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Increasing enzymatic saccharification of saccharide polymers in lignocellulosic biomass with subcritical water treatment

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Abstract

Oil palm trunk (OPT) is one of the wastes from logging oil palm trees that are no longer productive. OPT contains cellulose, hemicellulose, and lignin, which can be hydrolyzed to produce fermentable sugar and further converted into biofuel and value-added chemicals for food ingredients such as xylitol, lactic acid, dietary fiber, prebiotics, and compounds for food packaging. The OPT pretreatment aims to break down the recalcitrant by breaking down the lignin bonds to increase the efficiency of enzymatic hydrolysis of polysaccharides. This study aimed to apply the pretreatment with the subcritical water (SCW) method at 170℃, 400 psi, for 20 mins on OPT samples and confirm the achievement of the delignification objective in the samples by conducting chemical component analysis and observing changes in the X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR) spectra of raw OPT samples and which has been given pretreatment and enzymatic saccharification analysis. The findings from the study revealed that pretreatment with the SCW method reduced the levels of lignin and hemicellulose by 30.43% and 42.53%, respectively, while the percentage of cellulose increased by 50.34%. XRD and FTIR spectroscopy analysis also revealed the change in the structure of the pretreated OPT. The total reducing sugar of the OPT samples after enzymatic hydrolysis increased in the pretreated sample, which was 11 g/g, compared to the raw OPT, which was 8 g/g. SCW pretreatment method at 170°C, 400 psi, for 20 mins was able to give a delignification effect and increase saccharification in enzymatic hydrolysis of OPT.

1. Introduction

Indonesia is the world's biggest producer of palm oil. Empty fruit bunches (EFB), palm kernel shells (PKS), mesocarp fiber (MF), oil palm fronds (OPF), and oil palm trunks (OPT) are all forms of solid waste generated by the oil palm industry (plantation and milling). These remaining waste materials, known as oil palm biomass (OPB), cause significant environmental degradation, jeopardizing the oil palm industry's viability. Employing thermochemical methods (torrefaction and pyrolysis) to enhance OPB has generated considerable attention to reuse leftovers while limiting environmental impact (Nabila *et al*., 2023). The natural lignocellulosic OPT biomass comprises a high concentration of polymeric carbohydrates, primarily cellulose, hemicellulose, and lignin. Cellulose and hemicellulose are fermentable sugar sources. Due to its high carbohydrate content, lignocellulosic biomass (LCB) is the most potential, cost -effective, and sustainable substrate for biofuel production. Sugar produced from the hydrolysis of lignocellulosic biomass also plays an important role in producing some chemicals for food ingredients. Some of the lignocellulosic raw materials include corncobs, wheat straw, corn stover, wheat bran, and miscanthus, which are used in the production of xylitol (Rao *et al*., 2016). Low-priced lignocellulosic biomass was used as a substrate to produce organic acid with high yield, concentration, and productivity (Akhtar *et al*., 2014).

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The conversion of lignocellulosic biomass to prebiotic oligomers via controlled enzymatic saccharification, including lignocellulose-derived prebiotic oligosaccharides (LDOs), gives an alternative to functional foods (Saini *et al*., 2022).

Lignin is essential for avoiding microbial invasion, protecting plants from diseases and insect invasion, and improving plant structural support. However, research has demonstrated that lignin inhibits the cellulase hydrolysis process. Furthermore, the lignin-derived degradation created during the pretreatment process may restrict cellulase function further.

Pretreatment of the biomass is required to remove lignin and increase the amount of hydrolysis using enzymes and overall sugar production. The optimal pretreatment should have the following effects: (1) enhance sugar formation or capacity to produce sugars after enzymatic digestion, (2) prevent carbohydrate degradation or loss, (3) avoid the formation of residues that restrict subsequent hydrolysis and fermentation processes, (4) cost-effectiveness, and (5) environmental sustainability. The decreased access of cellulose to enzymes can be caused by the adsorption of cellulase by lignin, making it ineffective in hydrolysis process activity. The complex and resistant structure of lignin-carbohydrate also reduces the accessibility of cellulose to enzymes (Li *et al*., 2022).

Cellulose molecules can be transformed into fermentative sugar by chemical reaction (e.g., acid hydrolysis) or enzymatic saccharification. The resulting sugar then changes to bioethanol and other products with added value. Enzymatic reaction, in contrast to acid hydrolysis, offers several benefits, including high product yield, gentle reaction conditions, low energy needs, and reduced pollution. However, the presence of naturally resistant components limits the enzymatic saccharification of lignocellulosic substrates. The complex cellulose-hemicellulose-lignin structure broke during the preparation procedure.

Lignocellulosic biomass in oil palm trunks is recalcitrant. In general, lignocellulosic biomass conversion into bioproducts includes three main stages: pretreatment, hydrolysis, and fermentation by microorganisms to produce specific products. The pretreatment aims to break down the resistance of the biomass compounds. The pretreatment stage attempts to maximize enzyme exposure to sugar polymers (cellulose and hemicellulose) to produce efficient hydrolysis with the least energy expense, inhibitor chemicals generated, and maximum sugar recovery. Sun *et al*. (2016) describe pretreatment as the process of transforming lignocellulosic biomass from its original form to a more accessible state for efficient enzymatic hydrolysis.

One method for pretreatment that is widely applied is the hydrothermal process, which is an approach in which water in the liquid phase or the vapor phase is used for the treatment of lignocellulosic biomass (Hu and Ragauskas, 2012). Subcritical water is an alternative pretreatment that is widely used. With this method, lignocellulosic biomass is heated with water at high temperature and pressure, causing water molecules to become radicals, which will react with molecules in the biomass. Because of its simplicity and lack of a chemical reagent, liquid hot water pretreatment is offered as a cost -effective green pretreatment approach (Li *et al*., 2022). This method is relatively mild, does not require a catalyst, and does not cause significant corrosion problems. Water enters the biomass under high pressure, hydrates the cellulose, and removes considerable amounts of hemicellulose and a minor fraction of lignin. The solubility of hemicellulose is catalyzed by hydronium ions resulting from the auto-ionization of water. Controlling the pH around neutral can minimize the formation of inhibitors.

Subcritical water is one of the most widely used pretreatment alternatives. With this method, lignocellulosic biomass is heated with water at high temperature and pressure, causing water molecules to become radicals, which will react with molecules in the biomass. Utilization of high-temperature water is a pretreatment primarily aimed at removing lignin from lignocellulosic biomass. With this method, lignocellulosic biomass contact with hot water at temperatures ranging from 150 to 260°C at a pressure of up to 3 MPa (Pangsang *et al*., 2019). Pressure is applied to prevent the evaporation of water and is not immediately released so that an explosion does not occur. Cellulose becomes more susceptible to enzymatic hydrolysis because the pretreatment hydrolyzes hemicellulose and removes lignin (Cardona *et al*., 2018). Under these reaction conditions, H_3O^+ acts as an autocatalyst to break glycosidic bonds and increase the accessibility of enzymes for efficient hydrolysis (Nadia *et al*., 2018). Muda *et al*. (2019) have studied the application of the subcritical water method to palm oil trunks, but this research aims to use pyrolysis to produce bio-oil. In this research, the subcritical water method was studied for the pretreatment of oil palm trunk waste to see the effect of this treatment on the delignification of oil palm trunk so that it is expected to increase exposure to enzymes during the hydrolysis stage of the saccharification process.

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2. Materials and methods

2.1 Materials

The sample in this research was obtained from chopped OPT from a 27-year-old plant in an oil palm estate in Riau, Indonesia. Samples from oil palm plantations were taken and put in plastic bags, then sent from the plantation to the laboratory location at room temperature (28–31°C) within 48 hrs.

2.2 Subcritical water pretreatment

The apparatus consists of a reactor with a heating element, a thermocouple and a pressure gauge. The reactor was constructed of stainless steel and has a capacity of 200 mL. It can withstand pressures of up to 100 MPa. Nitrogen gas (99.9% purity) was utilized to maintain reactor pressure during SCW treatments. Nitrogen gas was injected at a pressure of 20 kg/cm^2. The experiment was carried out at 170°C, 400 psi (2.758 MPa) for 20 mins, and the ratio of dry sample to water was 7:1 (mL/g). Following pretreatment, the reactor was cooled to ambient temperature, and the solid was collected by filtering with Whatman paper to separate liquids and solids. The solids were washed with distilled water three times for 100 mL each. The solid washing setting was dried at 60°C for 24 hrs to produce OPT powder and stored in the freezer before being used in the study.

2.3 Analysis of oil palm trunk component

Analysis of the components of lignocellulosic biomass of oil palm trunks is based on the assumption that the overall structure of oil palm trunks is only composed of cellulose, hemicellulose, lignin, and extractives as was done according to the method described by Lin *et al*. (2010). The extractives were analyzed by measuring the weight of the OPT lost after a mixture of 1 g of dried OPT with 60 mL of acetone and was incubated at 90℃ for 2 hrs. Subsequent analysis used OPT that had been treated with acetone to prevent interference from extractives which resulted in measurement errors of lignin and carbohydrates. Strong alkaline aqueous solutions can dissolve lignin. The hemicellulose composition was determined by measuring the weight lost after the OPT (which was free of extractives) was treated with 10 mL of 0.5 M NaOH and then heated to 80℃ for 3.5 hrs. To measure the lignin content, 1 gram of OPT (which is free from extractives) is mixed with 98% (v/v) H_2SO_4 and left at room temperature for 24 hrs then heated at 100℃ for 1 hr, which causes the lignin to precipitate while the cellulose and hemicellulose are completely hydrolyzed. The weight of the precipitate after drying at 105℃ is the weight of lignin in oil palm trunks. Cellulose content was calculated based on the difference between the total weight of lignin, hemicellulose, and extractives in 1 g of OPT.

2.4 X-ray diffractometer analysis

X-ray Diffractometer (Rigaku D/Max 2500, Japan) was used to assess the crystallinity indices of raw and processed OPT samples. The sample was initially firmly packed in a rectangular glass cell with dimensions of 15×10 mm and a thickness of 1.5 mm. Cu Kα (λ = 0.154 nm) radiation was employed at 40 kV and 30 mA. The grade range was 5 to 40, with a 0.02° step size. The cellulose crystallinity is calculated using the equation below:

$$TPC= (I_{002} - I_{am})/(I_{002})$$

Where I_{002} is the intensity of the crystalline part at 2θ = 22° and I_{am} is the peak for the amorphous part at 2θ = 18.7°

2.5 Fourier transform infrared analysis

FTIR spectra were recorded using a FTIR spectrometer (FTS3500, Bio Rad). Samples and KBr pellets for analysis were mixed at a ratio of 1:100. Each sample was recorded from 4000 to 400 cm^{-1} with a resolution of 2 cm^{-1} in transmission mode. One set of 64 scans was taken for each sample.

2.6 Enzymatic hydrolysis

Enzymatic hydrolysis was performed on 100 mL of 0.1 M sodium acetate buffer at pH 5.0 with a concentration of 10% (w/v, dry weight basis) OPT. The biomass was hydrolyzed in a shaking water bath (150 rpm) for 48 hrs at 50℃ using 20 FPU Cellic® CTec3 HS enzyme/g cellulose. After incubation at certain times: 10, 24, 34, and 48 hrs, the biomass mixture in the buffer was filtered using Whatman filter paper and the filtrate obtained was a sample for reducing sugar analysis. The effectiveness of saccharification was determined by measuring the reducing sugar concentration with a spectrophotometer at 546 nm and the 3.5-dinitrosalicylic acid (DNS) technique, with glucose as the standard.

3. Results and discussion

3.1 The effect of subcritical water pretreatment on the chemical composition of oil palm trunk

The chemical composition of OPT before and after the pretreatment is shown in Table 1. The data in the table revealed that the SCW pretreatment can reduce lignin and hemicellulose in OPT waste, so the percentage of cellulose increased. Water molecules at high temperature and pressure can become radicals which will

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Table 1. Chemical composition of OPT before and after pretreatment with the SCW method.

Sample	Content (%)				
OPT	Cellulose	Hemicellulose	Lignin	Ash	Moisture
Raw	44.22±1.21	26.03±1.40	16.76±1.13	7.89±0.99	5.10±0.70
Pretreated	66.48±0.12	14.96±0.12	11.66±0.27	0.87±0.02	6.02±0.03

Values are presented as mean±SD of triplicates.

react with molecules in the biomass, causing hemicellulose hydrolysis and removing lignin (Cardona *et al*., 2018). Under these reaction conditions, H_3O^+ acts as an autocatalyst to break glycosidic bonds. At 25°C, the dielectric constant ε of water is 78.5. This value decreases to 43.95 at 150°C/50 bar because the hydrogen bonds weaken with increasing temperature. Therefore, the polarity of water decreases with increasing temperature, and water becomes more non-polar and functions as a suitable solvent for organic compounds. In addition, when the temperature increases, the density of water decreases, which causes an increase in diffusivity, thereby increasing the degradation, dissolution of hemicellulose, and removal of some of the lignin in a dense biomass matrix (Nabila *et al*., 2023).

The results of this study are in line with several other studies that resulted in a decrease in lignin (delignification) and/or hemicellulose and an increase in the percentage of cellulose in biomass after pretreatment using the SCW or liquid hot water (LHW) method (Cardona *et al*., 2018; Mahmood *et al*., 2018; Nabila *et al*., 2023). Treatment with the SCW method at 165°C for 20 mins at 50 bar on water hyacinth (*Eichhornia crassipe*) caused an increase in cellulose content and a decrease in hemicellulose and lignin, respectively, from 25.0, 11.0, and 2.5% to 68.2, 0 and 0.9% (Thi *et al*., 2017). Empty palm fruit bunch (EPFB) biomass pretreated with LHW at 185°C for 30 mins resulted in a solid fraction with hemicellulose content decreasing to around 73.6% compared to pretreatment, while the cellulose content was relatively low, unchanged at 99.9% (Cardona *et al*., 2018). Pretreatment in an autoclave at 150°C for 20 mins at 400 rpm caused delignification of 23% in OPF (Mahmood *et al*., 2018). The loss of large amounts of lignin and hemicellulose will cause cellulose to be more open, making it more accessible to enzymes during the hydrolysis stage (Lai and Idris, 2016). Changes in the chemical composition of OPT compared to other studies that carried out pretreatment of OPT using various methods other than SCW are shown in Table 2.

The data in Table 2 reveals that different pretreatment methods result in varying chemical composition changes. Pretreatment with the SCW method on OPT has the same effect as pretreatment using Mw-A (Lai and Idris, 2016), 10% NaOH, and SSE -AA (Thamsee *et al*., 2018) which causes a decrease in hemicellulose and lignin content so that the percentage of cellulose increased markedly. Treatment with SSE did not cause significant changes in the content of cellulose, hemicellulose, and lignin, whereas treatment with the SAC method instead caused a decrease in cellulose and an increase in the percentage of hemicellulose and resulted in a very large reduction in the lignin content (Lai and Idris, 2016).

As previously explained, SCW treatment causes a decrease in hemicellulose and lignin and increases cellulose markedly because water molecules at high temperature and pressure can become radicals which will react with molecules in the biomass causing hemicellulose hydrolysis and remove lignin (Cardona et al., 2018). Pretreatment with Mw-A was also effective in releasing cellulose content from pests. Mw-heating was carried out at 80°C and atmospheric pressure. Under microwave heating, microwaves can penetrate the lignocellulose of the OPT and directly vibrate the molecules. Molecular oscillation causes a rapid increase

Table 2. Changes in the composition of OPT with various pretreatment methods.

Method	Content changes due to pre-treatment (%)			References
	Cellulose	Hemicellulose	Lignin	
Subcritical Water/SCW (170°C, 2.758 MPa, 20 mins)	(+) 57.27	(-) 42.53	(-) 30.43	This Research
Microwave-alkaline/Mw-A, (NaOH 2.5 M + microwave at 700 W, 80°C, 60 mins)	(+) 41.55	(-) 61.23	(-) 14.90	Lai and Idris (2016)
Steam-alkaline-chemical/SAC (NaOH 2.5 M + autoclave at 121°C, 0.12 MPa, 15 mins; + CH3COOH to pH 3)	(-) 15.91	(+) 73.91	(-) 89.31	Lai and Idris (2016)
NaOH 10%	(+) 29.09	(-) 2.37	(-) 23.44	Thamsee *et al.* (2018)
Superheated Steam Explosion/SSE (180°C, 0.6 MPa, 5 mins)	(+) 1.83	(-) 1.78	(+) 1.26	Thamsee *et al.* (2018)
Superheated Steam Explosion-Alkaline Autoclaving/SSE-AA (180°C, 0.6 MPa, 5 mins + NaOH at 121°C, 10–60 mins in an autoclave)	(+) 39.24	(-) 32.84	(-) 26.83	Thamsee *et al.* (2019)

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in temperature and eventually damages the lignocellulosic OPT structure. Such rapid oscillations disrupt the intermolecular and intra-molecular hydrogen bonds embedded within the lignocellulosic complexes. The significant decrease in hemicellulose content can be attributed to the ability of microwave irradiation to depolymerize the building blocks of hetero-polysaccharide sugars into oligosaccharides. Microwave heating transfers and induces direct heat to lignocellulosic biomass (such as pests) and eventually disrupts the structure of the hetero-polysaccharide sugar building blocks of hemicellulose. Thus, the structure of lignin and hemicellulose is disrupted and releases more cellulose (Lai and Idris, 2016).

The addition of NaOH to lignocellulosic biomass removes bonds and increases the porosity of lignocellulosic biomass, which causes an increase in the internal surface area, as well as increases the breakdown of the structural cross-links between lignin and polymer complexes, resulting in dissolved lignin (Thamsee et al., 2018). Sodium hydroxide (NaOH) solution causes intra-crystalline swelling in the fiber, which contributes to its effective pretreatment. During treatment, NaOH penetrates and causes swelling of both the amorphous and crystalline cellulose areas accessible to the reagent. The interaction of bases and lignocellulosic biomass causes the saponification of intermolecular ester bonds in the biomass. This causes swelling of the biomass beyond the dimensions of aqueous swelling, and favors increased enzymatic and microbiological penetration into the fine structure of the cell wall. The removal of these bonds and inorganic elements increases the porosity or voids in the lignocellulosic biomass thereby increasing the internal surface area, which in turn helps break the structural links between lignin-carbohydrate bonds and disrupts the structure of the lignin.

Conventional heating such as the autoclave method is only able to heat the surface of the biomaterial through conduction and convection, thus explaining why the SAC method has a lower cellulose content. The lignin content in the SAC pretreatment was reduced the most. This can be explained by the fact that the SAC samples were exposed to autoclave conditions with high temperature and pressure, such as 121°C and 0.12 MPa, and then the samples were exposed to extreme changes in pH, from pH 13 to 3.5 using CH3COOH. The existence of conditions of temperature, pressure, and extreme changes in pH contribute to a very large level of lignin reduction (Lai and Idris, 2016).

3.2 Effect of subcritical water pretreatment on X-ray diffraction spectra of oil palm trunk

The XRD profiles of raw (blue graph) and pretreated OPT with the SCW method (red graph) are shown in Figure 1. In Figure 1 the XRD profile of OPT has the same pattern as cellulose biomass in general which at a diffraction angle of 2θ has peaks/maximum intensities of primary diffraction in the range of 22° and 23° (representing constituents with crystalline structures/ regions), while the peaks/secondary maximal intensity is in the range of 16-18° (representing constituents with amorphous structures/regions) (Chen *et al*., 2010; Mohan *et al*., 2015; Lai and Idris, 2016).

Figure 1 clearly shows the presence of peaks confirming the crystalline and amorphous structure of the OPT biomass. It can be seen that the primary peak of the red graph has a slight decrease in height and a narrowing of the area which represents a reduction in the crystallinity structure although it is very limited. The change in the width of the crystal peaks reflects the intensity transformation in the hydrogen bonding of the cellulose molecule. Hydrogen bonding in the treated sample subjected to heating in water at high pressure is disrupted after the pretreatment and finally, the crystal structure is changed to an amorphous state. Water with very high pressure and temperature can act as an intra-crystalline swelling agent that can penetrate and expand both amorphous and crystalline-accessible regions (Liu *et al*., 2012). Therefore, destruction of the crystalline structure of cellulose occurs and the sequence of fibers in cellulose is distorted. As a result, the microfibrils emerge from the connected structure and become fully exposed, thereby increasing the outer surface and porosity of the

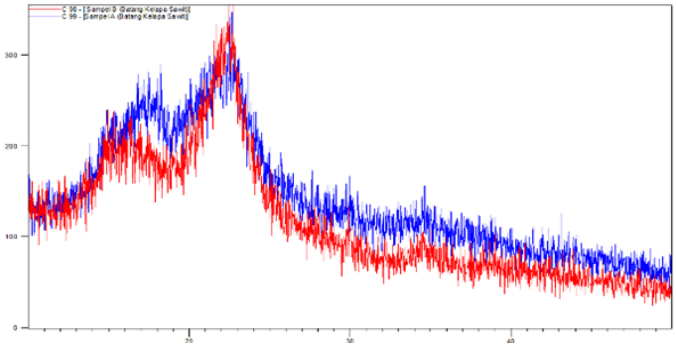

Figure 1. XRD spectra of raw (blue) and pretreated (red) OPT using SCW method.

The crystallinity index of the OPT sample from the XRD test results increased before the pretreatment of 20.99% to 50.00% after being pretreated with the SCW method at 170°C for 20 mins with a pressure of 400 psi. The results of this study are in agreement with the results of several studies (Mohan *et al*., 2015; Lai and Idris, 2016). Mohan *et al*. (2015) investigated the effect of pretreatment on bamboo samples using the SCW method at various temperatures ranging from 170-220°C for 40 mins. The crystallinity index of the bamboo samples increased from 65.83 to 71.9% with an increase in

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temperature from 170°C to 210°C for 40 mins. The results of the research by Lai and Idris (2016) also showed an increase in the crystallinity index in OPT that were pretreated with the microwave-alkali (Mw-A) and steam-alkaline-chemical (SAC) methods, from the initial 32.21% to 44.20 and 46.34%, respectively.

The increase in the crystallinity index of the pretreated samples indicated that the hydrolysis effect in the amorphous zone was greater than that of the crystalline zone, which resulted in an effective decrease in the amount of lignin and hemicellulose from the biomass structure, while the crystal structure did not change much. The loss of large amounts of lignin and hemicellulose causes a decrease in the diffraction peaks in the amorphous areas while the peaks in the crystalline areas tend to remain constant so that the ratio of the intensity of the primary and secondary peaks will increase resulting in a greater crystallinity index. The results of the analysis with XRD are in accordance with the results of the analysis of the chemical composition of OPT which showed a significant decrease in the levels of hemicellulose and lignin so that the percentage of cellulose increased.

3.3 The effect of subcritical water pretreatment on the Fourier transform infrared spectroscopy spectra profile of oil palm trunk

FTIR spectroscopy is used as an analytical tool to determine chemical changes in the structure of OPT and to understand the process of solubility of compounds in it during pretreatment. The FTIR spectra of raw and pretreated OPT with the SCW method are shown in Figure 2. The FTIR peaks related to the structure of cellulose, hemicellulose, and lignin were confirmed by comparing the research data with data available from the literature. Significant differences in the transmission and band shapes and locations of the functional groups in lignocellulosic biomass can be detected in the FTIR spectra.

In Figure 2 there appear to be several signals in the FTIR spectrum which show several peaks at certain wave numbers, as follows: peaks in the range 3200 – 3400 cm^{-1} which show strong bonds associated with the O–H hydrogen groups are present in both OPT samples. The peak in the range of 3200-3600 cm^{-1} indicates stretching of the O-H bond originating from the hydroxyl group of the hemicellulose residue. Peaks in the range of 2850 – 2970 cm^{-1} show that C-H stretching absorption is present in both OPT samples. Kubo and Kadla (2005) concluded that the peaks at wave numbers 2840 and 2937 cm^{-1} indicate C-H stretching associated with the presence of wood lignin. The peak in the range of 2054 – 2260 cm^{-1} indicates the presence of C=C groups present in both OPT samples. Peaks in the range of 1730 and 1735 cm^{-1} were present in the raw OPT samples but were absent after pretreatment. The peaks at wave numbers 1730 and 1735 cm^{-1} were obtained from the C=O strain in xylan in hemicellulose (Pandey and Pitman, 2003; Sills and Gosset, 2012). The disappearance of these peaks after pretreatment with SCW was caused by the presence of high-temperature water which could dissolve hemicellulose in the biomass. The peak at around 1600 cm^{-1} indicates the presence of aromatic C=C groups. These peaks were found in both OPT samples, with the intensity in the raw samples being greater than after pretreatment. Peaks at 1504, 1514, and 1593 cm^{-1} were present in the raw samples but were absent after pretreatment. The peaks at wave numbers 1500 and 1505 cm^{-1} were obtained from the vibration of the framework of the aromatic ring in lignin (Pandey and Pitman, 2003; Sills and Gosset, 2012). The peaks around 1594 and 1595 cm^{-1} also indicate vibration of the aromatic ring framework and stretching of the C=O group of lignin compounds (Sills and Gossett, 2012). The disappearance of the peaks in the wave numbers in the pretreated OPT samples proved that delignification had occurred due to the SCW treatment on the OPT causing damage to the chemical bonds between lignin and carbohydrates. Peaks in the range of 1050 – 1300 cm^{-1} indicate the presence of the C-O group from alcohol/ether/carboxylic acid/ester.

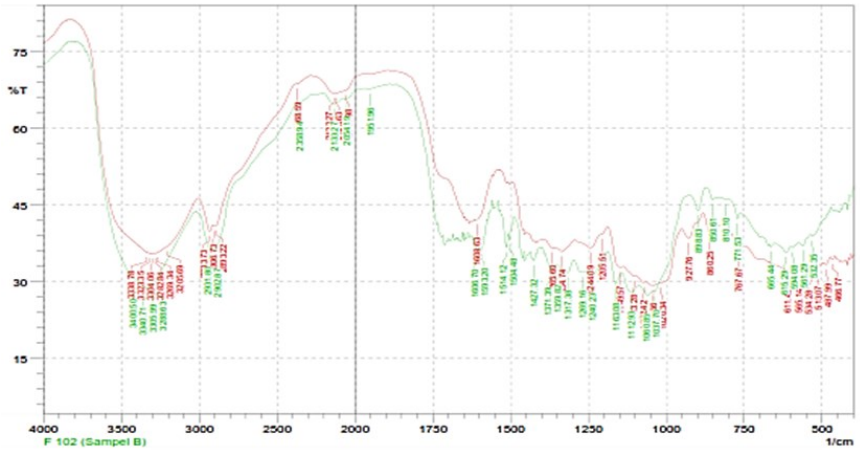

Figure 2. FTIR spectra of raw (green) and pretreated (red) OPT using SCW method.

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These peaks were found in both OPT samples, with the intensity in the raw samples being greater than after pretreatment.

3.4 Effect of subcritical water pretreatment on enzymatic saccharification of oil palm trunk

OPT is made of cellulose, hemicellulose, lignin, ash, and extractives, which are densely linked, resulting in a hard and resistant structure. Lignin is considered a major factor in limiting the enzymatic hydrolysis of lignocellulose. Lignin physically blocks enzymes from reaching cellulose and permanently adsorbs cellulase, decreasing its activity and reducing the hydrolysis process. The enzymatic breakdown of lignocellulosic biomass starts with the action of enzyme adsorption on the fiber surface. Enzymes could not access the carbohydrates in their natural state; thus pretreatment is essential to break down the highly structured lignocellulosic biomass structure and expose the cellulose and hemicellulose for enzymatic hydrolysis and the release of fermentable sugars. After hydrolysis with enzymes, the reducing sugar produced from the pretreated sample is greater than that of the raw OPT (Figure 3). Pretreatment with the SCW method applied to OPT samples caused lignin decomposition and hemicellulose dissolution causing cellulose to be more exposed to enzymes during the hydrolysis stage.

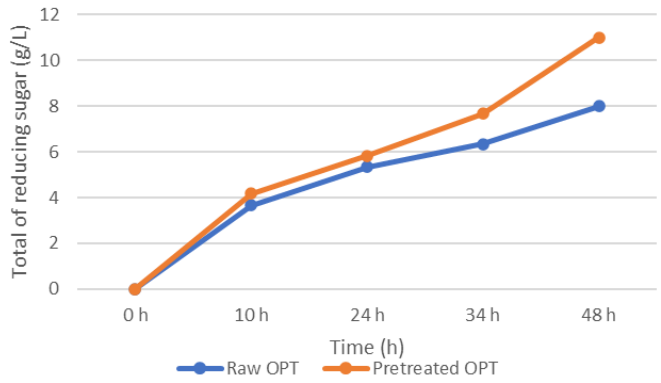

Figure 3. Total reducing sugar obtained from the enzymatic hydrolysis of raw and pretreated OPT.

Several studies have also reported an increase in sugar resulting from enzymatic hydrolysis after pretreatment with the hydrothermal method. When rice straw was processed for 5 hrs at 180°C, the hydrolysis rate rose from 14.7% to 72.9% (Hashemi *et al*., 2019). Nadia *et al*. (2018) investigated the effect of pretreatment on OPF at temperatures ranging from 170 to 210°C for 30 mins in batch autoclaves on the degree of saccharification after enzymatic hydrolysis. The amount of reducing sugars after enzymatic hydrolysis increased significantly from 24% for raw samples to 48.8% for pretreated samples at 210°C. The higher the temperature, the greater the degree of saccharification after enzymatic hydrolysis. The study by Thamsee *et al*. (2018) who gave superheated steam explosion (SSE) treatment with a temperature of 180°C, 0.6 MPa for 5 min on OPT showed an increase in saccharification efficiency after enzymatic hydrolysis. The yield of reducing sugar after enzymatic hydrolysis of raw and pretreated samples was 0.03 and 0.104 (g/g), respectively.

4. Conclusion

The results showed that pretreatment with the SCW method reduced the content of lignin and hemicellulose in OPT while the proportion of cellulose increased. XRD and FTIR analyzes also revealed changes in the structure of the pre-treated OPT. The SCW pretreatment method at 170°C, 400 psi, for 20 mins caused delignification and increased saccharification efficiency after enzymatic hydrolysis of OPT. Pretreatment with the SCW method has the potential to be applied to OPT to produce sugar which can be further fermented into biofuels and value-added chemicals for food ingredients.

Conflict of interest

The authors declare no conflict of interest.

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References

Akhtar, J., Idris, A. and Lai, L.W. (2017). Pretreatment of lignocellulosic biomass for organic acid production, In Biotechnology Development in Agriculture, Industry and Health – Advanced Conversion Technologies for Lignocellulosic Biomass Edition. Vol 3. Malaysia: Penerbit UTM Press

Cardona, E., Liano, B., Penuela, M., Pena, J. and Rios, L.A. (2018). Liquid-hot-water pretreatment of palm oil residues for ethanol production: An economic approach to the selection of the processing conditions. *Energy*, 160, 441-451. https://doi.org/10.1016/j.energy.2018.07.045

Chen, X., Jiang, Z-H., Chen, S. and Qin, W. (2010). Microbial and bioconversion production of d-xylitol and its detection and application. *International Journal of Biological Sciences*, 6(7), 834-844. https://doi.org/10.7150/ijbs.6.834

Hashemi, S.S., Karimi, K. and Mirmohamadsadeghi, S.

(2019). Hydrothermal pretreatment of safflower straw to enhance biogas production. *Energy*, 172, 545–554. https://doi.org/10.1016/j.energy.2019.01.149

Hu, F. and Ragauskas, A. (2012). Pretreatment and Lignocellulosic Chemistry. *BioEnergy Research*, 5, 1043-1066. https://doi.org/10.1007/s12155-012-9208-0

Kubo, S. and Kadla, J.F. (2005). Hydrogen bonding in lignin: a Fourier transform infrared model compound study. *Biomacromolecules*, 6, 2815-2821. https://doi.org/10.1021/bm050288q

Lai, L.W. and Idris, A. (2016). Comparison of steam-alkali-chemical and microwave-alkali pretreatment for enhancing the enzymatic saccharification of oil palm trunk. *Renewable Energy*, 99, 738-746. https://doi.org/10.1016/j.renene.2016.07.059

Li, X., Shi, Y., Kong, W., Wei, J., Song, W. and Wang, S. (2022). Improving enzymatic hydrolysis of lignocellulosic biomass by bio-coordinated physicochemical pretreatment—A review. *Energy Reports,* 8, 696-709. https://doi.org/10.1016/j.egyr.2021.12.015

Lin, L., Yan, R., Liu, Y. and Jiang, W. (2010). In-depth investigation of enzymatic hydrolysis of biomass wastes based on three major components: cellulose, hemicelluloses and lignin. *Bioresource Technology*, 101(21), 8217–8223. https://doi.org/10.1016/j.biortech.2010.05.084

Liu, J.G., Wang, Q.H., Wang, S., Dexun, Z. and Sonomoto, K. (2012). Utilization of microwave-NaOH pretreatment technology to improve performance and L-lactic acid yield from vinasse. *Biosystems Engineering*, 112(1), 6-13. https://doi.org/10.1016/j.biosystemseng.2012.01.004

Mahmood, H., Moniruzzaman, M., Yusup, S. and Akil, H.M. (2018). Inic liquid pretreatment at high solids loading: A clean approach for fabrication or renewable resource based particulate composites. *Polymer Composites*, 39(6), 1994-2003. https://doi.org/10.1002/pc.24159

Mohan, M., Banerjee, T. and Goud. V.V. (2015) Hydrolysis of bamboo biomass by subcritical water treatment. *Bioresource Technology*, 191, 244–252. https://doi.org/10.1016/j.biortech.2015.05.010.

Muda, N.A., Yoshida, H., Ishak, H., Ismail, M.H.S. and Izhar, S. (2019). Conversion of oil palm trunk into bio-oil via treatment with Subcritical Water. *Journal of Wood Chemistry and Technology,* 39, 255-269. https://doi.org/10.1080/02773813.2019.1578375

Nabila, R., Hidayat, W., Haryamto, A., Hasanudin, U., Iryani, D.A., Lee, S., Kim, S., Chun, D., Choi, H., Im, H., Lim, J., Kim, K., Jun, D., Moon, J. and Yoo, J. (2023). Oil palm biomass in Indonesia: Thermochemical upgrading and its utilization. *Renewable and Sustainable Energy Reviews*, 176, 113193. https://doi.org/10.1016/j.rser.2023.113193

Nadia, A., Rodiansono, R. and Sunardi, S. (2018). Hydrothermal pretreament of oil palm fronds for increasing enzymatic saccharification. *AIP Conference Proceedings*, 2021, 030002. https://doi.org/10.1063/1.5062726

Pandey, K.K. and Pitman, A.J. (2003). FTIR studies of the changes in wood chemistry following decay by brown-rot and white-rot fungi. *International Biodeterioration and Biodegradation*, 52(3), 151-160. https://doi.org/10.1016/S0964-8305(03)00052-0

Pangsang, N., Rattanapan, U., Thanapimmetha, A., Srinoppkakhun, P., Liu, C.-G., Zhao, X.-Q., Bai, F.-W. and Sakdaronnarong, C. (2019). Chemical-free fractionation of palm empty fruit bunch and palm fiber by hot-compressed water technique for ethanol production. *Energy Reports*, 5, 337-348. https://doi.org/10.1016/j.egyr.2019.02.008

Rao, L.V., Goli, J.K., Gentela, J. and Koti, S. (2016). Bioconversion of lignocellulosic biomass to xylitol: An overview. *Bioresource Technology*, 213, 229-310. https://doi.org/10.1016/j.biortech.2016.04.092

Saini, R., Patel, A.K., Saini, J.K., Chen, C.-W., Varjani, S., Singhania, R.R. and Dong, C.D. (2022). Recent advancements in prebiotic oligomers synthesis via enzymatic hydrolysis of lignocellulosic biomass. *Bioengineered*, 13(2), 2139-2172. https://doi.org/10.1080/21655979.2021.2023801

Sills, D.L. and Gossett, J.M. (2012). Using FTIR to predict saccharification from enzymatic hydrolysis of alkali pretreated biomasses. *Biotechnology and Bioengineering*, 109(2), 353-362. https://doi.org/10.1002/bit.23314

Sun, S.N., Sun, S.L. Cao, X.F. and Sun, R.C. (2016). The role of pretreatment in improving the enzymatic hydrolysis of lignocellulosic materials. *Bioresource Technology*, 199, 49-58. https://doi.org/10.1016/j.biortech.2015.08.061

Thamsee, T., Choojit, S., Cheirsilp, B., Yamseangsung, R., Ruengpeerakul, T. and Sangwichien, C. (2018). Combination of superheated steam explosion and alkaline autoclaving pretreatment for improvement of enzymatic digestibility of the oil plm tree residues as alternative sugar sources. *Waste and Biomass Valorization*, 10, 3009-3023. https://doi.org/10.1007/s12649-018-0292-z

Thi, B.T.N., Ong, L.K., Thi, D.T.N. and Ju, Y.-H. (2017). Effect of subcritical water pretreatment on cellulose recovery of water hyacinth (*Eichhornia crassipe*). *Journal of the Taiwan Institute of Chemical Engineers*, 71, 55-61. https://doi.org/10.1016/j.jtice.2016.12.028

