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Antioxidant Profile in Pineapple Peel Fermentation by *Lactobacillus plantarum* and *Lactobacillus casei*

Sri Hartini[1*], Margareta N. Cahyanti[1], Dewi K. A. Kusumahastuti[1,2], Indah T. Susilowati[3], Y. M. Anggara Mahardika[4]

[1] Department of Chemistry, Science and Mathematics Faculty, Satya Wacana Christian University, Salatiga, Indonesia
[2] Medicine Study Programme, Tallinn Health Care College, Tallinn, Estonia
[3] Medical Laboratory Technology Diploma 3, STIKES Nasional, Sukoharjo, Indonesia
[4] Digital Business Study Programme, Insitute Teknologi Sains dan Kesehatan Sugeng Hartono, Sukoharjo, Indonesia

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Corresponding Author:
Sri Hartini
sri.hartini@uksw.edu

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Abstract: Pineapple peels are a potential food waste capable of being repurposed as a substrate for producing high-antioxidant probiotic beverages. Therefore, this study aimed to determine the feasibility of using pineapple peels as a substrate for producing high-antioxidant probiotic beverages through fermentation by *Lactobacillus plantarum* and *Lactobacillus casei*. Pineapple peels juice was fermented for 48 hours and the growth of microorganisms, reducing sugars, pH, titratable acid, polyphenol compounds, and antioxidant activity were periodically analyzed. The results showed that after 24 hours, the growth of *L. plantarum* and *L. casei* gradually slowed down. As fermentation time increased, the levels of reducing sugars and the pH decreased. *L. casei* reached the highest titratable acidity of 0.90% equivalent to lactic acid after 48 hours of fermentation, while *L. plantarum* reached 1.21%. The percentage of radical inhibition and phenolic compound increased between 9 and 30 hours of fermentation. The highest inhibition activity of 39% was achieved after 30 hours of fermentation for *L. casei*, while for *L. plantarum*, it reached 55% after 9 hours. This study showed that pineapple peels could produce probiotic beverages with high antioxidant. It was a suitable alternative for vegetarians or individuals who were allergic to dairy products.

Keywords: Antioxidant; Lactic acid bacteria; Pineapple peels; Probiotics

Introduction

Pineapple (Ananas comosus Mill.) is a tropical fruit with a unique flavor, taste, and multiple nutritional benefits. It is widely cultivated in tropical regions around the world for products such as canned pineapple and juice, which are very popular among consumers (Leneveu-Jenvrin et al., 2020). Pineapple is readily available in Indonesia and has a sweet, slightly acidic flavor, as well as rich in nutrients including vitamin A, vitamin C, fiber, vitamin B1, and B6 (Ancos et al., 2016). Indonesia produced 3,203,775 tons of pineapple in 2022 (BPS, 2023) and the increasing demand has resulted in large-scale production, leading to a significant amount of waste. Pineapple has about 60% of its weight as peels, and this waste is currently underutilized, leading to environmental pollution (Roda & Lambri, 2019).

One way to address this waste problem is by processing pineapple peels to derive added value. Pineapple peels contain many beneficial phytochemical components namely carbohydrates, vitamins, polyphenols, and minerals, which can be used as a source of antioxidant compounds, dietary fiber, and nutrition for microorganisms (Cornelia & Kristyanti, 2021). Therefore, studies on the use of pineapple peels as recyclable waste and functional foods are highly needed (Roda & Lambri, 2019). Functional food is one of the latest trends in the industry, affirming that food serves as an energy source and can provide additional health benefits. Pineapple peels are a potential source of functional food products that are delicious and

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beneficial for human health (A.R. et al., 2023; Aparecida Damasceno et al., 2016). The use can be optimized by examining the composition of bioactive compounds and exploring ways to incorporate the use into high value-added foods.

Fermentation of pineapple peels using bacteria such as *Lactobacillus casei* and *Lactobacillus plantarum* is consistent with the principles of sustainable agriculture and circular economy, which are increasingly important in a resource-limited world. Product of fermentation such as antioxidant can be used in various ways in the pharmaceutical, food, and cosmetics industries (Abdullah, 2017; Abraham et al., 2023). *L. casei* and *L. plantarum* are known to have efficient fermentation capabilities, as well as reduce food waste and enhance safety by extending the shelf life. Therefore, this study aimed to investigate the feasibility of using pineapple peels as a substrate for growth and probiotic production. The bioactive compounds and activity produced during fermentation were also evaluated.

Method

Materials

Pineapple peels used in this study were obtained at a traditional market in Salatiga, while commercial *L. plantarum* and *L. casei* were acquired from the Integrated Research and Testing Laboratory, Gadjah Mada University. All the chemicals and reagents used were of analytical grade and provided by Satya Wacana University, Department of Chemistry. These materials were of excellent purity (>90%), thereby requiring no additional purification.

Sample Preparation

After separating the flesh of pineapple, peels were subjected to bleaching at a temperature of 85°C for five minutes, followed by a reduction to room temperature. A total of 1000 g peels were combined with 400 g water and crushed in a blender. Using filtration, the solid part was separated from the liquid part, and then the liquid part was sterilized at a temperature of 121°C for 15 minutes. The substrates for fermentation process were enriched with 1 g yeast extract, 1.6 g NH4Cl, and 1 g MgSO4, and 0.1 g sucrose for every 30 ml juice.

Culture Preparation

L. casei and *L. plantarum* culture was inoculated into MRSA and incubated at 37°C for 48 hours. Subsequently, it was transferred to MRSB medium and incubated for 48 hours at 37°C.

Fermentation

Pineapple peels were inoculated with culture at a 10% (v/v) ratio and subjected to incubation at 37°C for 48 hours. Periodic sampling was used in this study and the liquid obtained from fermentation process was used for the assessment of many parameters, including microbial growth, reducing sugar, pH and titratable acid, total phenolic, and antioxidant activity.

Moisture Content

Moisture content was determined using the Ohaus MB25 moisture analyzer. Pineapple peels were crushed with a blender without the addition of water, then 1 g sample was placed on an aluminum plate and heated in the moisture analyzer.

Ash Content (Cahyanti et al., 2021)

Ash content was determined gravimetrically using the Vulcan A-550 muffle furnace. Crushed pineapple peels were weighed in a known mass porcelain crucible. The crucible containing sample was heated in the muffle furnace at 575°C for 3 hours. The ash content was calculated using Equation (1).

$$\%\ Ash\ = \frac{weight_{crucible+sample} - weight_{empty\ crucible}}{weight_{crucible+ash} - weight_{empty\ crucible}} \times 100 \quad (1)$$

Fat Content (Hasan, 2022)

Crushed sample was weighed as 10 g in a Soxhlet thimble and hexane, used as the extraction solvent, was placed in a fat flask. The apparatus was assembled, and then the heating process was carried out for 15 hours. Subsequently, the solvent was evaporated using a rotary evaporator. Fat content was calculated using Equation (2).

$$\%\ fat\ = \frac{weight\ of\ extracted\ fat}{weight\ of\ sample\ used} \times 100 \quad (2)$$

Protein Content (Hasan, 2022)

Crude protein content was determined using the macro Kjeldahl method. A 0.3 g sample was mixed with 0.35 g copper sulfate and 7.5 g sodium sulfate in a Kjeldahl digestion flask. About 10 mL concentrated sulfuric acid was added, the mixture was digested, and a clear light blue color appeared. After cooling, the digest was diluted to 100 mL in a volumetric flask with 30 ml distilled water. For distillation, the diluted mixture was placed in the apparatus with 75 mL of 40% sodium hydroxide. In a receiving flask, 20 mL of 4% boric acid and mixed bromocresol green and methyl red indicators were added. Distillation continued until the boric acid solution indicated a color change. The distillate was titrated with 0.2 N hydrochloric acid and a blank was prepared with distilled water. The protein content was calculated using Equation (3).

$$\%\ protein\ = \frac{(V_s - V_b) \times N\ HCl \times Ar\ N \times 6{,}25}{massa\ sampel} \times 100\% \quad (3)$$

Carbohydrate Content (BeMiller, 2017)

Carbohydrate content was determined using the difference method based on Equation (4).

Carbohydrate content = 100% - (moisture content + ash content + fat content + protein content). (4)

Chemical Composition Analysis (modified from(Zulfikar et al., 2020))

A total of 1 g sample (mass a) was refluxed with 120 ml distilled water at 100°C for 1 hour, separated by filtration, and subsequently dried (mass b). The dried biomass was refluxed with 150 ml of 0.5M H_2SO_4 for 1 hour, separated again by filtration, rinsed with distilled water until neutral, and dried (mass c). The biomass was soaked in 10 ml of 72% H_2SO_4 for 4 hours, separated by filtration, rinsed with distilled water until neutral, and dried (mass d). The product obtained was then pulverized (mass e). The calculation of fiber composition was performed based on Equation (5)-(8).

$$Hot\ water\ soluble\ compound\ = \frac{a-b}{a} \times 100 \quad (5)$$

$$Hemicellulose\ = \frac{b-c}{a} \times 100 \quad (6)$$

$$Cellulose\ = \frac{c-d}{a} \times 100 \quad (7)$$

$$Lignin\ = \frac{d-e}{a} \times 100 \quad (8)$$

Microbial Growth

The growth of *L. casei* was measured based on optical density (OD), which was assessed using a UV-Vis spectrophotometer. About 0.5 g sample was diluted to 50 ml bacteria suspension in a volumetric flask and the absorption was measured at a wavelength of 600 nm.

Reducing Sugar

The reducing sugar was measured based on a previous study (Hidayat & Yunita, 2022) with modification in the amount of sample. About 0.5 g sample were combined with 5 ml distilled water and thoroughly mixed until homogeneous. The liquid phase was placed into a volumetric flask with a capacity of 10 ml, and the volume was adjusted with distilled water. About 0.5 ml sample solution was combined with 0.5 ml aquades and 1 ml DNSA reagent. The mixture was boiled for 10 minutes and the volume was adjusted to 10 ml. The solution was then scanned to find the maximum wavelength, and the absorbance was measured at this point.

pH and Titratable Acid

The Hanna HI-9812-5 pH meter was used for the pH measurement and titratable acid was determined based on a previous study (Batali et al., 2021). Sample weighing 2.5 g was combined with 25 ml distilled water and subsequently titrated with a 0.1 M solution of sodium hydroxide (NaOH) until a pH of 8.2±0.1 was reached.

Antioxidant Activity

Antioxidant activity of crude extracts was evaluated by measuring the radical scavenging activity (RSA) using the DPPH method based on a previous study (Aryal et al., 2019) with modification on sample preparation. In a concise manner, 0.1 mL sample, dissolved in methanol to make 1 ml solution, was introduced into 2 mL DPPH solution at a concentration of 0.1 mM. The mixture was incubated in a light-restricted environment for 30 minutes. Subsequently, the absorbance was determined at a wavelength of maximum absorption (λmax) of 517 nm, using an equivalent quantity of DPPH and methanol as a reference blank.

The percentage scavenging of DPPH• (RSA %) was calculated using the equation:

$$\%\ scavenging\ of\ DPPH\ \bullet = \frac{A_0 - A_1}{A_0} \times 100 \quad (9)$$

Where A_0 is the absorbency of the control (solvent without sample) and A1 is the absorbency of sample.

Polyphenol Compounds

The chemical constituents in the extract of fermented pineapple peels were analyzed using High-Performance Liquid Chromatography (HPLC) Knauer GmBH-Jerman Model Smart Line Series, and the chemicals subjected to analysis included gallic acid and catechin. High-performance liquid chromatography (HPLC) used phosphoric acid, water, acetonitrile, and methanol in a ratio of 14:7:3:1 as the mobile phases. The stationary phase used was Eurosphere 100-5 C18 (250x4.6mm with pre-column) GJ95.

Result and Discussion

Proximate and Chemical Composition of Pineapple Peels

As shown in Table 1, moisture content in pineapple peels was estimated at 86%. This was consistent with a previous study conducted by (Kodagoda, 2017), which reported moisture content of 87%. The results showed that ash content was approximately 0.61%. This was higher compared to the value obtained by (Romelle et al., 2018), namely 0.40%. Protein content was also higher at 5.83% compared to previous studies. According to (Kodagoda, 2017), fat content of pineapple outer peels was 0.66%, while moisture content of the inner peels was 0.44%. (Christopoulos & Ouzounidou, 2021) stated that variations in the quality and nutrition of fruits were attributed to a complex interplay of factors including

environmental conditions and genetic variations (species and varieties) during cultivation.

Table 1. Proximate and chemical composition of sweet pineapple peels

Compositions		Percentage (x̄±SD)
Proximate	Moisture	86.32±0.35
	Ash	0.61±0.00
	Lipid	5.83±0.11
	Protein	2.25±0.07
	Carbohydrate	5.00±0.39
Chemical composition	Water soluble compound	54.11±1.79
	Hemicellulose	24.25±2.03
	Cellulose	15.14±0.25
	Lignin	3.02±0.40

Table 1 shows the chemical composition of pineapple peels, with water-soluble compounds constituting the largest proportion at 54.11%. This composition was associated with the presence of polar compounds such as phenolic (Saraswaty et al., 2017). Based on the results, pineapple peels were found to contain 27% hemicellulose, 15% cellulose, and 3% lignin. This was consistent with a previous study conducted by (S. Bhat et al., 2022), which reported values of 22%, 19%, and 5%, respectively. The high cellulose and hemicellulose content provide a basis for the use of pineapple peels as a fermentation substrate.

The Growth of Microorganisms and the Reducing Sugar Content During Fermentation

The growth profiles of *L. plantarum* and *L. casei* are presented in Figure 1, both showing nearly identical phase behaviors. However, the growth dynamics of *L. plantarum* were slower compared to *L. casei*. The initial 10 hours show a lag or adaptation phase followed by a log phase, with a slight decline before increasing again. *L. plantarum* requires more time to adapt, as shown in (Śliżewska & Chlebicz-Wójcik, 2020). The study was carried out to observe the growth of five Lactobacillus strains when transitioning from MRS to semi-solid media. The results showed that *L. plantarum* had a slower response towards the log phase compared to the other strains. Similarly, in this study, the bacteria required more time due to the transition from starter to growth media.

As shown in Figure 1, the reduced sugar consumption was high during the lag phase. The availability of sugar during the fermentation process is a crucial factor. (Irmayanti, et al., 2020) showed that providing sufficient sucrose content in probiotic drinks production derived from tofu waste resulted in a noticeable rise in the concentration of *L. casei*. Specifically, increasing the sucrose content from 10% to 14% led to an increase in *L. casei* concentration from 4.55 x 10^6 to 5.45 x 10^6. The reducing sugar slightly increased before decreasing logarithmically in line with the growth of *L. plantarum*. The increase was attributed to the contribution of dissolved compounds supporting its existence, such as the hydrolysis of cellulose into glucose.

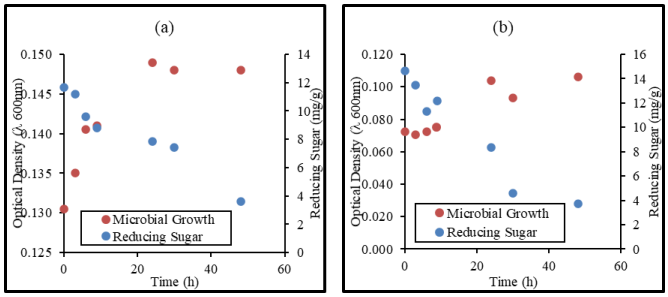

Figure 1. The microbial growth and reducing sugar concentration during fermentation (a) *L. casei* and (b) *L. plantarum*

Dynamics of pH and Titratable Acidity During Fermentation

Figure 2 shows the dynamics of changes in pH that occur in fermentation. The results showed a decline in pH levels, reaching the minimum value of 3.20 after 48 hours of fermentation for *L. casei* and 3.10 for *L. plantarum* after the same period. This was consistent with previous studies that observed a significant decline in pH in the initial 12-hour period, followed by a gradual decrease resulting in a final value of 3 after 48 hours of fermentation (Mirmohammadi et al., 2021). Lactic acid bacteria have a high capacity to grow in a wide range of dietary substrates, particularly those rich in sugar content, leading to the production of acid that rapidly reduces the pH of the surrounding medium (Kondybayev et al., 2022). Parhi et al., (2021) provided a more in-depth explanation regarding the various forms of sugar that *L. casei* could metabolize. These bacteria have excellent glucose metabolism while lacking the ability to use galactose and lactose as carbon sources. In addition, Cui et al., (2021) stated that *L. plantarum* could use cellobiose, mannose, D-ribose, and L-fucose, ferment prebiotics such as fructooligosaccharides (FOS) and galactooligosaccharide (GOS). The acids generated through sugar metabolism primarily include lactic acid, along with succinic acid (non-volatile), as well as acetic and propionic acids (volatile) (Urdaneta et al., 1995).

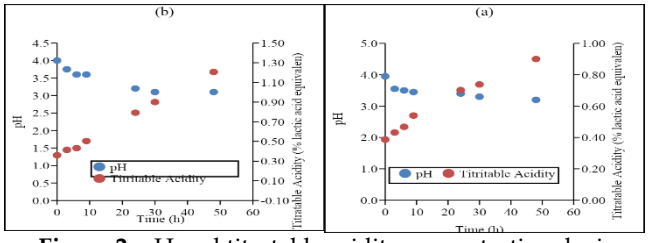

Figure 2. pH and titratable acidity concentration during fermentation (a) *L. casei* and (b) *L. plantarum*

When fermentation extended over a longer period, the amount of titratable acid increased accordingly. This result was consistent with Malik et al., (2019) which used beetroot juice as a substrate. It was found that the titratable acid content increased from 0.22% to 0.43% equivalent to lactic acid after 24 hours of fermentation, and further by 0.54% after 48 hours for *L. casei*. In *L. plantarum*, the titratable acidity increased from 0.20% to 0.51% equivalent to lactic acid after 24 hours, and further by 0.64% equivalent to lactic acid after 48 hours. Both *L. casei* and *L. plantarum* reached the highest titratable acidity of 0.91% and 1.21% equivalent to lactic acid, respectively, after 48 hours of fermentation. This value was higher than the 0.6% equivalent to lactic acid found in cabbage juice inoculated with *L. casei* (Yoon et al., 2006). The titratable acid in fermented white and red potato juice from the Pyeongchang area, Korea, inoculated with *L. casei* had a low value of 0.20-0.25% equivalent to lactic acid (Kim et al., 2012). For *L. plantarum*, the titratable acidity was also higher than the 0.88% equivalent to lactic acid found in carrot juice (Irmayanti, et al., 2020). The observed increase in concentration during fermentation showed that the substrate offered sufficient nutrients and lacked any inhibitory substances capable of impeding the growth of *L. casei* (Yoon et al., 2006). Phenolic molecules were produced in response to the acidic environment through the formation of hydroxy and ferulic acid. The accumulation of phenolic compounds led to an elevation in antioxidant content (Zubaidah et al., 2023).

The increase in acid production and the decrease in pH occurred in harmony. According to (Bisson et al., 2023), in general, Lactobacilli under sub-lethal conditions can develop adaptive stress responses, resulting in an increased ability to grow at the same stress level. Bacteria strains are capable of developing cross-tolerance, showing that the adaptation of cells to mild stress conditions also enhances the tolerance to various harsher conditions such as high temperature, acidity, and the presence of oxygen.

Dynamics of Antioxidant Activity During Fermentation

The various stages observed during fermentation showed the complex interaction between the release of nutrients into the surrounding water-based environment and the active microbial metabolism of lactic acid bacteria. Both the nature and the length of the process affected the metabolism of the microbial community, as evidenced in this study by changes in reducing sugar concentration, total acid, and pH. Figure 3 shows antioxidant activity as radical inhibition. In the 0 h of fermentation, the presence of antioxidant resistant to initial heat treatment was observed even after bleaching at 85°C for 5 minutes followed by material sterilization. Polyphenols in pineapple peels facilitated the formation of complexes with bromelain, resulting in resistance at high temperatures treatment. Jagannath & K, (2020) provided valuable insights into the heat resistance of bromelain-polyphenol complexes compared to free bromelain. The complexes enhanced thermal stability, retaining the activity even after exposure to temperatures between 25°C - 85°C. Meanwhile, free bromelain experienced a 40% decrease in activity under the same conditions. At temperatures above 85°C, the activity of bromelain-polyphenol complexes and free bromelain sharply decreased, specifically in the range of 85°C - 95°C.

The dynamics of radical inhibition in fermentation products by *L. casei* and *L. plantarum* were different. The radical inhibition by *L. casei* decreased after a 3-hour fermentation, followed by a gradual rise, and the maximum level was reached after 30 hours. The results were in line with (Jin et al., 2018) which used mango pulp as a growth substrate for *L. casei*. Radical inhibition activity decreased after 8 hours of fermentation then slowly increased and reached a maximum value at 32 hours. On the other hand, fermentation products by *L. plantarum* showed radical inhibition percentage that increased after 3 hours. The percentage thereafter declined at 6 hours and the maximum value was reached after 30 hours. The results were consistent with (R. Bhat et al., 2015) which used guava pulp as a growth substrate for *L. plantarum*. Radical inhibition activity increased after 4 hours of fermentation and then declined at 8-12 hours. The maximum value was finally reached after 16 hours.

The percentage of radical inhibition in *L. plantarum* fermentation products was higher compared to *L. casei*. This was attributed to the ability of *L. plantarum* to produce a wide spectrum of enzymes including b-glucosidase, p-coumaric acid decarboxylase, and decarboxylase. These enzymes can degrade polyphenolic compounds into simple phenolic compounds (R. Bhat et al., 2015).

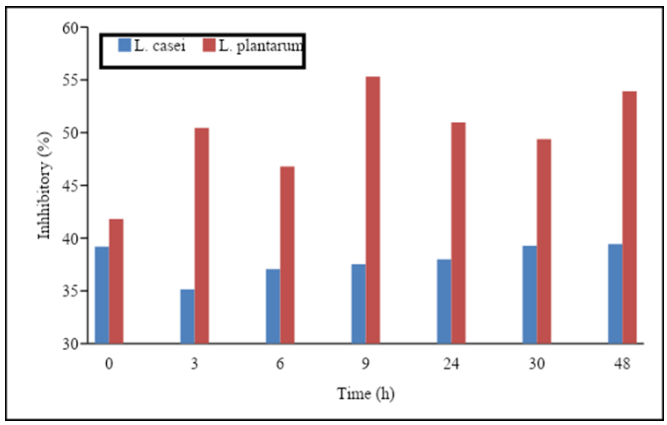

Figure 3. The dynamics of radical inhibition during fermentation

Dynamics of Phenolic Content During Fermentation

Phenolic compounds and flavonoids constitute the largest group of secondary metabolites found in plants (Cosme et al., 2020). The main phenolic compounds and flavonoids detected in fermentation product were gallic acid and catechin. This result was consistent with Li et al. (2014) that found gallic acid (31.76 mg/100 g dry extracts), catechin (58.51 mg/100 g), epicatechin (50.00 mg/100 g), and ferulic acid (19.50 mg/100 g) as the main polyphenols in pineapple peels.

As shown in Figure 4, the level of gallic acid and catechin in fermentation products by *L. plantarum* was higher compared to *L. casei*. Gallic acid is a phenolic compound with the highest DPPH radical inhibition capacity followed by catechin (Li et al., 2021). This disparity in phenolic content contributed to the higher radical inhibition observed in *L. plantarum* fermentation products compared to *L. casei*.

The production of flavonoids and phenols occurred during the early growth phase of *L. casei* and *L. plantarum*. The result reinforced a previous study showing that pineapple peels contained a high number of polyphenolic compounds rich in antioxidant (Kumar et al., 2021). He et al., (2022) also stated that the form of polyphenols and flavonoids could change during fermentation, depending on the specific strain used. In general, fermentation can also alter the phenolic content in sample. In which fermented jujube juice with L. plantarum and *L. casei*, found an increase in total phenols (Li et al., 2021). Report by Rivera et al., (2023) added that the optimal fermentation time to maximize phenolic content and antioxidant activity was 24 hours, with a subsequent decline occurring after this point.

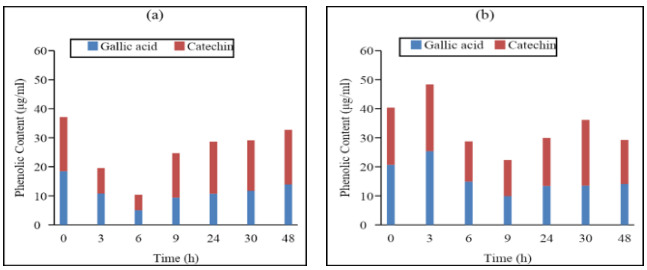

Figure 4. Dynamics of phenolic content during fermentation (a) *L. casei* and (b) *L. plantarum*

Conclusion

In conclusion, *L. casei* reached the highest titratable acidity of 0.90% equivalent to lactic acid after 48 hours of fermentation, while *L. plantarum* reached 1.21%. An increase in the percentage of radical inhibition was observed between 9 and 30 hours of fermentation with a similar dynamic between phenolic compounds and inhibition activity. The highest inhibition activity of 39% was achieved after 30 hours for *L. casei*, while for *L. plantarum*, it reached 55% after 9 hours. Based on the results, pineapple peels have great potential as a suitable substrate for the development of probiotic beverages characterized by high levels of antioxidant. This innovation is a suitable alternative for vegetarians or individuals who are allergic to dairy products.

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Author Contributions

Conceptualization, Sri Hartini, Margareta N. Cahyanti, Dewi K. A. Kusumahastuti, Indah T. Susilowati, and Y. M. Anggara Mahardika; methodology, Sri Hartini, Margareta N. Cahyanti, and Dewi K. A. Kusumahastuti; validation, Sri Hartini and Dewi K. A. Kusumahastuti; formal analysis, Sri Hartini; investigation, Margareta N. Cahyanti, Indah T. Susilowati, and Y. M. Anggara Mahardika; resources, Sri Hartini, Dewi K. A. Kusumahastuti.; data curation, Margareta N. Cahyanti, Indah T. Susilowati, and Y. M. Anggara Mahardika; writing—original draft preparation, Sri Hartini, Margareta N. Cahyanti, Dewi K. A. Kusumahastuti; writing—review and editing, Indah T. Susilowati, and Y. M. Anggara Mahardika; visualization, Sri Hartini, and Margareta N. Cahyanti.; supervision, Sri Hartini.; project administration, Sri Hartini and Margareta N. Cahyanti.; funding acquisition, Sri Hartini. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

References

A.R., S., M., S., H.Z., U., Koh, S. P., & Shukor, M. Y. A. (2023). Local pineapple waste as potential bio-ingredient. *Food Research*. https://doi.org/10.26656/fr.2017.6(s3).002

Abdullah, A. (2017). Solid And Liquid Pineapple Waste Utilization For Lactic Acid Fermentation. *Reaktor*. https://doi.org/10.14710/reaktor.11.1.50-52

Abraham, R. A., Joshi T, J., & Abdullah, S. (2023). A comprehensive review of pineapple processing and its by-product valorization in India. In *Food Chemistry Advances*. https://doi.org/10.1016/j.focha.2023.100416

Ancos, B., Sánchez-Moreno, C., & González-Aguilar, G.

A. (2016). Pineapple composition and nutrition. In *Handbook of Pineapple Technology: Postharvest Science, Processing and Nutrition*. https://doi.org/10.1002/9781118967355.ch12

Aparecida Damasceno, K., Alvarenga Gonçalves, C. A., Dos Santos Pereira, G., Lacerda Costa, L., Bastianello Campagnol, P. C., Leal De Almeida, P., & Arantes-Pereira, L. (2016). Development of Cereal Bars Containing Pineapple Peel Flour (Ananas comosus L. Merril). *Journal of Food Quality*. https://doi.org/10.1111/jfq.12222

Aryal, S., Baniya, M. K., Danekhu, K., Kunwar, P., Gurung, R., & Koirala, N. (2019). Total Phenolic content, Flavonoid content and antioxidant potential of wild vegetables from western Nepal. *Plants*. https://doi.org/10.3390/plants8040096

Batali, M. E., Cotter, A. R., Frost, S. C., Ristenpart, W. D., & Guinard, J.-X. (2021). Titratable Acidity, Perceived Sourness, and Liking of Acidity in Drip Brewed Coffee. *ACS Food Science & Technology*. https://doi.org/10.1021/acsfoodscitech.0c00078

BeMiller, J. N. (2017). Carbohydrate Analysis. In *Food Analysis. Food Science Text Series* (pp. 330–360). Springer, Cham.

Bhat, R., Suryanarayana, L. C., Chandrashekara, K. A., Krishnan, P., Kush, A., & Ravikumar, P. (2015). Lactobacillus plantarum mediated fermentation of Psidium guajava L. fruit extract. *Journal of Bioscience and Bioengineering*. https://doi.org/10.1016/j.jbiosc.2014.09.007

Bhat, S., Suthar, P., Rafiq, S., Farooq, A., & Sheikh, T. (2022). Pineapple Wastes and By-Products: Chemistry, Processing, and Utilization. In *Handbook of Fruit Wastes and By-Products: Chemistry, Processing Technology, and Utilization*. https://doi.org/10.1201/9781003164463-19

Bisson, G., Maifreni, M., Innocente, N., & Marino, M. (2023). Application of pre-adaptation strategies to improve the growth of probiotic lactobacilli under food-relevant stressful conditions. *Food and Function*. https://doi.org/10.1039/d2fo03215e

BPS. (2023). *Produksi Tanaman Buah-buahan 2022*. https://www.bps.go.id/Indicator/55/62/1/Produksi-Tanaman-Buah-Buahan.Html.

Cahyanti, M. N., Doddapaneni, T. R. K. C., Madissoo, M., Pärn, L., Virro, I., & Kikas, T. (2021). Torrefaction of agricultural and wood waste: Comparative analysis of selected fuel characteristics. *Energies*. https://doi.org/10.3390/en14102774

Cerning, J., Renard, C. M. G. C., Thibault, J. F., Bouillanne, C., Landon, M., Desmazeaud, M., & Topisirovic, L. (1994). Carbon source requirements for exopolysaccharide production by Lactobacillus casei CG11 and partial structure analysis of the polymer. *Applied and Environmental Microbiology*. https://doi.org/10.1128/aem.60.11.3914-3919.1994

Christopoulos, M., & Ouzounidou, G. (2021). Climate Change Effects on the Perceived and Nutritional Quality of Fruit and Vegetables. *Journal of Innovation Economics and Management*. https://doi.org/10.3917/jie.034.0079

Cornelia, M., & Kristyanti, T. (2021). *Utilization of Pineapple's (Ananas comosus L. Merr) Peel Waste as Raw Material in Cider Making*. https://doi.org/10.5220/0010041402580263

Cosme, P., Rodríguez, A. B., Espino, J., & Garrido, M. (2020). Plant phenolics: Bioavailability as a key determinant of their potential health-promoting applications. In *Antioxidants*. https://doi.org/10.3390/antiox9121263

Cui, Y., Wang, M., Zheng, Y., Miao, K., & Qu, X. (2021). The carbohydrate metabolism of lactiplantibacillus plantarum. *International Journal of Molecular Sciences*. https://doi.org/10.3390/ijms222413452

Hasan, K. (2022). Determination of Protein Content by Spectrophotometric Method and Fat Content by Soxhletation Method on Purple Kopek Eggplant and Green Kopek Eggplant. *Jurnal Matematika Dan Ilmu Pengetahuan Alam LLDikti Wilayah 1 (JUMPA)*. https://doi.org/10.54076/jumpa.v2i2.229

He, Z., Zhang, H., Wang, T., Wang, R., & Luo, X. (2022). Effects of Five Different Lactic Acid Bacteria on Bioactive Components and Volatile Compounds of Oat. *Foods*. https://doi.org/10.3390/foods11203230

Hidayat, U., & Yunita, N. P. (2022). Penentuan Kadar Gula Reduksi dan Kadar Protein secara Spektrofotometri, serta Uji Organoleptik Produk Nata de Leri Hasil Optimalisasi Asam Asetat Glasial. *Jurnal Penelitian Inovatif*. https://doi.org/10.54082/jupin.76

Irmayanti, I., Hakim, L., Makmur, T., Rizki, P.; Ar, C. (2020). The Effect of Lactobacillus Casei and Sucrose Concentrations on the Microbiology and Chemical Characteristics of Tofu Waste-Based Probiotic Drinks. *Proceedings Of International Conference On Multidiciplinary Research*, 80–86.

Jagannath, A., & K, S. (2020). Multi Target Preservation as an Effective Post-harvest Processing Technology for the Chemical and Microbiological Stability of Pineapple (Ananus Comosus). *International Journal of Fruit Science*. https://doi.org/10.1080/15538362.2020.1755411

Jin, X., Chen, W., Chen, H., Chen, W., & Zhong, Q. (2018). Comparative Evaluation of the Antioxidant Capacities and Organic Acid and Volatile Contents of Mango Slurries Fermented with Six Different Probiotic Microorganisms. *Journal of Food Science*. https://doi.org/10.1111/1750-3841.14373

Kim, N. J., Jang, H. L., & Yoon, K. Y. (2012). Potato juice fermented with Lactobacillus casei as a probiotic functional beverage. *Food Science and Biotechnology*. https://doi.org/10.1007/s10068-012-0171-5

Kodagoda, K. (2017). Development of non-alcoholic wines from the wastes of Mauritius pineapple variety and its physicochemical properties KHGK Kodagoda and RAUJ Marapana. *Journal of Pharmacognosy and Phytochemistry*.

Kondybayev, A., Konuspayeva, G., Strub, C., Loiseau, G., Mestres, C., Grabulos, J., Manzano, M., Akhmetsadykova, S., & Achir, N. (2022). Growth and Metabolism of Lacticaseibacillus casei and Lactobacillus kefiri Isolated from Qymyz, a Traditional Fermented Central Asian Beverage. *Fermentation*. https://doi.org/10.3390/fermentation8080367

Kumar, P., Tanwar, R., Gupta, V., Upadhyay, A., Kumar, A., & Gaikwad, K. K. (2021). Pineapple peel extract incorporated poly(vinyl alcohol)-corn starch film for active food packaging: Preparation, characterization and antioxidant activity. *International Journal of Biological Macromolecules*. https://doi.org/10.1016/j.ijbiomac.2021.07.136

Leneveu-Jenvrin, C., Quentin, B., Assemat, S., & Remize, F. (2020). Maintaining physicochemical, microbiological, and sensory quality of pineapple juice (Ananas comosus, Var. 'Queen Victoria') through mild heat treatment. *Processes*. https://doi.org/10.3390/PR8091186

Li, T., Jiang, T., Liu, N., Wu, C., Xu, H., & Lei, H. (2021). Biotransformation of phenolic profiles and improvement of antioxidant capacities in jujube juice by select lactic acid bacteria. *Food Chemistry*. https://doi.org/10.1016/j.foodchem.2020.127859

Malik, M., Bora, J., & Sharma, V. (2019). Growth studies of potentially probiotic lactic acid bacteria (Lactobacillus plantarum, Lactobacillus acidophilus, and Lactobacillus casei) in carrot and beetroot juice substrates. *Journal of Food Processing and Preservation*. https://doi.org/10.1111/jfpp.14214

Mirmohammadi, R., Zamindar, N., Razavi, S. H., Mirmohammadi, M., & Paidari, S. (2021). Investigation of the possibility of fermentation of red grape juice and rice flour by Lactobacillus plantarum and Lactobacillus casei. *Food Science and Nutrition*. https://doi.org/10.1002/fsn3.2461

Parhi, P., Song, K. P., & Choo, W. S. (2021). Effect of inulin and fructooligosaccharide supplementation on the growth and survival of Lactobacillus casei in model sugar systems. *Journal of Food Processing and Preservation*. https://doi.org/10.1111/jfpp.15228

Rivera, A. M. P., Toro, C. R., Londoño, L., Bolivar, G., Ascacio, J. A., & Aguilar, C. N. (2023). Bioprocessing of pineapple waste biomass for sustainable production of bioactive compounds with high antioxidant activity. *Journal of Food Measurement and Characterization*. https://doi.org/10.1007/s11694-022-01627-4

Roda, A., & Lambri, M. (2019). Food uses of pineapple waste and by-products: a review. In *International Journal of Food Science and Technology*. https://doi.org/10.1111/ijfs.14128

Romelle, F. D., Rani, P. A., & Manohar, R. S. (2018). Chemical Composition of Some Selected Fruit Peels. *European Journal of Food Science and Technology*.

Saraswaty, V., Risdian, C., Primadona, I., Andriyani, R., Andayani, D. G. S., & Mozef, T. (2017). Pineapple peel wastes as a potential source of antioxidant compounds. *IOP Conference Series: Earth and Environmental Science*. https://doi.org/10.1088/1755-1315/60/1/012013

Śliżewska, K., & Chlebicz-Wójcik, A. (2020). Growth kinetics of probiotic lactobacillus strains in the alternative, cost-efficient semi-solid fermentation medium. *Biology*. https://doi.org/10.3390/biology9120423

Urdaneta, D., Raffe, D., Ferrer, A., de Ferrer, B. S., Cabrera, L., & Pérez, M. (1995). Short-chain organic acids produced on glucose, lactose, and citrate media by Enterococcus faecalis, Lactobacillus casei, and Enterobacter aerogenes strains. *Bioresource Technology*. https://doi.org/10.1016/0960-8524(95)00103-4

Yoon, K. Y., Woodams, E. E., & Hang, Y. D. (2006). Production of probiotic cabbage juice by lactic acid bacteria. *Bioresource Technology*. https://doi.org/10.1016/j.biortech.2005.06.018

Zubaidah, E., Arum, M. S., Dewanti, T., Rahayu, A. P., Srianta, I., & Tewfik, I. (2023). Sauerkraut inoculated with Lactobacillus casei as a potent immunomodulator in Escherichia coli infected mice. *Food Research*. https://doi.org/10.26656/fr.2017.7(2).586

Zulfikar, A., Putri, N. P. S. N. K., & Tajalla, G. U. N. (2020). Studi Pengaruh Waktu Alkalisasi pada Ekstraksi Selulosa Berbasis Serat Eceng Gondok (Eichhornia crassipes). *SPECTA Journal of Technology*. https://doi.org/10.35718/specta.v4i2.182