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EFFECTS OF PRETREATMENT METHODS ON THE LIGNOCELLULOSIC COMPOSITION OF SALACCA MIDRIBS

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ABSTRACT

This study aims to determine the effect of pretreatment on hemicellulose, cellulose, and lignin content of Salacca midrib. Unlike previous studies, this study was designed to evaluate the effects of single-, double-, and triple-stage pretreatments on the sallaca midrib. The study was also designed to evaluate the differences in the physical and biological pretreatment methods. The pretreatments applied consist of physical, chemical, biological, physicochemical, chemical-biological, physical-biological, and physicochemical-biological pretreatments. Physical pretreatment is carried out in an autoclave at 121 °C, chemical pretreatment using Sodium Hydroxide (NaOH) 4%, and biological pretreatment using Debaryomyces hansenii. Based on the ANOVA test, the various pretreatment methods have a significant effect on lignocellulose content. The highest hemicellulose content was the chemical pretreatment at 34.13%, the cellulose content was the chemical pretreatment at 40.85%, and the highest lignin reduction was the physical-chemical-biological at 27.15%

INTRODUCTION

Salacca midribs are a byproduct of salak plants. According to data from the Central Statistics Agency (BPS), in 2022, North Sumatra was the province with the highest salak production, totaling 265,180 tons. This is in line with the increase in waste produced, which ranges from 260 to 310 tons annually, with the largest amount coming from the midrib and old leaves, accounting for 63.54% (Silaban & Harahap, 2021). Based on the study of Triyastiti and Krisidiyanto (2017), salacca midrib contains fiber, namely 33.9% hemicellulose, 31.7% cellulose, 17.4% lignin, and 0.6% silica, while according to Widyorini et al. (2015), salacca midrib contains 53% alpha cellulose, 35% hemicellulose, and 29% lignin. Salacca midrib also contains 9.24 (w/w) water content (Harahap et al., 2020). Based on the compositions, this lignocellulose content can be utilized as a substrate in the production of bioprocess products using microorganisms, yielding high-value products such as lignin, which can be used as a biopolymer (Abolore et al., 2025). Cellulose can be converted to bioethanol (Zhang et al., 2019), and hemicellulose can be used to produce xylose, which can be converted to xylitol (Pramasari et al., 2023) and biodiesel (Ruli, 2025).

To use agricultural waste as a substrate for fermentation, it needs to be treated to improve the accessibility of the catalyst to lignin, cellulose, and hemicellulose content (Hernandez et al., 2024). The result of pretreatment is called a hydrolysate. Pretreatment can be carried out in three ways: physical, chemical, biological, or combined (Chen et al., 2024). The physical pretreatment is carried out not only to reduce the size of the substrate particles and to heat pretreat (Atidhira and Noviansyah, 2017), but also to use carbon as an active agent (Rahayu et al., 2022). The chemical pretreatment is carried out using acids, such as H2SO4 (Chen et al., 2024; Naufala and Pandebesie, 2016), and alkaline (Ruli, 2025), and biological pretreatment is carried out using microorganisms or enzymes (Fan et al., 2025).

Devi et al. (2019) studied the effects of physical, chemical, and biological pretreatments on the salacca midrib. For physical pretreatment, steam explosion temperatures of 120, 140, and 160 °C were tested, with the optimal result at 160 °C (the highest lignin reduction, from 20.05 to 15.90%). In these conditions, the highest increase in cellulose was also observed, from 35.32% to 51.09%. Meanwhile, the optimal increase in hemicellulose was achieved through biological pretreatment with *Trichoderma reesei*, with a fermentation time of 15 days (fermentation was conducted at 5, 10, and 15 days), resulting in a 30.04-36.21% increase. In chemical pretreatment, sodium hydroxide (NaOH) 2, 4, 6 % were used. The study showed that chemical pretreatment is not optimal for reducing lignin or increasing cellulose and hemicellulose.

Based on the results of the study, the objective of this study was to evaluate the effect of single, double, and triple stages of pretreatments on the lignocellulose content, mainly hemicellulose, as a substrate of xylitol production for the next study. For physical pretreatment, an autoclave at 121 °C will be used because, according to Devi et al. (2019), the hemicellulose content did not increase at 120 and 140 °C, but increased by 1% at 160 °C. In the chemical treatment, 4% NaOH will be used because the hemicellulose content at 4% and 6% NaOH did not increase, and in the biological treatment, the fermentation time was determined to be 10 days because the difference in hemicellulose content between 10 days and 15 days of fermentation was only 1%, from 4.62% to 5.73%. Meanwhile, *D. hansenii* was used because it has a high level of halotolerance (Ming et al., 2020) and the ability to produce xylanolytic enzymes (Wang et al., 2020).

MATERIALS AND METHODS

This study began with the preparation of salacca midrib powder, yeast, peptone, and Glycerol (YPG) media, and the inoculum. The next step was doing pretreatment and analysis of lignocellulose content. This study was replicated twice for the control (untreated) and each of the pretreatment methods. Control was used to compare the effect of pretreatment on the lignocellulose content of salaca midrib.

Experimental Design

The study was conducted in a completely randomized design (CRD) with one treatment factor: control, physical, chemical, biological, physicochemical, chemical-biological, physical-biological, and physical-chemical-biological. Each pretreatment was replicated twice, yielding 16 experimental data points. The results were then analyzed using ANOVA.

Salacca midrib preparation

Salacca midribs are obtained from salak plantations in Pakkat, North Sumatra. The thorns are removed, then cut into several pieces and soaked for 24 hours. Next, the salacca midrib is washed and dried in an oven at 50°C for 5 hours (Devi et al., 2019). After the salacca midrib is dry, it will be ground to 60 mesh. Before use, the sallaca midrib powder can be stored at room temperature in a dry location. The salacca midrib powder used for each pretreatment was 20 g.

YPG Media preparation

20 g peptone, 10 g yeast extract, 20 mL glycerol, mixing in 1 L of distilled water.

Inoculum preparation

Inoculum used in this study was obtained from the culture stock at the Department of Chemical Engineering, Institut Teknologi Bandung, Indonesia. For inoculum preparation, 12 loops of *D. hansenii* were added to 100 mL YPG medium. The culture was incubated for 28 hours at 37°C.

Salacca midrib physical pretreatment

Salacca midrib powder was added to an Erlenmeyer flask containing distilled water at a ratio of 1:7. The mixture was placed in an autoclave at 121°C, cooled to 60°C, removed, allowed to reach room temperature, then filtered to obtain a slurry, which was dried in an oven. The dried substrate was analyzed for its lignocellulose content.

Salacca midrib chemical pretreatment

Salacca midrib powder was added to 4% NaOH at a 1:5.4 ratio (Devi et al., 2019). The mixture was stirred for 4 days, then filtered to obtain a slurry, which was dried in an ov-en. The dried substrate was analyzed for its lignocellulose content.

Salacca midrib biological pretreatment

Salacca midrib powder was added to the YPG medium and inoculum at a ratio of 1:2.39:0.18, and the mixture was fermented for 10 days at 200 rpm and 37°C (Devi et al., 2019). After that, the slurry was filtered to obtain a dry slurry, which was then dried in an oven. The dried substrate was analyzed for its lignocellulose.

Salacca midrib physical-chemical pretreatment

Salacca midrib powder was added to an Erlenmeyer flask containing distilled water at a ratio of 1:7. The mixture was placed in an autoclave at 121 °C, cooled to 60 °C, removed, and allowed to reach room temperature. After that, the substrate is subjected to chemical pretreatment by mixing it with 108 mL of 4% NaOH, stirring with a magnetic stirrer for 4 days, and then filtering the slurry to obtain a dried product. The dried substrate is then analyzed for its lignocellulose content.

Salacca midrib chemical-biological pretreatment

Salacca midrib powder was added to 4% NaOH at a 1:5.4 ratio (Devi et al., 2019). The mixture was then stirred using a magnetic stirrer for 4 days. After that, the substrate was subjected to biological pretreatment by adding 47.8 mL of YPG medium and 3.6 mL of inoculum, then fermented for 10 days at 200 rpm and 37 °C. The substrate was filtered to obtain a slurry, which was then dried in an oven. The dried substrate was then analyzed for its lignocellulose content.

Salacca midrib physical-biological pretreatment

Salacca midrib powder was added to an Erlenmeyer flask containing distilled water at a ratio of 1:7. The mixture was placed in an autoclave at 121 °C, cooled to 60 °C, removed, and allowed to reach room temperature. After that, the substrate was subjected to biological pretreatment by adding 47.8 mL of YPG medium and 3.6 mL of inoculum, and then fermented for 10 days at 200 rpm and 37°C. The substrate was filtered to obtain a slurry, which was then dried in an oven. The dried substrate was then analyzed for its lignocellulose content.

Salacca midrib physical-chemical-biological pretreatment

Salacca midrib powder was added to an Erlenmeyer flask containing distilled water at a ratio of 1:7. The mixture was placed in an autoclave at 121°C, cooled to 60°C, removed, and allowed to reach room temperature. After that, the substrate was subjected to chemical pretreatment by mixing the substrate with 107.2 mL of 4% NaOH and stirring with a magnetic stirrer for 4 days. Next, the substrate was subjected to biological pretreatment by adding 47.8 mL of YPG medium and 3.6 mL of inoculum, then fermented for 10 days at 200 rpm and 37°C. The substrate was filtered to obtain a slurry, which was then dried in an oven. The dried substrate was then analyzed for its lignocellulose content.

Lignocellulose content assay of Salacca midrib

This assay was performed on untreated substrates (control) and on pretreated substrates. The method used to determine lignocellulose content is the Chesson-Datta method (1981). This method consists of four steps. In the first step, the analysis begins by mixing 1 gram of dry sample (salacca midrib powder pretreated and dried) with 150 mL of distilled water, then refluxing for 2 hours at 100°C (a). The sample was then filtered to obtain the filtrate and the residue. Then, the filtered residue was transferred to a porcelain dish, dried in an oven for 24 hours at 60°C, and weighed until its mass was constant (b). The porcelain dish has been measured before. In the second step, after the hot, soluble water component was obtained, the dried residue was hydrolyzed by refluxing it with 150 mL of 1 N H2SO4 at 100°C for 2 hours. After that, the sample is washed with hot water (boiling water) and dried in an oven for 24 hours (c). In the third stage, the sample was then added 10 ml of 72% (v/v) H2SO4 and allowed to stand at room temperature for 4 hours. Then, the sample was hydrolyzed with 150 mL of 1N sulfuric acid for 1.5 hours at 100°C. After that, the residue was filtered with a crucible filter to obtain the filtrate and residue. Then, the residue was washed with hot distilled water until the volume reached 300 ml. After washing, the samples and crucible filters were dried in an oven at 60°C and weighed until a constant weight (d) was obtained. The fourth stage is carried out to obtain the ash content. The sample is incinerated at 575 ± 25°C until it becomes ash (± 10 seconds). Then the ash was cooled using a desiccator and weighed (e). These equations are used to calculate the hemicellulose, cellulose, and lignin content.

Hemicellulose content = $\frac{b-c}{a} x100\%$ (1)

Cellulose content = $\frac{c-d}{a} x100\%$ (2)

Lignin content = $\frac{d-e}{a} x100\%$ (3)

RESULTS AND DISCUSSION

The effects of salacca midrib pretreatment on lignin content (Figure 1), hemicellulose content (Figure 2), and cellulose content (Figure 3) are shown.

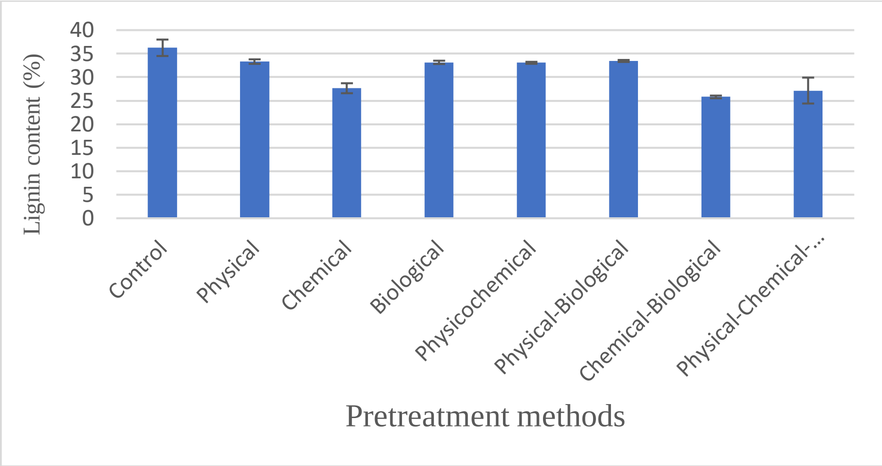

Figure 1. The lignin content from pretreatment method

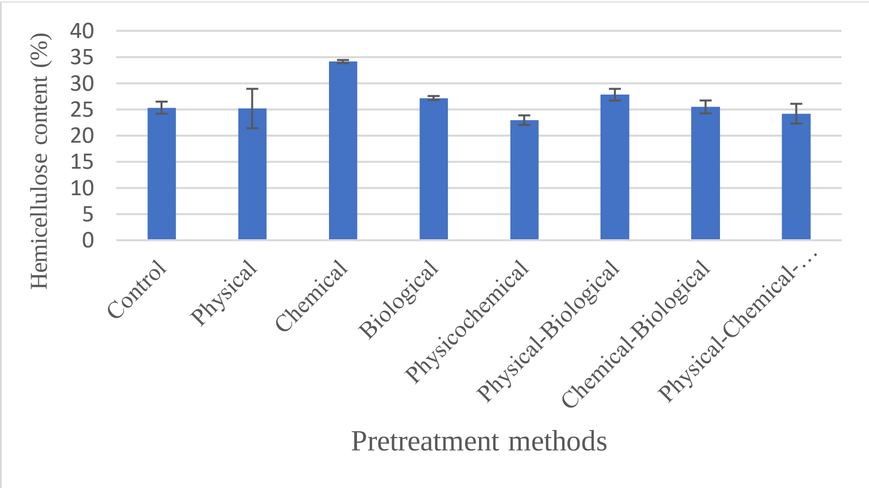

Figure 2. The hemicellulose content from pretreatment method

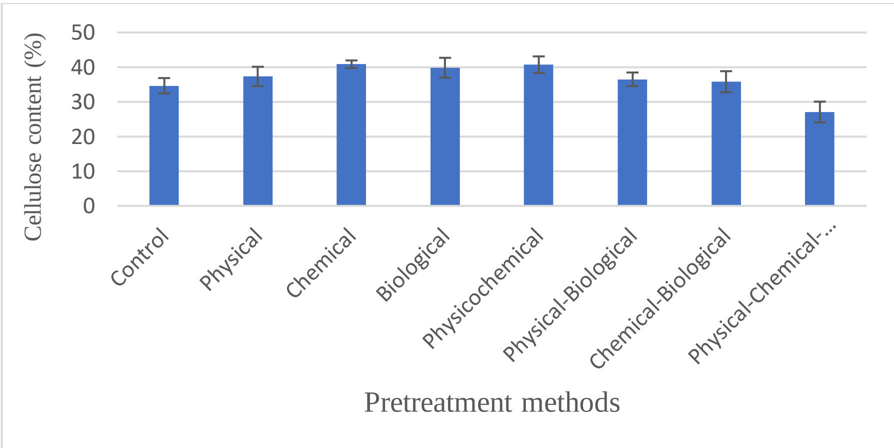

Figure 3. The cellulose content from pretreatment method

Based on Figure 1-3, the pretreatment methods have a significant effect on lignocellulose content. This statement is supported by the ANOVA significance test, expressed by a p-value $\leq \alpha$, where $\alpha = 0.005$ (the p-value is 0.000093), where chemical-biological was the optimal pretreatment for the reduction of lignin, from 36.25±1.75 to 25.80±0.28 (%). Meanwhile, the optimal pretreatment to increase the hemicellulose and cellulose content was chemical pretreatment, from 25.33±1.15 to 34.13±0.30 (%) and from 34.64±2.21 to 40.85±1.10 (%), respectively. This study was not aligned with Devi et al. (2019), which showed that physical pretreatment was optimal for reducing lignin and increasing cellulose content, whereas biological pretreatment was optimal for increasing hemicellulose. Table 1 compares lignocellulosic composition with previous studies.

Table 1. The comparison of pretreatment methods on the lignocellulosic composition

lignocellulosic composition	**Pretreatment method**								**Reference**
	Control	**Physical**	**Chemical**	**Biological**	**physicochemical**	**Physical-biologcal**	**Chemical biologcal**	**Physical-chemical-biological**	
Hemicellulose	30.49	35.00	33.00	36.21	-	-	-	-	Devi et al. (2019)
	25.33	25.16	34.13	27.17	22.95	27.80	25.47	24.18	This study
Cellulose	35.32	51.09	40.00	45.00	-	-	-	-	Devi et al. (2019)
	34.64	37.32	40.85	39.83	40.71	36.51	35.81	27.07	This study
Lignin	20.05	15.90	17.00	16.50	-	-	-	-	Devi et al. (2019)
	36.25	33.31	27.65	33.15	33.05	33.45	25.80	27.15	This study

Based on the table, Devi et al. (2019) showed that the physical pretreatment reduced lignin by only 4.15%, whereas this study found that the chemical-biological pretreatment reduced lignin by up to 10.45%. In biological pretreatment, Devi et al. (2019) showed that the hemicellulose content increased by 5.72%, whereas in this study, the chemical pretreatment was 8.8%; however, with physical pretreatment, Devi et al. (2019) showed an increase in cellulose content by 15.77%, and this study was 6.21% by chemical pretreatment.

Pretreatment was used to break down the complex lignocellulose structure into simpler components such as cellulose, hemicellulose, and lignin. In physical pretreatment, the difference in temperature and pressure will affect the number of lignin molecules that are depolymerized, where the higher the temperature and pressure, the more lignin will be depolymerized because the substrate will have thermal softening (thermal softening is the softening of lignin content with high temperature) (Harmsen et al., 2010; Jacquet et al., 2015). In the previous study by Devi et al. (2019), the highest lignin reduction and highest hemicellulose content were obtained when the substrate was obtained at160°C and a pressure of 6 bar, while in this study, physical pretreatment was carried out using an autoclave (121°C and 2 bar), so the reduction lignin was obtained lower, even, the hemicellulose content after physical pretreatment was lower than the control, but in another study conducted by Atidhira and Noviansyah (2017) showed that the optimal pretreatment results were obtained from physical pretreatment using an oven at 100 °C when compared to physical pretreatment using an autoclave and hot water bath, chemical pretreatment, and physicochemical pretreatment.

The chemical pretreatments include alkaline, acid, oxidative, and organosolv pretreatments. In chemical pretreatment, the addition of NaOH increases the porosity and internal surface area of the substrate, reduces the degree of polymerization and crystallinity, enabling the lignin contained in the substrate to be destroyed and its concentration reduced (Rafidah et al., 2020). The addition of NaOH can also degrade hemicellulose and cellulose into sugar monomers, including glucose, xylose, and arabinose (Lismeri et al., 2018). Based on Figure 4, hydroxide ions from NaOH will break the bonds of the basic structure of lignin (Rafidah et al., 2020). The cleaved lignin will bond with Na+ ions to form phenolic salts that are easily soluble in water (Lestari et al., 2018), so in this study, the cellulose and hemicellulose content were increased, and lignin content was decreased.

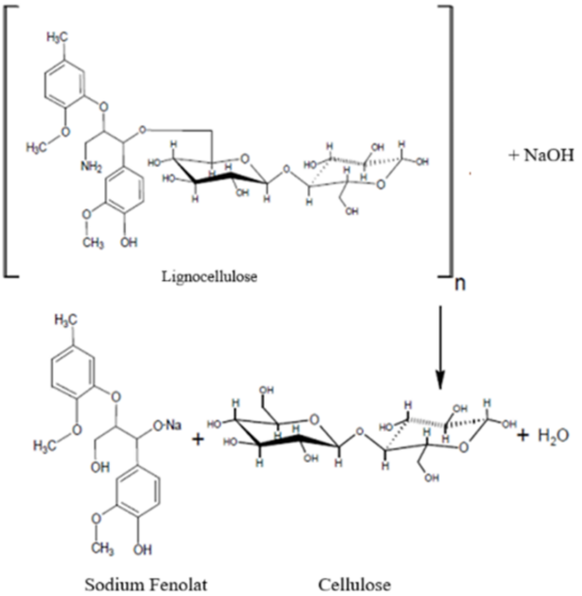

Figure 4. Reaction of lignocellulose breaking with NaOH

The study by Naufala and Pandebesie (2016) showed that acid pretreatment of a mixture of water hyacinth and rice husks with H_2SO_4 increased the cellulose content from 27.82% to 37.21%. Meanwhile, the hemicellulose content decreased from 30.38% to 21.66%, and the lignin content increased from 15.05% to 21.67%, so a base pretreatment can be obtained from Naufala and Pandebesie's (2016) study to increase hemicellulose and decrease lignin.

In biological pretreatment, *D. hansenii* can assist in breaking glycosidic bonds in cellulose and hemicellulose. Based on the study of Devi et al. (2019), the biological pretreatment using *Trichoderma reseii* FNCC 6012 with varying pretreatment times, namely 5, 10, and 15 days, was found that the longer the pretreatment time, the higher the hemicellulose yield, but the increase in hemicellulose content was only around 0.325-0.346. Based on the above data, we can conclude that the type of microorganism and pretreatment time do not significantly affect hemicellulose content because the reduction in lignin was not optimal with *D. hansenii*, as it cannot produce lignolytic enzymes. Lignolytic enzymes are enzymes that can degrade lignin. However, *D. hansenii* is capable of breaking the glycosidic bonds in the lignin-carbohydrate complex (LCC). In lignin-carbohydrate complex bonds, there are several different bonds, including benzyl-ether, benzyl ester, glycosidic or phenyl glycosidic, hemiacetal or acetal, and ferulate or diferylate ester (Albersheim et al., 2011). Under these conditions, *D. hansenii* is only capable of breaking glycosidic bonds, so the increase in hemicellulose and reduction in lignin will not be optimum.

In this study, a combination of two and three pretreatment methods was also carried out, and chemical-biological and physical-chemical-biological pretreatments were found to be optimal for reducing lignin compared to physical-biological and physical-chemical pretreatments, because in physical pretreatment, steam explosion was not used. Meanwhile, the best pretreatment method to increase hemicellulose content is chemical pretreatment. This is in contrast to the study by Devi et al. (2019), which showed that physical pretreatment of the salacca midrib substrate was the best for reducing lignin, whereas biological pretreatment was the best for increasing hemicellulose. In chemical pretreatment, Devi et al. (2019) applied NaOH concentrations of 2, 4, and 6%. The results showed that the higher the NaOH concentration, the greater the lignin reduction and the higher the hemicellulose concentration produced (the increase ranged from 0.30 to

6.77%), but at 4% and 6% NaOH, the increase in hemicellulose content was only 0.30%. Based on the data, it can be inferred that increasing NaOH concentration does not significantly increase hemicellulose content. In this study, the hemicellulose content produced was 0.342 higher than that reported by Devi et al. (2019).

CONCLUSION

Pretreatment has a significant effect on the resulting cellulose, hemicellulose, and lignin content, where the highest hemicellulose and cellulose contents were obtained in the chemical, from 25.33 to 34.13% and from 34.64 to 40.85%, respectively, and the highest lignin reduction was obtained in the chemical-biological, from 36.25 to 25.80%. This hemicellulose can be used to produce xylose, xylitol, and xylanase, while cellulose is used to produce bioethanol; cellulase is used as a substrate in the fermentation process. In this study, hemicellulose will be applied as a xylitol for the next study.

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