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Effect of Fermentation Time and *Lactobacillus plantarum* Inoculum Concentration on Lactic Acid Production from Durian Peel Waste

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ABSTRACT

Lactic acid is an important chemical whose demand continues to increase every year. Therefore, alternative innovations are needed to meet this demand. One potential raw material that can be utilized is durian peel waste, which is high in cellulose and can be converted into reducing sugar through pretreatment and hydrolysis as a fermentation substrate to produce lactic acid. This study aims to determine the optimal conditions for fermentation time (5 hours; 6.5 hours; 8 hours; 9.5 hours and 11 hours) and *Lactobacillus Plantarum* inoculum concentration (5%, 10%, 15%, 20%, 25%) in producing high concentrations of lactic acid. The research was conducted through Alkali Hydrogen Peroxide (AHP) delignification, acid hydrolysis, and fermentation processes. The lactic acid content was analyzed using the acid-base titration method (Total Titratable Acidity). The results showed that the highest lactic acid content was obtained at a fermentation time of 11 hours with an inoculum concentration of 20%, which reached 0.135%. Thus, fermentation time and inoculum concentration affect the lactic acid content produced, and durian peel waste has the potential to be used as an alternative raw material in lactic acid production.

INTRODUCTION

Indonesia is a tropical country with abundant fruit diversity, one of which is durian. According to data from the *Badan Pusat Statistik*, Indonesia produced approximately 1,852,045 tons of durian in 2023 (Badan Pusat Statistik, 2023). However, only about 20.52% of the fruit is edible, while the remaining 79.48% consists of peel and seeds that are generally discarded and become waste (Kusumaningtyas & Syah, 2020). The large amount of durian peel waste has the potential to cause environmental problems if not properly managed (Gamay et al., 2024). One potential approach to utilizing this waste is converting it into value-added products, such as lactic acid.

Lactic acid is an important organic acid widely used in the food, pharmaceutical, and chemical industries (Ojo & de Smidt, 2023). It is commonly applied in the production of yogurt, cheese, and fermented vegetables, and is also used as a raw material for biodegradable polymers such as polylactic acid (PLA) (Kim et al., 2022). In Indonesia, the demand for lactic acid continues to increase, with production growing at an average rate of 4.04% per year (Agustin et al., 2022). Lactic acid can be produced through chemical synthesis or microbial fermentation. Currently, nearly 90% of commercial lactic acid production is derived from microbial fermentation, while the chemical route contributes only a small portion of the total production (Díaz-Orozco et al., 2025).

Fermentation processes offer several advantages, including lower operating temperatures, the ability to utilize renewable biomass resources, and the production of high-purity lactic acid when appropriate microorganisms are used (Huang et al., 2023).

Durian peel contains a high amount of lignocellulosic components, including cellulose (55.3%), lignin (19.3%), and ash (6.1%) (Putra et al., 2024). The high cellulose content can be hydrolyzed into reducing sugars that can serve as a carbon source in lactic acid fermentation (Abedi & Hashemi, 2020). Several pretreatment and hydrolysis processes have been studied to increase the availability of fermentable sugars from lignocellulosic biomass. From (Afifah et al., 2022) reported that delignification using the Alkaline Hydrogen Peroxide (AHP) method effectively reduces lignin content in biomass. Delignification is an important step in lignocellulosic biomass pretreatment to remove lignin and improve cellulose accessibility for enzymatic or acid hydrolysis (Han et al., 2020). Furthermore, hydrolysis using acid catalysts can produce significant amounts of reducing sugars from durian peel (Obed et al., 2015).

Lactic acid bacteria (LAB) are the most commonly used microorganisms for lactic acid production due to their ability to efficiently convert sugars into lactic acid (Bintsis, 2018). Various microorganisms have been studied for lactic acid production through fermentation. From (Nandini et al., 2021) reported that among several bacterial strains tested, *Lactobacillus Plantarum* produced the highest lactic acid concentration under controlled fermentation conditions. Other studies have shown that inoculum concentration and fermentation time significantly influence lactic acid production (Triovanta et al., 2020). Higher inoculum concentrations can increase lactic acid production because a greater number of active cells are available to convert the substrate into the desired product (Nurfuzianti & Nurfuzianti, 2021). In addition, *Lactobacillus Plantarum* exhibits several growth phases, with optimal growth occurring during the logarithmic phase (Hidayatulloh et al., 2019) before declining due to nutrient depletion, which may subsequently reduce lactic acid production (Pehulisa et al., 2019)

These parameters influence microbial growth and metabolic activity, which ultimately determine the amount of lactic acid produced during fermentation. Considering the high cellulose content of durian peel waste that can be converted into reducing sugars and further utilized for lactic acid production, as well as the increasing demand for lactic acid in Indonesia, this study aims to produce lactic acid from reducing sugars derived from durian peel waste and to determine the optimal conditions by varying fermentation time and *Lactobacillus Plantarum* inoculum concentration.

RESEARCH METHODS

This study employed an experimental method using durian peel waste obtained from Nyidam Duren Wisata Kulit Durian, Surabaya, as the raw material. The durian peel waste was processed through a delignification stage to remove lignin, followed by acid hydrolysis to produce reducing sugars, which were subsequently used as a substrate in the fermentation process to produce lactic acid using *Lactobacillus plantarum*.

This study used a factorial experimental design to evaluate the combined effect of fermentation time and inoculum concentration on lactic acid production. The fermentation time variations were 5, 6.5, 8, 9.5, and 11 hours, while the inoculum concentrations used were 5%, 10%, 15%, 20%, and 25% (v/v). The experimental data obtained were analyzed descriptively by comparing the lactic acid concentration produced at different fermentation times and inoculum concentrations to determine the conditions that produced the highest lactic acid concentration.

The materials used in this study included *Lactobacillus plantarum*, MRS broth media, H_2SO_4, H_2O_2, NaOH, and phenolphthalein indicator. The main equipment used in the experiment included a shaker incubator, autoclave, centrifuge, and laminar air flow cabinet. The parameter observed in this study was the lactic acid content produced from the fermentation process, which was analyzed using the Total Titratable Acidity (TTA) method.

Preparation of raw materials

Durian peels were separated from the inner white portion and washed to remove impurities such as dust and sand. The cleaned peels were cut into small pieces and dried in an oven at 120 °C

for 1 hour. The dried peels were then ground using a grinder to obtain durian peel powder and subsequently sieved to obtain an 80-mesh particle size. The resulting powder was analyzed to determine its lignocellulosic content.

Delignification

Delignification was carried out using the Alkali Hydrogen Peroxide (AHP) method. Durian peel powder was treated with a mixture of 8.5% H_2O_2 and 1.5% NaOH until the solution reached pH 11. The mixture was stirred at 80 °C for 180 minutes to remove lignin components. After the reaction was completed, the mixture was filtered and the solid residue was washed with H_2O until neutral pH was achieved. The obtained solids were then dried in an oven at 90 °C until constant weight to obtain cellulose powder. The resulting cellulose was further analyzed to determine its lignocellulosic composition.

Hydrolysis

A total of 100 g of cellulose powder obtained from the delignification process was subjected to acid hydrolysis using 1000 mL of 3.5 N H_2SO_4 and 500 mL of distilled water. The hydrolysis reaction was carried out at 90 °C for 75 minutes to convert cellulose into reducing sugars. After the hydrolysis process was completed, the solution was cooled to approximately 28–31 °C and filtered to obtain the filtrate containing reducing sugars. The pH of the filtrate was then adjusted to neutral (pH 7) using 0.5 N NaOH solution before being used in the fermentation process.

Fermentation

All equipment used in the fermentation process was sterilized using 70% alcohol followed by autoclave sterilization at 121 °C for 30 minutes. MRS broth medium was prepared by dissolving 55.15 g of MRS broth in 1000 mL of distilled water and sterilized at 121 °C for 15 minutes. A total of 2 mL of *Lactobacillus Plantarum* culture was inoculated into 200 mL of MRS broth medium and incubated at 37 °C for 14 hours to prepare the starter culture. The culture was then centrifuged to separate the cells, and the obtained suspension was used as the fermentation inoculum. The fermentation process was carried out by adding *Lactobacillus Plantarum* inoculum at concentrations of 5%, 10%, 15%, 20%, and 25% (v/v) into 100 mL of reducing sugar solution in fermentation bottles. The fermentation was conducted at 37 °C with fermentation time variations of 5, 6.5, 8, 9.5, and 11 hours.

Total Titratable Acid Analysis

The lactic acid concentration was determined using the Total Titratable Acidity (TTA) method through acid–base titration (Mawarni & Fithriyah, 2015). A total of 5 mL of fermentation sample was placed in an Erlenmeyer flask and titrated using 0.1 N NaOH solution with phenolphthalein as the indicator until the endpoint was indicated by a stable pink color. Lactic acid reacts with NaOH in a 1:1 molar ratio during the neutralization reaction. Therefore, the volume of NaOH required to reach the titration endpoint represents the amount of lactic acid present in the sample. The lactic acid concentration was calculated using the following equation:

$$\text{Lactic Acid Concentration } \% = \frac{V_{NaOH} \times N_{NaOH} \times \text{MW}_{LA}}{V_s \times 1000} \quad (1)$$

Where:

V_{NAOH} = Volume of NaOH used to neutralize the acid (ml)
N_{NaOH} = Normality of NaOH solution (0,1 N)
MW_{LA} = Molecular weight of lactic acid (90 g/mol)
V_S = Volume of sample used for titration (ml)

The overall research procedure for lactic acid production from durian peel waste is illustrated in Figure 1. The process includes durian peel preparation, delignification, acid hydrolysis to produce reducing sugars, and fermentation using *Lactobacillus plantarum*. The lactic acid produced from the fermentation process was analyzed using the Total Titratable Acidity (TTA) method.

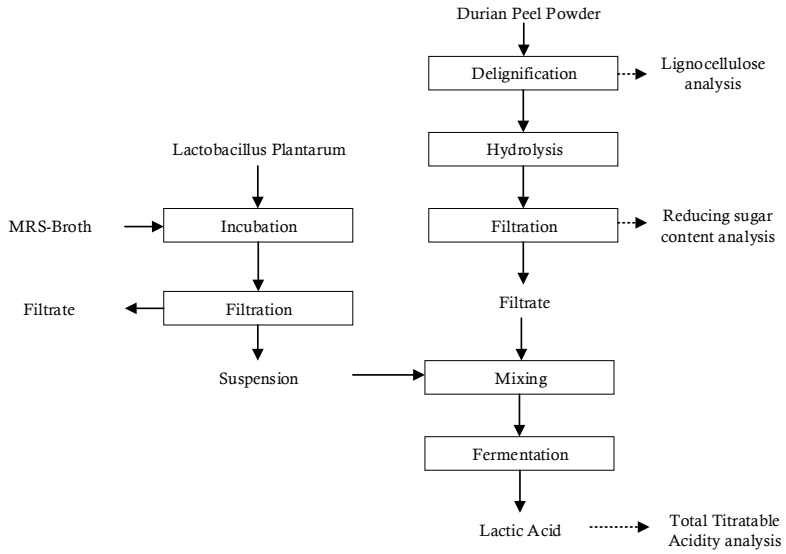

Figure 1. Research Process Flowchart

RESULTS AND DISCUSSION

Delignification Process Results

The delignification process was conducted to reduce the lignin content in durian peel powder and increase the cellulose content as the main substrate for further processing. The lignocellulosic composition of the durian peel waste was analyzed before and after the delignification process. The delignification process utilized hydrogen peroxide (H_2O_2) with a concentration of 8.5%. The results of the lignocellulose content analysis are presented in Table 1.

Table 1. Lignocellulose Content Before and After Delignification of Durian Peel

Component	Content Before (%)	Content After (%)
Cellulose	63.02	75.02
Lignin	4.02	0.02

Source: Penelitian dan Konsultasi Industri Laboratorium Surabaya

Based on Table 1, the delignification process resulted in an increase in cellulose content and a significant decrease in lignin content. The cellulose content increased from 63.02% to 75.02%, while the lignin content decreased from 4.02% to 0.02%. This indicates that the delignification process effectively removed lignin from the durian peel biomass. According to (Afifah et al., 2022), the decrease in lignin content occurs due to the action of the Alkali Hydrogen Peroxide (AHP) method, which oxidizes lignin structures. Under alkaline conditions, hydrogen peroxide forms reactive oxidizing species such as hydroperoxyl ions (HOO^-), hydroxyl radicals (OH), and superoxide anions (O_2^-). These reactive species break down the structural bonds within lignin, including phenolic bonds, Cα–Cβ bonds, and alkyl–aryl ether bonds, resulting in the degradation of lignin into smaller molecules that are more easily dissolved in water.

Hydrolysis Process Results

The hydrolysis process was carried out using 3.5 N sulfuric acid (H_2SO_4) for 75 minutes to convert cellulose into reducing sugars. The reducing sugar content of the hydrolysis product was analyzed. The results are presented in Table 2.

Table 2. Reducing Sugar Content Analysis After Hydrolysis Process

Material	Sulfuric Acid Concentration (N)	Hydrolysis Time (Minutes)	Reducing Sugar Content (%)
Durian Peel Powder	3.5	75	4.22

Source: Penelitian dan Konsultasi Industri Laboratorium Surabaya

Based on Table 2, the reducing sugar content obtained from the hydrolysis of durian peel powder was 4.22%. This result indicates that the acid hydrolysis process was able to convert part of the cellulose contained in the biomass into reducing sugars. The use of 3.5 N H_2SO_4 enabled the hydrolysis reaction to occur effectively without causing excessive degradation of the produced sugars. According to (Obed et al., 2015), hydrolysis under overly harsh conditions or excessive reaction times can cause reducing sugars to degrade into by-products such as furfural, hydroxy methyl furfural (HMF), acetic acid, and formic acid. These compounds may inhibit microbial activity in subsequent fermentation processes. Therefore, the obtained reducing sugar concentration of 4.22% indicates that the hydrolysis conditions used were still within an appropriate range for producing fermentable substrates.

Fermentation Process Results

The fermentation process was carried out using *Lactobacillus Plantarum* with variations in inoculum concentration (5%, 10%, 15%, 20%, and 25%) and fermentation time (5, 6.5, 8, 9.5, and 11 hours). The concentration of lactic acid produced during fermentation was determined using the acid–base titration method with 0.1 N NaOH as the titrant and phenolphthalein as the indicator. The lactic acid content was calculated based on the volume of NaOH required to neutralize the sample until the endpoint of the titration was reached, which was indicated by a color change of the solution to pink. The neutralization reaction occurring during the titration is presented as follows:

$$C_3H_6O_3 + NaOH \rightarrow C_3H_5O_3Na + H_2O$$

The results of lactic acid analysis obtained using the titration method are presented in Table 3, while the relationship between fermentation time, inoculum concentration, and lactic acid concentration is illustrated in Figure 2.

Table 3. Lactic Acid Level Analysis Using Acid-Base Titration

Fermentation Time (hours)	Inoculum Concentration	Lactic Acid Concentration (%)
5	5%	0.0900
	10%	0.0960
	15%	0.1020
	20%	0.1080
	25%	0.1170
6,5	5%	0.0863
	10%	0.0960
	15%	0.1035
	20%	0.1148
	25%	0.1148
8	5%	0.0806
	10%	0.0960
	15%	0.1050
	20%	0.1215
	25%	0.1125
9,5	5%	0.0788
	10%	0.0960
	15%	0.1065
	20%	0.1283
	25%	0.1103
11	5%	0.0750
	10%	0.0960
	15%	0.1080

20%	0.1350
25%	0.1080

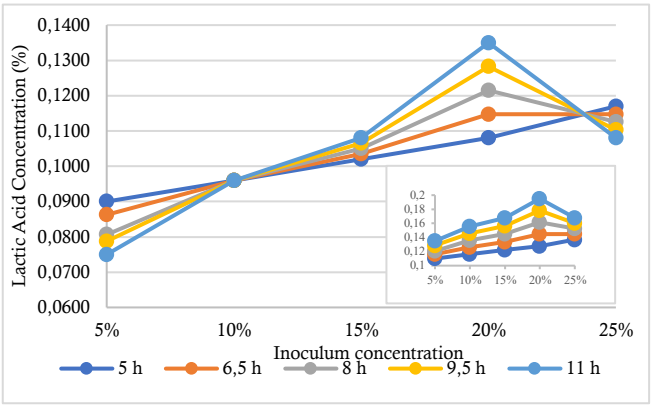

Figure 2. Effect of Inoculum Concentration and Fermentation Time on Lactic Acid Content (**Inset:** representation of the main plot shown separately)

Based on Figure 2, it can be observed that the lactic acid concentration generally increased with increasing fermentation time. This indicates that longer fermentation periods allow *Lactobacillus plantarum* to convert more reducing sugars into lactic acid. According to (Hidayatulloh et al. (2019), during the early stage of fermentation, microorganisms typically enter the logarithmic growth phase, in which cell division occurs rapidly and metabolic activity increases significantly. As the bacterial population increases, more enzymes involved in carbohydrate metabolism are produced, thereby enhancing the conversion of reducing sugars into lactic acid. These findings are consistent with those reported by (Saraswati et al., 2021), who observed that at fermentation times of 8–12 hours, microorganisms are in the logarithmic phase, resulting in increased lactic acid production due to higher microbial activity.

In terms of inoculum concentration, the results show that lactic acid concentration increased up to an inoculum concentration of 20%, but decreased at 25%. This indicates that there is a critical inoculum concentration that produces the highest lactic acid production. Increasing the inoculum concentration initially increases the number of active microbial cells, thereby accelerating substrate utilization and enhancing lactic acid production.

However, at excessively high inoculum concentrations (25%), lactic acid production decreased. This phenomenon is not only caused by nutrient limitation but also by increased metabolic competition and product inhibition. An excessive number of microorganisms leads to rapid nutrient consumption, while the accumulation of lactic acid can lower the pH and inhibit further microbial activity. This is consistent with the findings of (Kacaribu and Darwin, 2022), who reported that excessively high inoculum concentrations can reduce fermentation efficiency due to nutrient competition and metabolic stress in microbial cells.

Overall, these results indicate that the balance between fermentation time and inoculum concentration plays a crucial role in determining the efficiency of lactic acid production, where imbalanced conditions may lead to reduced fermentation performance.

CONCLUSION

Based on the results of this study, it can be concluded that inoculum concentration of *Lactobacillus Plantarum* and fermentation time significantly influence the production of lactic acid during the fermentation process. The highest lactic acid concentration of 0.135% was obtained at an inoculum concentration of 20% with a fermentation time of 11 hours. Increasing the inoculum concentration initially enhanced lactic acid production due to the higher number of active microbial cells involved in the fermentation process. However, further increases in inoculum concentration resulted in a decrease in lactic acid concentration, indicating the existence of an optimum inoculum level. Additionally, longer fermentation time led to higher lactic acid

production, suggesting that the microorganisms were still in the exponential growth phase where metabolic activity occurs intensively. The results of this study also highlight the potential utilization of durian peel waste as a sustainable and environmentally friendly raw material for lactic acid production. This approach not only enhances the value of agricultural waste but also contributes to waste reduction and the development of environmentally friendly bioprocesses.

For further research, it is recommended to investigate other factors affecting lactic acid production, such as pH, substrate concentration, and longer fermentation time, in order to obtain a more comprehensive understanding of the optimal conditions for lactic acid production.

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