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Application of Subcritical Water Hydrolysis (SCW) in Producing Reducing Sugar for Biofuel Production

Dian Ranggina[1]*, Maria Assumpta Nogo Ole[1], Hanny F. Sangian[2], Arief Widjaja[3] , Hijrah Amaliah Azis[4]
[1]*Department of Chemical Engineering, Ujung Pandang State Polytechnic, Makassar, Indonesia 90245*
[2]*Department of Physics, Sam Ratulangi University, Manado, Indonesia 95115*
[3]*Department of Chemical, Engineering Institut Teknologi Sepuluh Nopember, Surabaya, Indonesia 60111*
[4]*Department of Chemical, Faculty of Engineering, Sulawesi University of Technology, Indonesia 90221*

*Corresponding author: dian.ranggina.dr@poliupg.ac.id

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ABSTRACT. This work aimed to produce reducing sugar from coconut husk using subcritical water hydrolysis. Coconut husk contains cellulose and hemicellulose that can be converted into reducing sugars and then fermented into biofuel. In this study, the subcritical water hydrolysis was carried out in a batch reactor at temperatures 120-260 °C and pressures, 40, 80, and 160 bar for 1 h. Subcritical water method obtained two products, namely (a) liquid containing sugar and (b) solid containing cellulose, hemicellulose and lignin. The liquid sugars were analyzed by HPLC and DNS, while crystal structure was characterized by XRD and SEM. The highest yield of reducing sugar obtained was 0.25 g/g cellulose+ hemicellulose at 150 °C and 80 bar.

1. INTRODUCTION

Biofuel is one of alternative energy to overcome problem of fossil fuel supplies, which are depleting annually. Furthermore, fossil-based fuels emit pollution that are not environmentally friendly. An alternate energy source that can help reduce our dependence on fossil fuels is lignocellulose biomass. A common source of lignocellulosic biomass is agricultural waste, such as coconut husk, which contains 17.73% hemicellulose, 26.72% cellulose, and 41,19% lignin [1]. Due to its high cellulose and hemicellulose content, coconut husk has the ability to fermented into biofuel by reducing sugar. However, in order to produce the reducing sugars to be converted into biofuels, a pretreatment is required that can release the lignin bonds from the lignocellulose structure. This will allow cellulose and hemicellulose to be extracted, reducing the crystalline characteristics and degree of polymerization of cellulose [2].

Hydrolysis is a commonly used method for breaking down lignocellulose. This approach is frequently employed due to its considered simple, affordable, and low-risk in its application. Many forms of hydrolysis exist, including enzymatic, acidic, and alkaline processes as well as ones that employ surfactants and ferric chloride as reagents [3]. Acid pretreatment has been conducted and manipulated to cellulose crystalline structure and improved the digestibility of the enzymatic hydrolysis. On the other hand, the pretreatment of lignocellulose using this method has disadvantages, including the use of significant amounts of acid-base, which complicates recycling and has an impact on the environment, a long pretreatment process, and the production costs were high[4-5].

Subcritical water (SCW) hydrolysis contributed to improve the conversion of biomass into reducing sugar by conditioning lignocellulose in terms of structure and size. Subcritical water (SCW) has a unique characteristic in which the concentrations of H^+ and OH^- increase with temperature, allowing water to serve as both an acid and base catalyst [6]. In a subcritical system, a variety of chemicals and bioproducts can be synthesized using water as a solvent. The subcritical state refers to conditions where the temperature and pressure are above the boiling point but below the critical point of the substance. Additionally, the SCW process is characterized by minimal waste production, non-corrosiveness, and rapid processing times. Subcritical conditions cause a decrease in water density, which increases the diffusion rate while decreasing surface tension and viscosity [7].

Prado et al., (2014) evaluated the usage of subcritical water method under different temperatures at constant pressure. The industrial residues (coconut husk, grape seeds and pressed palm) were employed to produce reducing sugars using SCW. The reactor used was a semi-batch pressurized by steam at 208 °C to 257 °C and 20 MPa [8]. This work was to prepare reducing sugar of coconut husk using SCW by altering the pressure from 40-160 bars, which differed from previous report by Prado et. al. (2014) Moreover, temperature was conditioned at 120 to 260 ºC employing a batch reactor pressurized by carbon dioxide [8]

2. MATERIALS AND METHODS

2.1 Raw Material Preparation

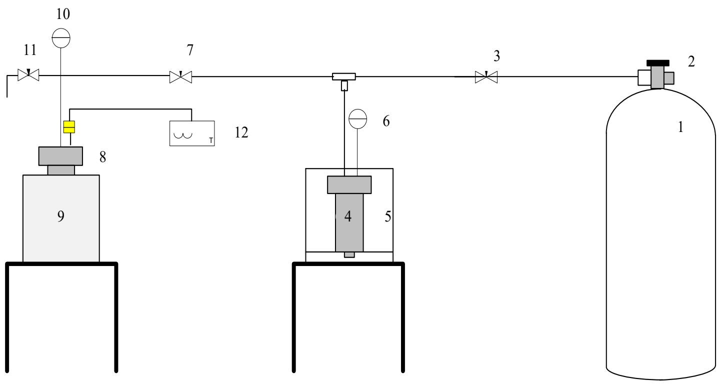
Copra coconut husk used in this study was collected in the city of Manado, North Sulawesi. Prior to mechanical pretreatment, material was dried under sunlight for about 2 days. The size of material was reduced by milling process. The particles were screened using screener (Retsch GmBh Rheinische Strade 36 4278, Haan, Germany) with size 100-120 mesh powders.

The chemicals used in this study are citric acid (Merck), sodium metabisulfite (Sigma Aldrich, USA), sodium potassium tartrate (Merck, Germany), sulfuric acid H2SO4 97-95% (Sigma Aldrich, USA), and 3,5-dinitrosalicylic acid (Sigma Aldrich, USA).

2.2 Subcritical Water Hydrolysis

The reactor design used in this study is shown schematically in Figure 1. The design of the equipment was adapted from work by Ju et al., (2011). It consists of a tube of carbon dioxide, heater, temperature controller (PID), pressure regulator valve, subcritical reactor, and pressure gauges. Temperature controller (PID) and pressure indicator were connected to the reactor [2].

Six grams of coconut husk was added into 120 mL distilled water and put inside subcritical reactor. CO_2 was supplied to the reactor by setting at 40, 80, and 160 bars. The pressure was maintained at 40 bars and temperatures were varied at 120, 130, 140 and 150 ºC. When pressure was at 80 bars, temperatures could be changed at 150, 160,180 and 200 ºC. Finally, one hundred and sixty bars pressing the broth inside reactor, resulted the alteration temperatures at 200, 220, and 260 ºC and conducted for 1h for all variables. After hydrolysis the reactor was cooled to 30 ºC. The fraction of solid was separated from liquid by using filter paper and dried for 2 days at 60 ºC inside oven prior to analysis of chemical composition.



Note:
1 : CO_2 cylinder
2.3.7.11 : Valves
4 : Cooling Reactor
5 : Cooling Bath
6 & 10 : Pressure Gauge
8 : SCW Reactor
9 : Heater
12 : PID

Figure 1. The Design Of SCW Reactor Used in This Work

2.3 Analysis of the Product of SCW Hydrolysis

Analysis of composition of cellulose, hemicellulose and lignin from coconut husk was conducted by using chesson method, which was previously reported by Datta & Rathin (1981). While analysis of reducing sugar used DNS method [11]. A microtube is filled with 2 mL of the liquid sample, then centrifuged for 10 min at 1000 rpm. The one 0.2 mL sample was removed and mixed into1.8 mL of distilled water and 3 ml DNS solution. The five-milliliter sample was heated inside boiling water and cooled with ice water for 10 min. The absorbance was measured by a spectrophotometer (CECIL 1001, Cambridge, United Kingdom) at 540 nm wavelength. The yield of reducing sugar was calculated with the following equations [12]:

$$Yield = \frac{mass\ of\ total\ reducing\ sugar\ ,TRS}{mass\ of\ dried\ substrate\ coconut\ coir\ dust,CCD} \quad (1)$$

$$Yield = \frac{mass\ of\ total\ reducing\ sugar\ ,TRS}{mass\ of\ celluose+hemicelluse} \quad (2)$$

The measurement of sugars fractions (glucose, xylose and galactose) used HPLC technique, Isocratic HPLC pump Waters 1515, detector refractive index 2414 and column Aminex HPX87P (Bio-Rad, CA). The crystal structure of pre-treated coconut husk was analyzed by XRD (Philips X'Pert X-Ray Diffractometer) according to the previous work (Park et al., 2010). The crystalline index was calculated as proposed by authors [12] as follows:

$$Cr.I = \frac{I_{002} - I_{am}}{I_{002}} \times 100 \quad (3)$$

Where Cr.I is the crystalline index, I_{002} is crystalline area, while I_{am} is the amorphous area. The SEM was performed to analyze the morphology of coconut husk before and after SCW hydrolysis.

3. RESULTS AND DISCUSSION

3.1 Chemical Composition of Coconut Husk

The chemical compositions of coconut husk untreated and SCW-pretreated obtained by chesson method are presented in Table 1. The contents of substrate without SCW-treatment including 15.31 % hemicellulose, 23.43 % cellulose, 48.81 % lignin and 12.45 % for others components, respectively. The results were slightly different from those reported in previous studies, particularly cellulose, hemicellulose, and lignin. Sangian, et. al. (2015) reported chemical compositions of coconut coir were cellulose (26,72 %), hemicellulose (17,73 %), lignin (41,19 %). Differences in content were affected by type, maturity and origin of raw material [4].

The purpose of determining chemical composition was to know the influence of pressure and temperatures of SCW hydrolysis. When pressures were set at 40 bars (isobar condition), weight of hemicellulose and lignin changed after SCW-hydrolysis. This effect could be explained by the solubility of solid into water. The hemicellulose has an amorphous structure so that it can be effectively degraded into reducing sugar. Meanwhile, content of cellulose increased to 26.19% from control that recorded at 23.43%. The increase was caused by the inter and intra-molecular bonds of cellulose so it was difficult to decompose into monomers. Furthermore, cellulose is more crystalline structure than those hemicellulose and lignin [4]

When pressure increased above 40 bars, the compositions of cellulose, hemicellulose, and lignin were fluctuating. Compositions of cellulose and lignin increased to 29.96%, 53.77%, while hemicellulose decreased to 4.93%, respectively at 150 ºC and 80 bars. At range 180 ºC to 260 ºC, cellulose and lignin decreased, however, hemicellulose inclined as temperature increased. The decrease of cellulose was indicative that cellulose, or products was degraded into organic acid, furfural and 5-HMF, which were toxic to the fermentation [8].

Table 1. Chemical compositions of substrates, control and SCW-treated solids

Pretreated	Content (%)			
	Hemicellulose	Cellulose	Lignin	Others
Untreated (Control)	15.31	23.43	48.81	12.45
Variable I (40 bar, 120 ºC)	13.92	26.19	47.39	12.50
Variable II (40 bar, 130 ºC)	9.42	31.53	39.88	19.16
Variable III (40 bar, 140 ºC)	7.52	31.95	47.05	13.48
Variable IV (40 bar, 150 ºC)	9.80	26.54	51.04	12.61
Variable V (80 bar, 150 ºC)	4.93	29.96	53.77	11.34
Variable VI (80 bar, 160 ºC)	3.99	30.42	55.51	10.08
Variable VII (80 bar, 180 ºC)	6.09	28.91	55.05	9.94
Variable VIII (80 bar, 200 ºC)	6.15	26.12	54.78	12.95
Variable IX (160 bar, 200 ºC)	6.63	24.31	47.11	21.95
Variable X (160 bar, 220 ºC)	8.78	21.43	46.16	23.62
Variable XI (160 bar, 260 ºC)	12.62	19.55	49.57	21.35

3.2 Characterization of an SCW-Treated Solids

3.2.1 Scanning Electron Microscopy (SEM) Images

Differences of the surface morphology between untreated and SCW-treated (80 bar; 150 ºC) are presented in SEM image as shown in Fig. 2. The SEM image could be useful tool obtaining an information about the morphological structure of substrates after subcritical water hydrolysis.The untreated substrate showed the surface structure of coconut husk exhibited rigid and highly organized (Fig. 2A). However, SCW-treated solid at 80 bars, 150 ºC shown in Fig. 2B, clearly showed the breakage of structure. The treated coconut husk was rougher, more porous and not arranged. These structural change was due to some cellulose and hemicellulose dissolved in water.

The results of this study are comparable to previous studies Öztürk et al. (2010), using SCW hydrolysis on kenaf samples, at a temperature of 250ºC the cell wall structure is degraded and distorted, so that cellulose and hemicellulose are released [14].

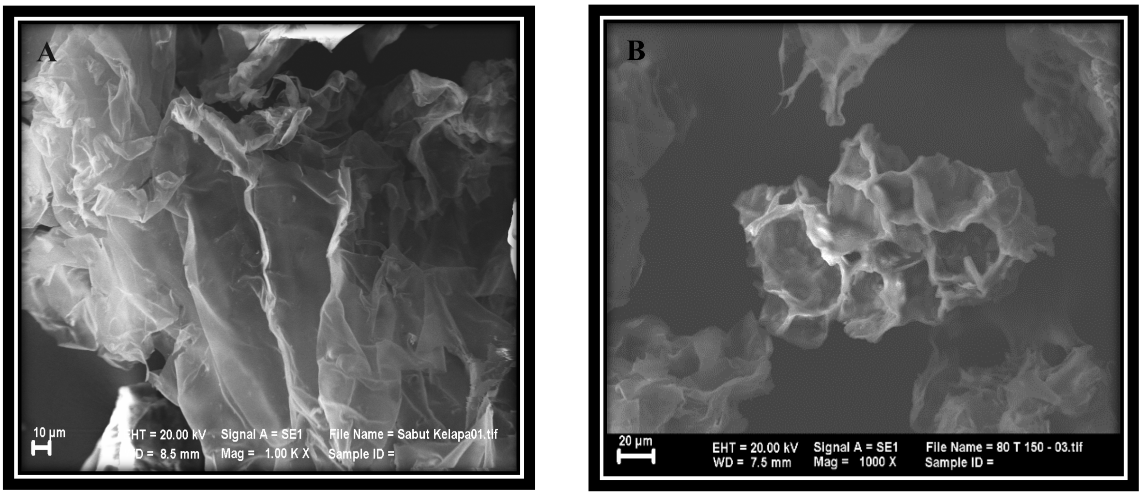


Figure 2. The SEM images of untreated (a) and subcritical water- treated coconut husk at 150 ºC 80 bars (b).

3.2.2 X-ray Diffraction Analysis

Fig. 3 shows the diffraction patterns of coconut husk before and after treatment. The crystal index (CrI), which gave crystalline degree, was calculated by the height of intensities ratio of crystalline (I_{002}) and amorphous (I_{am}). Calculation of Cr.I conducted in this study using the intensity method, which is shown in the Table.2. It was found that the crystalline index of untreated was relatively high, which was recorded at 45.79%. On the other hand, the index of substrate treated at 80 bars, 150 ºC was 42.5%, which was lower than control. This decrease could be explained the change the structure of substrate from high- to lower crystallinity, that was more amorphous. [14]

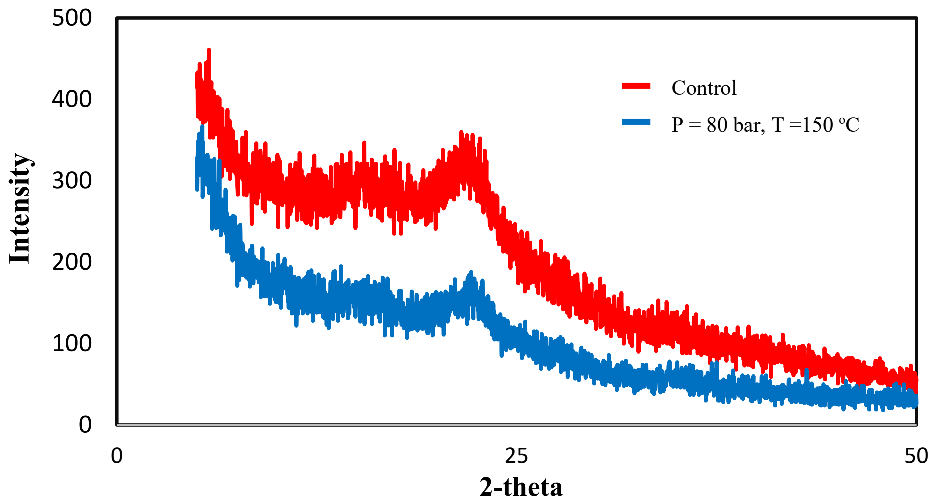


Figure 3. X-ray diffraction pattern of control and SCW-treated Substrate

This study shows the impact of high temperature and pressure on the subcritical water method, which helps break down intra- and intermolecular hydrogen bonds during hydrolysis. In addition, the hydrolysis of SCW with CO_2 as a pressure gas can produce H_2CO_3 by reacting with water, and it also functions as a catalyst to reduce the crystallinity of cellulose [15]

Table 2. Intensity Data of Crsytalline Curve (I_{002}) and Intensity Data of Noncrystalline/ Amorphous Curve (I_{am}) in Cr.I Calculation Use The Intensity Method

Sample	2θ	Crystalline			2θ	Noncrystalline/ Amorf			Cr.I (%)
		I_{002}	Back-ground (BG)	I_{oo2}-I_{BG}		Iam	Back-ground (BG)	I_{am}-I_{BG}	
Control	21,89	356	83	273	17,76	235	87	148	45,79
80 bar, 150 ºC Treated	22,29	180	60	120	19,43	107	38	69	42,5

3.2.3 Amount of Total Reducing Sugars Released by SCW-Hydrolysis

Reducing sugar produced by SCW- hydrolysis was analyzed using DNS (dinitrosalicylic acid) method. Table 3 presents the effects of temperature and pressure on sugar released after SCW-hydrolysis. As shown in Table 2, reducing sugar increased from 0.99 g/L (40 bar, 120 ºC) to 3.73 g/L (40 bar, 150 ºC). And the increase of pressure 40 to 80 bar, concentration of reducing sugar declined to 1.33 g/L from 4.78 g/L, which was conducted at 80 bar, 150 ºC. If pressure was set to 160 bar, reducing sugars released of substrate were fluctuating for different temperatures. The highest sugar obtained was 4.78 g/L from SCW-hydrolysis at 80 bar and 150 ºC for 1h. Subcritical water hydrolysis is a process in which water is used as reactants, solvent and catalysts at temperatures 100 – 374 °C and pressure high enough to maintain water in its liquid phase.

Table 3. Sugar Concentration and Yield of SCW-Hydrolysis of substrates used Eq. (1) and Eq. (2)

P (bar)	T (ºC)	Sugar Concentration	Volume	Mass	Yield	
		(g/L)	(mL)	(g)	Yield (1)	Yield (2)
					g.sugar/g.solid	g.sugar/cell.+hemi.
40	120	0.99	120	0.12	0.02	0.05
40	130	2.72	120	0.33	0.05	0.14
40	140	3.48	120	0.42	0.07	0.18
40	150	3.73	120	0.45	0.07	0.19
80	150	4.78	120	0.57	0.10	0.25
80	160	3.27	120	0.39	0.07	0.17
80	180	1.72	120	0.21	0.03	0.09
80	200	1.33	120	0.16	0.03	0.07
160	200	1.68	120	0.20	0.03	0.09
160	220	3.77	120	0.45	0.08	0.19
160	260	1.83	120	0.22	0.04	0.09

In these subcritical conditions, the physical and chemical properties of water change, making it a more reactive solvent for breaking down complex carbohydrates into simple sugars, including reducing sugars. Subcritical water behaves like a weak acid, which can help break glycosidic bonds in polysaccharides (such as starch or cellulose) without the need for additional acid. Under these conditions, polysaccharides such as starch or cellulose can be broken down into oligosaccharides, disaccharides, and eventually into monosaccharides such as glucose, fructose, and galactose, which are reducing sugars. When water reaches subcritical conditions, hydrogen bonds weaken, causing water to dissociate into H_3O^+ and OH^-, which hydrolyze cellulose and hemicellulose into reducing sugars, which hydrolyzed the cellulose and hemicellulose into reducing sugars as follows [2]:

$(H_6H_{10}O_5)n$ (*cellulose*) + $nH_2O \longrightarrow nC_6H_{12}O_6$

$(H_5H_8O_4)m$ (*hemicellulose*) + $mH_2O \longrightarrow C_5H_{10}O_5$

The percentage biomass converted into reducing sugar after SCW-hydrolyisis can be determined by calculate the yield of sugar as shown Table 2. The mass sugar obtained per mass of coconut husk was the first yield (1), meanwhile the second yield was defined as the mass of sugar per mass (cellulose + hemicellulose). The data showed that the highest of sugar liberated at 80 bar and 150 ºC was 0.1 g.sugar/g.solid,or 0.25 g. sugar/ g. cellulose +hemicellulose. If the pressure incresed up to 80 bar and 160 ºC, the amount of sugar liberated from biomass was significantly declined. The cellulose, hemicellulose, or products (sugars) were degraded into HMF, furfural an other water soluble materials (Prado et al., 2014).

Prado et al. (2014) reported that yield of reducing sugar of SCW-hydrolysis for coconut husk was 0.12 g.sugar/g.dried solid at 212 ºC, 20 MPa for 30 min. The results were higher than the present work due to the differences of operation.The different origin and matuarity of coconut husk may affect the chemical composition and influenced to yield sugars as previously published [4].

4. CONCLUSION

Subcritical water method has been successfully used for producing the reducing sugar from high lignin lignocellulose, coconut husk. The SEM measurement showed that the surface structure of substrate changed into more porous compared with control. Meanwhile, XRD revealed the substrates transformed from high (cellulose I)- to low crystallinity (cellulose II). The amount of sugar released of substrate was significantly affected by temperature and pressure. The optimum condition was attained at 80 bar and 150 ºC, which resulted the maximum yield of sugar.

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