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# Integrated alkaline delignification and enzymatic hydrolysis of durian peel: A promising route for sustainable cellulosic sugars production

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## ABSTRACT

Durian peel is an underutilized solid waste with low biodegradability, leading to its accumulation and pollution of the environment. This waste has been reported to contain 55.3% cellulose and 23.11% lignin. The cellulose possesses significant potential to be converted into glucose through hydrolysis, as cellulose is a polysaccharide comprising glucose monomers. However, the high lignin content hinders enzyme access to cellulose, necessitating delignification to improve glucose yield. Therefore, this study aims to investigate the synergistic effects of sodium hydroxide (NaOH) concentration and enzyme concentration on hydrolysis during glucose production. The presence of lignin that inhibited hydrolysis was removed through delignification involving NaOH as a catalyst at various concentrations and reaction times. Lignin content was analyzed using the SNI 0492: 2008 method, while cellulose was analyzed with the SNI 0444: 2009 method. The results showed that 6% NaOH used for 105 minutes was able to concentrate cellulose up to 81.31% and remove lignin by 95.67%. The reducing sugar concentration was analyzed with a sugar refractometer. The results also showed that concentrated cellulose was converted to 122 g/L reducing sugar with the addition of 1.25% (% v/w) cellulose enzyme for 48 h at 40 °C.

**Keywords:** durian peel, cellulose, delignification, enzymatic hydrolysis, reducing sugar.

## INTRODUCTION

The global demand for renewable energy sources and environmentally friendly chemical feedstocks continues to rise due to the depletion of fossil resources and increasing environmental concerns (Magazzino et al., 2022; Wang and Azam, 2024). Concurrently, agricultural and agro-industrial residues are accumulating, specifically in tropical countries, as primary commodity production expands (Lopes and Ligabue-Braun, 2021). One abundant underutilized waste is durian peel, which is often discarded or incinerated, posing potential environmental hazards. The durian peel waste accounts for approximately 40% of the total weight of the durian fruit per 1 kg of durian. This indicates a substantial abundance of durian skin available as biomass waste from durian processing. Durian peel waste can cause environmental issues such as odor pollution and organic waste accumulation if improperly managed, leading to landfill overload and water pollution. However, durian peel contains a high content of lignocellulosic biomass that consist of 66.08% cellulose, 20.77% hemicellulose and 13.15% lignin (Savitri et al., 2023), making it a promising candidate for conversion into value-added products, such as glucose. Several studies have shown that glucose is a key compound widely used as a raw material in bioenergy, biochemical, and food industries (Brown, et al., 2024; Li et al., 2025; Yerizam, et. al., 2023). This indicates that valorizing durian peel waste as a glucose source not only contributes to effective waste management

but also supports the development of sustainable biorefineries. NaOH delignification is a highly effective strategy for improving the conversion efficiency of lignocellulosic biomass to fermentable sugars by removing lignin, increasing porosity, and facilitating enzymatic access (Sharma et al., 2023; Yadav et al., 2024; Yang et al., 2019). Previous studies have shown that mild NaOH delignification at 2% concentration and 110 °C autoclaving significantly improves glucose recovery by up to 6-fold compared to untreated biomass (Obeng et al., 2021). Durian peel has a complex fibrous structure and high lignin content, significantly hindering biodegradability and enzymatic accessibility (Yan Sim et al., 2024). Alkaline delignification can effectively delignify the waste by increasing cellulose content from approximately 66% to over 90% (Savitri et al., 2023) as well as reducing lignin and hemicellulose fractions, thereby facilitating subsequent enzymatic hydrolysis (Premjet and Premjet, 2025). Despite the existing literature, most studies have only focused on characterizing delignification processes or applying single methods without thoroughly investigating the integrated effects of alkaline delignification combined with enzymatic hydrolysis, specifically tailored for durian peel. Enzymatic hydrolysis has emerged as a pivotal technology for the efficient conversion of lignocellulosic biomass into glucose, serving as a cornerstone for the sustainable production of biofuels and biochemicals (Guo et al., 2023; Li et al., 2022). The process operates under mild conditions (Saorin Puton et al., 2025; Serrano-Febles et al., 2025), typically at moderate temperatures and near-neutral pH (Ruan et al., 2024). This helps to minimize energy consumption (Saini et al., 2022; Simon et al., 2025), reduce equipment corrosion (Baena et al., 2022; Sapatinha et al., 2025; Zheng et al., 2025), and lower operational costs compared to conventional acid hydrolysis (Climent Barba et al., 2022; Sahu et al., 2025). Enzymatic hydrolysis is highly selective, enabling the targeted breakdown of cellulose and hemicellulose into fermentable sugars with minimal formation of inhibitory by-products (Ghinea et al., 2025; Lourens et al., 2025). The specificity not only enhances glucose yields but also facilitates downstream fermentation by reducing the presence of toxic compounds. In addition, the process supports high-solids loadings, leading to concentrated sugar streams that improve economics and scalability. Therefore, this study aims to investigate the synergistic effects of NaOH concentration and enzyme concentration on hydrolysis during glucose production from durian peel. The process involved alkaline delignification followed by enzymatic hydrolysis. Additionally, the study seeks to determine the pretreatment kinetics involved in this process. The effectiveness of NaOH delignification in disrupting the lignocellulosic matrix of durian peel to enhance enzymatic accessibility was also assessed. The results are expected to help in optimizing key process parameters, such as alkali concentration, reaction time, and enzyme concentration involved in hydrolysis. This study filled existing gaps by concurrently fine-tuning both alkali and enzyme dosages to enhance the efficiency of hydrolysis and maximize sugar production. The lignocellulose content of both raw material and delignification product was analyzed to enhance the optimum delignification process. Lignin content was analyzed using the SNI 0492: 2008 method, while cellulose was analyzed using the SNI 0444: 2009 method. The reducing sugar as a hydrolysis product was analyzed using a glucose refractometer, and water content was assessed with SNI 01-2354.2: 2006.

## MATERIALS AND METHODS

### Materials

Durian peel used as a raw material was sourced from the South Sumatra region. Cellulase enzymes, specifically Sigma-Aldrich cellulase from *Trichoderma reesei* with an activity of ≥700 units/g, were procured from Merck Indonesia. All chemicals such as NaOH, $H_2O_2$, potassium dichromate, ferrous ammonium sulfate, sulfuric acid, ammonium sulfate, benzene, ethanol, distilled water, buffer, citric acid, and dinitro salicylic acid were of analytical grade and used without further purification. The cellulase enzyme was stored at 4 °C to maintain stability and activity before use. Enzymatic hydrolysis experiments were conducted under controlled conditions, typically at pH 5.0 and 50 °C, with enzyme loadings optimized based on preliminary assays to ensure efficient substrate conversion.

### Preparation materials

During sample preparation, durian peel was chopped into small, uniform pieces to promote even drying. The pieces were initially sun-dried

for approximately 6 hours using a commercial solar drying system (Model DR-6P(S), Dryfree), which maintains an average drying temperature of 40–50 °C with controlled airflow to optimize moisture removal. Subsequently, the peel underwent oven drying in a Memmert UN55 convection oven at a controlled temperature of 105 °C for 8 hours. The oven temperature was calibrated using a NIST-traceable thermometer to ensure accuracy within ±1 °C. Moisture content was monitored at regular intervals during drying by weighing samples until a constant mass was achieved, indicating residual moisture below 5%. Following complete drying, the peel was milled using a Retsch SM 100 cutting mill and passed through a 60-mesh stainless steel sieve (Endecotts, UK) to obtain a standardized particle size distribution suitable for further analysis.

## Delignification process

A total of 30 g of durian peel powder was mixed with 330 mL of NaOH solutions at concentrations of 2%, 3%, 4%, 5%, and 6% (w/v). The mixtures were heated in a thermostatically controlled water bath (Memmert WNB 7-45) maintained at 100 °C for reaction times of 65, 75, 85, 95, and 105 min, respectively, to investigate optimal delignification conditions. After reaction, the mixtures were vacuum-filtered, and solids were washed repeatedly with distilled water until a neutral pH was confirmed using a Hanna Instruments HI98103 pH meter calibrated daily with certified pH buffers (pH 4.01, 7.00, and 10.01). The washed residues were dried at 105 °C in a Memmert UN55 drying oven until constant weight to ensure complete moisture removal. Cellulose content was determined according to the Indonesian National Standard SNI 0444:2009, which quantifies α-cellulose by a redox back-titration method. The titration reagent preparation involved fresh standardization of potassium dichromate solution at 0.5 N, prepared by dissolving analytical 99% potassium dichromate in distilled water. The ferrous ammonium sulfate titrant at 0.1 N was standardized daily against the potassium dichromate primary standard to ensure precise normality. During analysis, 25 mL of pulp filtrate was mixed with 10 mL of potassium dichromate and carefully acidified with 50 mL concentrated sulfuric acid under cooling conditions to prevent decomposition. The mixture was then heated to 130 °C for 15 min to facilitate oxidation. After cooling, the residual Cr (VI) was back-titrated with standardized ferrous ammonium sulfate using ferroin as an indicator until a persistent purple endpoint was reached. The α-cellulose content (X) was calculated using the following formula:

$$X = 100 - \left(\frac{6.85\,(V_1 - V_2) \times N \times 20}{A \times W}\right) \quad (1)$$

where: $V_1$ denotes the volume of titrant utilized in the blank titration (mL), $V_2$ represents the volume of titrant consumed during the sample titration (mL), $N$ corresponds to the normality of the ferrous ammonium sulfate titrant (equivalents per liter), $A$ is volume of pulp filtrate analyzed (mL), and $W$ signifies the mass of the oven-dried pulp sample (g).

Lignin content analysis followed the SNI 0492:2008 standard, using the Klason method, which specifies the quantification of acid-insoluble lignin in pulp or wood samples. Initially, 10 g of air-dried pulp or 5 g of wood powder (particle size 0.25–0.40 mm) was extracted with a 2:1 mixture of benzene and ethanol to remove extractives. The extracted sample was hydrolyzed with 72% (w/w) sulfuric acid at 20 ±1 °C for 2 h under constant stirring with a glass rod to ensure uniform reaction. After hydrolysis, the acid concentration was reduced to approximately 3% by dilution with distilled water (300 mL for wood samples and 400 mL for pulp, adjusted to final volumes of 575 mL and 1540 mL, respectively). The sample was then heated to boiling and maintained at reflux for 4 h with a condenser to prevent evaporation. After cooling, the mixture was filtered through pre-weighed glass fiber filters to collect the acid-insoluble residue, which is primarily lignin. The residue was washed repeatedly with hot distilled water until a neutral pH was achieved, ensuring removal of residual acid and soluble carbohydrates. The filter and residue were dried at 105 °C to constant weight, and the lignin content (x) was calculated as:

$$x = \frac{oven\text{–}dry\ weight\ of\ lignin\ residue\ (g)}{oven\text{–}dry\ weight\ of\ original\ sample\ (g)} \times 100\% \quad (2)$$

All procedures were performed in triplicate to ensure reproducibility and accuracy, with equipment subjected to routine preventive maintenance and calibration verification quarterly using certified reference materials.

### Hydrolysis process

Approximately 5 g of delignified biomass was suspended in 250 mL of distilled water, to which cellulase enzyme was added at varying concentrations of 0.25%, 0.5%, 0.75%, 1.0%, 1.25%, and 1.5% (w/v). The enzymatic hydrolysis was carried out at 40 °C in a 50 mM citrate-phosphate buffer system adjusted to pH 5, which provides optimal pH stability for cellulase activity throughout the 48-h incubation period. The reaction mixture was continuously agitated at 150 rpm using an orbital shaker to ensure uniform substrate-enzyme interaction and minimize mass transfer limitations. The cellulase enzyme, derived from *Trichoderma reesei* and exhibiting an activity of approximately 700 U/g, was sourced from Sigma-Aldrich and stored at 4 °C before use to maintain enzymatic efficiency. Post-hydrolysis, samples were collected for quantitative analysis of reducing sugars to evaluate the hydrolytic performance relative to enzyme dosage and reaction duration. The citrate-phosphate buffer (50 mM, pH 5.0) used to maintain optimal pH during enzymatic hydrolysis was prepared by dissolving 18.15 g of sodium phosphate dibasic dihydrate and 9.61 g of citric acid in approximately 800 mL of distilled water. The pH was adjusted to 5 using 1 M HCl or 1 M NaOH, and the solution was diluted to a final volume of 1 L with distilled water, ensuring stable buffering capacity throughout the 48-h incubation. Enzyme activity was characterized using the filter paper unit (FPU) assay according to IUPAC standards, where one FPU corresponds to the amount of enzyme producing 1 µmol of reducing sugar per minute from filter paper substrate at 50 °C and pH 5. Glucose release during the assay was quantified by the dinitro salicylic acid (DNS) method, measuring absorbance at 540 nm. For sugar quantification in hydrolysis samples, aliquots were centrifuged at 10.000 x g for 10 min to remove solids. The supernatant was reacted with DNS reagent, boiled for 5 min, then cooled before absorbance measurement at 540 nm. Calibration curves constructed from glucose standards (0–1 mg/mL) enabled accurate determination of reducing sugars released. All measurements were replicated thrice to ensure statistical reliability.

## RESULTS AND DISCUSSION

### The effect of NaOH concentration and reaction time on the delignification process

This study showed that the concentration of NaOH in the delignification process of durian peel greatly affected lignin degradation. The decrease in lignin content caused cellulose content to be concentrated (Table 1). Furthermore, delignification of durian peel using 6% NaOH achieved an impressive lignin removal efficiency of 95.67% in a reaction time of 105 min (Figure 1), demonstrating the high effectiveness of moderate alkali concentrations under optimized conditions. This performance surpassed many previously reported studies where even higher NaOH concentrations, such as 10%, required extended reaction times up to 12 h to achieve significantly lower lignin removal efficiencies, approximately 40 to 50% (Rahmanto et al., 2022). The superior lignin breakdown at 6% NaOH suggested that factors beyond alkali concentration, such as temperature, biomass particle size, and mixing intensity, played significant roles in enhancing delignification efficiency. Literature indicated that using NaOH concentrations below 6% often resulted in moderate lignin removal and necessitated longer processing times or additional treatments to attain comparable cellulose purity. Pretreatment with 6% NaOH effectively and quickly removed lignin from the durian peel (Savitri et al., 2023). This aligns with other studies on alkaline pretreatment, which show the need to balance the chemicals used and processing time to protect the cellulose. These results are important for turning agricultural waste into valuable products, providing a better way to make bioethanol and cellulose more affordably and in higher quantities. Using NaOH to remove lignin is a strong pretreatment method for breaking up the complicated structure of lignocellulose. This process improves the availability of cellulose and breaks down lignin. It begins with NaOH dissolving in water, which releases hydroxide ions ($OH^-$). These ions then get into the biomass and specifically break the chemical links holding lignin to both hemicellulose and cellulose (Reyes et al., 2022).

The concentration of NaOH is crucial for removing lignin from durian peel and getting more cellulose. NaOH creates hydroxide ions ($OH^-$) that break the chemical bonds between lignin, hemicellulose, and cellulose. This includes breaking both ester and ether bonds (Falah et al.,

**Table 1.** The composition of samples after delignification

| NaOH concentration (%) | Reaction time (min) | Cellulose (%) | Lignin (%) |
|---|---|---|---|
| Untreated | 0 | 56.12 | 23.11 |
| 2 | 65 | 67.39 | 17.37 |
| | 75 | 70.86 | 15.85 |
| | 85 | 74.33 | 14.32 |
| | 95 | 76.82 | 13.16 |
| | 105 | 78.35 | 12.37 |
| 3 | 65 | 70.01 | 16.65 |
| | 75 | 73.00 | 15.10 |
| | 85 | 75.98 | 13.55 |
| | 95 | 78.06 | 12.37 |
| | 105 | 79.24 | 11.54 |
| 4 | 65 | 72.08 | 15.63 |
| | 75 | 74.63 | 14.28 |
| | 85 | 77.18 | 12.93 |
| | 95 | 78.96 | 11.80 |
| | 105 | 79.99 | 10.87 |
| 5 | 65 | 73.61 | 14.32 |
| | 75 | 75.77 | 13.39 |
| | 85 | 77.92 | 12.46 |
| | 95 | 79.53 | 11.45 |
| | 105 | 80.60 | 10.36 |
| 6 | 65 | 74.66 | 12.70 |
| | 75 | 76.63 | 12.41 |
| | 85 | 78.59 | 12.12 |
| | 95 | 80.15 | 11.32 |
| | 105 | 81.31 | 10.01 |

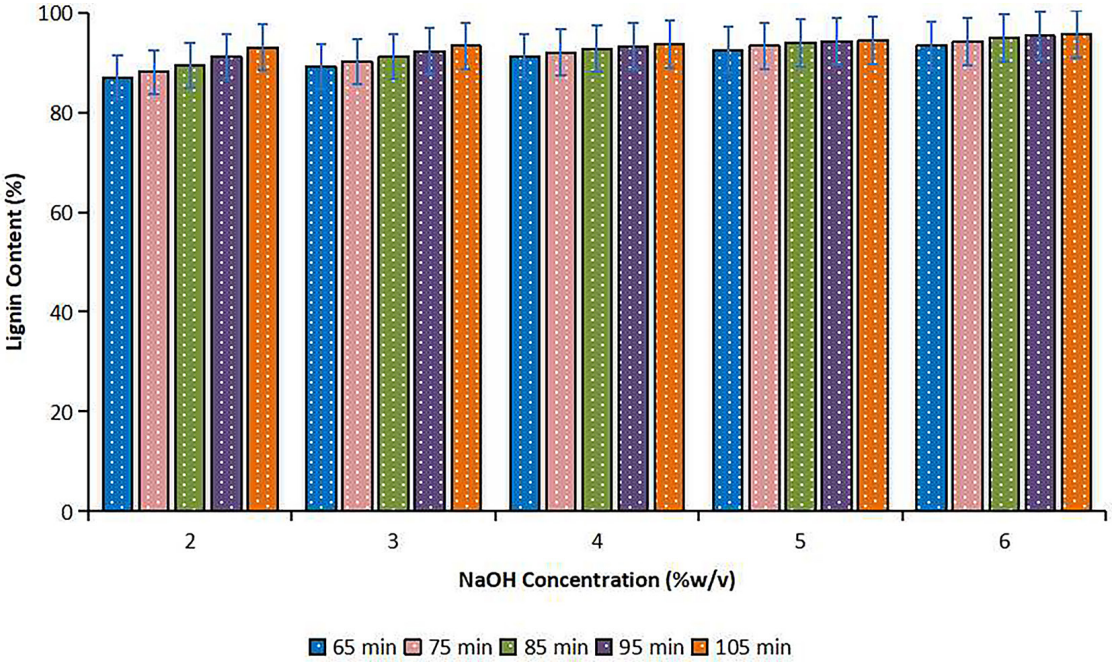


**Figure 1.** Lignin removal after delignification

2020; Pinto et al., 2022; Premjet and Premjet, 2025), which leads to the depolymerization and solubilization of lignin. Simultaneously, NaOH disrupts hydrogen bonds and swells the biomass fibers, thereby increasing porosity and reducing cellulose crystallinity (Gomes et al., 2021). These combined actions significantly enhance cellulose accessibility for further processing. During the NaOH delignification process, extending the reaction time promotes greater lignin degradation because it allows the alkaline solution to penetrate deeper into the biomass structure (Irawan et al., 2020; Shah et al., 2023). NaOH produces a high concentration of hydroxide ions ($OH^-$), which function as strong nucleophiles and bases that target the specific bonds linking lignin to hemicellulose and cellulose (Ben Mrad et al., 2021; Choudhury et al., 2022). While short reaction times primarily cleave easily accessible, surface-level bonds (Rahayu et al., 2022), longer durations allow these ions to diffuse deeper and disrupt buried bonds. This sustained exposure leads to the continuous cleavage of ester and ether bonds, along with further swelling of the plant matrix, ultimately enhancing the overall diffusion and accessibility of the chemical agents.

In this study, delignification using NaOH effectively degraded the lignin bond. Previous studies showed that the use of 2% NaOH at 120 °C for 60 min applied to rice straw removed about 42% lignin (Aziz et al., 2023). The 8% NaOH used in delignification of sorghum bagasse for 30 min decreased lignin by 10.12% (Utoro et al., 2023). Across various substrates—including wheat straw, oil palm empty fruit bunches, birchwood, eucalyptus, pinewood, sugarcane bagasse, grass residues, hybrid Pennisetum, and kapok wood—the reported lignin removal efficiencies generally ranged between 50% and 85%, often necessitating higher NaOH concentrations (up to 40%) and elevated temperatures (exceeding 130 °C) or more intensive pretreatment conditions. For instance, wheat straw treated with NaOH-based pretreatment at significantly elevated temperatures and microwave-assisted processes yielded lignin removals of approximately 74% (Mikulski and Kłosowski, 2022), while empty oil palm bunches exhibited efficiencies near 78% under alkaline conditions spanning 2% to 6% NaOH concentrations (Tayon et al., 2024). Similarly, grass waste compositions presented lignin removal efficiencies around 85% employing 4–10% NaOH with longer reaction durations (Yan et al., 2020). In light of these comparative benchmarks, the present study's lignin removal efficiency of 95.67% at a moderate alkali concentration of 6% and a relatively mild reaction temperature and time presents a compelling advancement. This superior efficacy underscores the inherent amenability of durian peel's lignocellulosic matrix to alkaline delignification, potentially attributable to its distinct lignin architecture and the optimized reaction conditions employed. Moreover, achieving such a high degree of lignin solubilization with relatively mild alkali concentration and moderate thermal exposure enhances the economic and environmental viability of the pretreatment process.

## Reducing sugar production

Hydrolysis of cellulose to produce reducing sugar was carried out with cellulose enzyme at

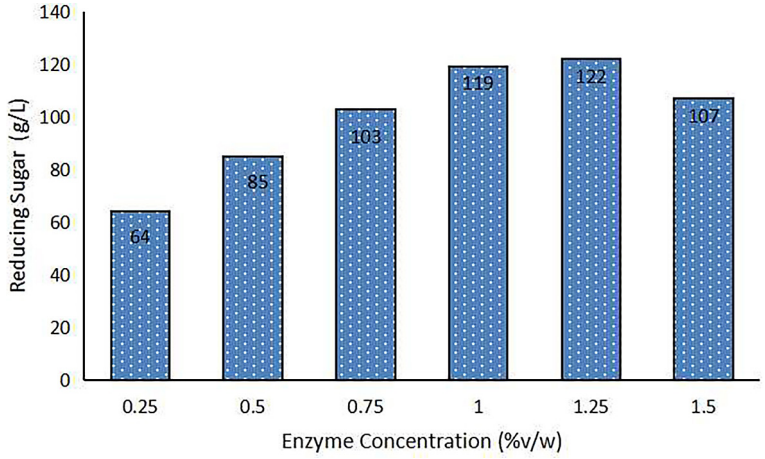


**Figure 2.** The effect of enzyme concentration on reducing sugar production

various concentrations (0.25, 0.5, 0.75, 1, 1.25, 1.5 % v/w). Figure 2 shows the effect of enzyme concentration on reducing sugar production. The result of this study revealed that the optimum enzyme in hydrolysis was 1.25% w/v, which produced 122 g/L reducing sugar. The decrease in reducing sugar production at an enzyme concentration of 1.5% can be attributed to enzyme inhibition or substrate saturation, where excessive enzyme molecules compete for limited substrate binding sites, leading to inefficient hydrolysis. Increasing the enzyme concentration in cellulose hydrolysis generally led to higher reducing sugar production because more enzyme molecules were available to bind to and break down cellulose chains into sugar. Cellulase enzymes catalyze the hydrolysis of β-1,4-glycosidic bonds in cellulose, cleaving long polymer chains into shorter sugar chains. When the enzyme concentration increased, there were more active sites to attack cellulose simultaneously, accelerating the rate of cellulose degradation and increasing the amount of sugar released in a given time. This higher availability overcame limitations such as enzyme saturation on the cellulose surface and increased conversion efficiency, specifically when sufficient cellulose substrate was present. Higher cellulose content and increased enzyme concentration generally exhibit a positive correlation with reducing sugar production, as more substrate availability and enzyme active sites enhance the hydrolysis rate and sugar yield. However, this proportionality is contingent upon effective delignification during the pretreatment stage, which removes lignin barriers, thereby increasing cellulose accessibility; without sufficient delignification, even high cellulose content and enzyme concentration may not translate to higher reducing sugar production due to limited enzyme penetration and substrate exposure. Thus, delignification is a crucial prerequisite to realize the full potential of cellulose content and enzyme loading for efficient sugar release. Several studies have shown that increasing cellulase concentration led to higher glucose yield, up to a point where the substrate became the limiting factor or enzyme inhibition occurred (Table 2). The results confirmed that higher enzyme loading enhanced cellulose breakdown efficiency and glucose release. Based on Table 2, a higher reduction of sugar in hydrolysis was achieved. In addition, the amount of reducing sugar produced was significantly influenced by the cellulose obtained during the delignification process. This indicated that the enzymes used in hydrolysis were effective in converting cellulose into reducing sugar.

The elevated lignin removal percentage observed here can be attributed to the synergy of optimized alkali concentration, sufficient reaction duration, and the morphological characteristics of durian peel that may favor alkali penetration and lignin solubilization. Moreover, the significant increase in cellulose content concomitant with lignin reduction highlights the efficiency of the delignification process to yield cellulose-rich substrates amenable to downstream enzymatic hydrolysis. The optimized alkali concentration of 6% NaOH effectively disrupts the complex lignin-carbohydrate matrix within the biomass. Alkali solutions selectively cleave ester bonds and ether linkages connecting lignin to hemicellulose and cellulose, thereby facilitating lignin solubilization (Puss et al., 2023). When the alkali concentration is optimized, as in this study, it achieves a balance whereby sufficient reactive species are present to maximize lignin removal without excessive degradation of cellulose or formation of inhibitory by-products (Chen et al., 2021; Gomes et al., 2021). This selective depolymerization is crucial for maintaining high cellulose integrity for subsequent applications. The significant concomitant increase in cellulose content following lignin removal underscores the process efficiency (Melro et al., 2020). As lignin is removed, the cellulose fraction becomes more

**Table 2.** Total reducing sugar production from various substances

| Substate | Cellulose enzyme concentration | Reaction time (h) | Reducing sugar (g/L) | Ref |
|---|---|---|---|---|
| Rice husk | 20 % v/w | 25 | 1.80 | (Efrinalia et al., 2022) |
| Rice straw | 50–200 FPU/mL | 20 | 27.89 | (Jin et al., 2020) |
| Potato peel waste | 92 U | 72 | 12.06 | (Chauhan et al., 2022) |
| Wheat straw | 0.866 ± 0.067 U/mL | 120 | 3.6 | (Danso et al., 2022) |
| Sisal cellulose | 0.9 mL/g | 15 | 27.7 | (Kaschuk et al., 2019) |
| Durian peel | 1.25 % v/w | 48 | 122 | This study |

exposed and enriched within the biomass residue. This cellulose-rich substrate exhibits enhanced amenability to enzymatic hydrolysis, a vital step for downstream production of fermentable sugars and biofuels. Retention of cellulose integrity is critical; thus, the selective delignification strategy preserves the polysaccharide fraction while effectively removing the phenolic lignin compounds (Savitri et al., 2023).

## CONCLUSIONS

In conclusion, pretreating durian peel with NaOH effectively degrades lignin while enriching cellulose content. Treatment with 6% NaOH for 105 minutes resulted in 95.67% lignin removal and an 81.31% increase in cellulose concentration. The substantial reduction in lignin content significantly enhanced the enzymatic hydrolysis of cellulose into reducing sugars. In the present study, cellulase enzyme was applied at varying concentrations relative to the cellulose content to determine the optimal yield. The highest reducing sugar concentration of 122 g/L was obtained using 1.25% (w/v) cellulase.

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