguyen et al., 2019).

Sensory Characteristics Based on Hedonic Tests

Based on the results of the hedonic test (Table 2), the aroma and color parameters of the three treatments were not significantly different. This means that the fermentation time does not affect the panelists' preferences for aroma and color. The color score in the hedonic test ranged from 2.90 to 3.40 with the treatment that had the highest color value being T3.

However, based on the Kruskal-Wallis test, there was no difference in color between samples. This may be due to the absence of a striking color in fermented pineapple juice. In the aroma parameter, the scores T1 and T3 were 2.85 and T2 was 2.75. A score of 2 indicates an unpleasant aroma towards a score of 3 which indicates neutral / does not give the impression of liking or disliking. So it can be concluded that the aroma of fermented pineapple juice tends to be less preferred. This may be due to the sour aroma produced by the fermentation process.

On the other hand, the fermentation time significantly affects the panelists' taste preferences. Based on taste parameter, T1 is the most preferred treatment compared to other treatments, with a value of 3.50 ± 0.761. This score is between scores 3 and 4, which means that the panelists' preference for the taste of T1 is neutral towards liking. Based on this, the most recommended treatment for making fermented pineapple juice is treatment 1 (16 h of fermentation). This may be due to the formation of organic acids, which causes a sour taste that some panelists may not like. According to Laosee et al., 2022, the most abundant acidic compound in fermented fruit juice is acetic acid, which causes a sour taste. Acetaldehyde is also present, which gives it a pungent aroma.

CONCLUSION

Fermentation time has a significant effect on pH and total LAB. The best treatment based on the hedonic test is the T1 treatment (16 hours), where the taste parameter score in this treatment is the highest, i.e. 3.50 ± 0.761 with a pH value of 4.12 and a total LAB of 11.22 log CFU/ml.

ACKNOWLEDGEMENT

The authors would like to acknowledge the Agavi Laboratory Bandung and STIKes Kuningan who have contributed to the process of research

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