

Characterization of Low Molecular Weight Organic Acids from Beech Wood Treated in Supercritical Water

KEI YOSHIDA, JUNKO KUSAKI, KATSUNOBU EHARA,
AND SHIRO SAKA*

*Graduate School of Energy Science, Kyoto University,
Yoshida-honmachi, Sakyo-ku, Kyoto 422, Japan,
E-mail: saka@energy.kyoto-u.ac.jp*

Abstract

Japanese beech (*Fagus crenata* Blume), its cell wall components, and model compounds were treated by supercritical water (380°C, 100 MPa) for 5 s using a batch-type reactor to investigate the production behavior of low molecular weight organic acids. It was found that cellulose and hemicellulose were decomposed to formic acid, pyruvic acid, glycolic acid, acetic acid, and lactic acid, whereas lignin was barely decomposed to such organic acids under the given conditions. However, after prolonged treatment (380°C, 100 MPa, 4 min) of lignin, some organic acids were recovered owing perhaps to the decomposition of the propyl side chain of lignin. It was additionally revealed that the predominant organic acid recovered was acetic acid, which might be derived from the acetyl group of hemicellulose in Japanese beech.

Index Entries: Biomass conversion; lignocellulosics; organic acid; supercritical water; Japanese beech.

Introduction

Because of the global warming caused by greenhouse gases derived from the combustion of fossil resources, renewable biomass resources will become more important in the future as new energy and chemical resources. Lignocellulosics are one of the most abundant biomass resources (1,2) and do not compete with food resources. Thus, efficient utilization of lignocellulosics is important. Some methods such as pyrolysis (3) and acid hydrolysis (4) have been investigated for the conversion of lignocellulosics. Besides these approaches, subcritical and supercritical water (>374°C, >22.1 MPa) treatments have been investigated (5–13).

Bobleter (5) proposed the hydrothermal treatment of lignocellulosics with subcritical water without using any catalyst. Recently, Sasaki et al. (9) conducted experiments investigating the supercritical water treatment

*Author to whom all correspondence and reprint requests should be addressed.

of cellulose and found that cellulose can be converted into water-soluble saccharides more effectively in supercritical water than in subcritical water. In addition, Sasaki et al. (14) and Antal et al. (15) conducted research on a decomposition mechanism of saccharides in subcritical and supercritical water. It was found that the main reactions of saccharides involve hydrolysis, dehydration, and fragmentation.

It has been reported that lignocellulosics can be separated into carbohydrate and lignin-derived products by supercritical water treatment (16). The former, which mainly consists of polysaccharides, oligosaccharides, monosaccharides, and their decomposition products, is an appropriate source for ethanol fermentation (11,13,17). The latter, which mainly consists of monomeric and oligomeric lignin-derived products, may be useful for developing substitution products of aromatic chemicals from fossil resources (12,18). Therefore, we proposed the ethanol-producing process from lignocellulosics using supercritical water technology followed by fermentation (19–21).

Organic acids were also obtained by supercritical water treatment of lignocellulosics when the treatment time was prolonged. Organic acids such as lactic acid can be converted into not only biodegradable polymer but also gases such as methane, carbon monoxide, and hydrogen by microorganisms. In addition, organic acids are thought to be one of the intermediates of the gas products during supercritical water gasification. Thus, identification and production of organic acids are very important when proposing a new system for efficient utilization of lignocellulosics. Therefore, in this study, we investigated the production behavior of organic acids from lignocellulosics.

Materials and Methods

Samples and Chemicals

Japanese beech (*Fagus crenata* Blume), microcrystalline cellulose (Avicel PH-101, Asahi Kasei), beech xylan (Sigma, St. Louis, mo), milled wood lignin (MWL), glucose, xylose, sinapyl alcohol, coniferyl alcohol, and guaiacol were used. Prior to supercritical water treatment, Japanese beech was extracted by a mixture of ethanol and benzene (1:2 in volume) for 12 h according to the standard method, and its lignin content was determined by a concentrated sulfuric acid method (22,23). MWL from Japanese beech was prepared by Bjorkman's (24) method.

High-performance liquid chromatography (HPLC)–grade water was used for the supercritical water treatment, and all other chemicals were reagent grade and used without purification.

Treatment and Separation

Supercritical water treatment was conducted using a biomass conversion system with a batch-type reaction vessel (25). Distilled water (4.95 mL)

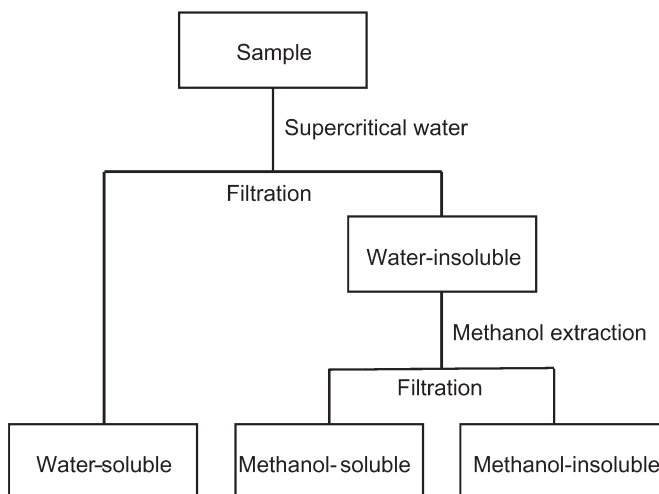


Fig. 1. Separation scheme of sample as treated in supercritical water.

was fully fed with 50 mg of the samples to a 5-mL reaction vessel made of Inconel-625. The reaction vessel was heated by immersing it into a molten tin bath preheated at 500°C. The temperature and the pressure increased to 380°C and 100 MPa, respectively, and were maintained for 5 s. The vessel was then immersed in a water bath to quench. During this treatment, the temperature and the pressure in the reaction vessel were monitored by an attached thermocouple and pressure gage. The treatment condition was defined as the maximum temperature and pressure during the experiment. For prolonged treatment in supercritical water, the temperature and pressure adopted were 380°C and 100 MPa, respectively, for 4 min.

Figure 1 shows the separation scheme of the samples as treated in supercritical water. The obtained reaction mixture was filtered through a 0.2- μ m membrane filter to separate the water-soluble portion from the water-insoluble residue. The water-insoluble residue was extracted by methanol, separating the methanol-soluble portion from the methanol-insoluble residue. Methanol-insoluble residue was dried and weighed to obtain yield.

Analytical Methods

The water-soluble portions were analyzed by HPLC and capillary electrophoresis (CE). HPLC analysis was conducted using a Shimadzu LC-10A HPLC equipped with a Shodex Sugar KS-801 (Showadenko) column and a refractive index detector. The carrier solvent was distilled water at a flow rate of 1.0 mL/min. CE analysis was conducted using HP3D CE systems (Agilent). A fused-silica capillary (75- μ m id, 104-cm total length, 95.5-cm effective length) from Agilent was used. Indirect ultraviolet detection at 270 nm (reference wavelength at 350 nm) was performed using 2,6-

pyridinedicarboxylic acid buffer (containing 0.5 mM cetyltrimethylammonium bromide, pH 5.6) for organic acid analysis (Agilent). Injection was carried out using 50-mbar pressure at the cathodic end for 4 s. The applied voltage was set at -30 kV, with a capillary temperature of 15°C.

The Fourier transform-infrared (FT-IR) spectra of beech and xylan were recorded by a Shimadzu IR-8300. The samples were pressed into KBr pellets (2 mg of sample/200 mg of KBr). A blank KBr disk was used as background.

Results and Discussion

Table 1 shows the yield of fractionated portions from Japanese beech and its cell wall components as treated in supercritical water. The yields of water-soluble, methanol-soluble, and methanol-insoluble portions from beech were 80.1, 18.3, and 1.6%, respectively. The yields of cellulose and xylan were decomposed to the water-soluble portion were 86.3 and 93.7%, respectively. MWL was mainly converted into the water-insoluble portion, with a yield of 70%, whereas its water-soluble portion was only 30%. It has been reported that cellulose and hemicellulose were mainly decomposed to a water-soluble portion and that lignin was decomposed to a methanol-soluble portion and methanol-insoluble residue (16). The obtained result in our study is therefore quantifiably consistent with previous work.

Lignin content was determined to be 26.5% (as a total of Klason lignin [23.0%] plus acid-soluble lignin [3.5%]) by a concentrated sulfuric acid method. The contents of cellulose, hemicellulose, and lignin in Japanese beech were assumed to be 50.0, 23.4, and 26.5%, respectively. The yield of the water-soluble portion, methanol-soluble portion, and methanol-insoluble residue can be calculated from the data for cellulose, xylan, and MWL using the values of 50.0, 23.4, and 26.5%, respectively, for the contents of cellulose, hemicellulose, and lignin in Japanese beech (26). As shown in the parentheses in Table 1, the water-soluble portion, methanol-soluble portion, and methanol-insoluble residue were calculated to be 73.0, 16.6, and 10.4%, respectively. The values were found to be close to the actual yield for Japanese beech, suggesting that the data obtained for cellulose, xylan, and MWL are valid except for the methanol-insoluble portion.

Figure 2 shows HPLC chromatograms and CE electropherograms of the water-soluble portion from Japanese beech and its cell wall components as treated in supercritical water. For the HPLC analysis, the hydrolyzed products such as oligosaccharides and monosaccharides could not be detected in all cases because the treatment time was too long to obtain the hydrolyzed products. The dehydrated products (levoglucosan, 5-hydroxymethylfurfural [5-HMF], furfural), fragmented products (glycolaldehyde, dihydroxyacetone), and organic acids were observed for beech, cellulose, and xylan, with lower yields observed for MWL.

Table 1
Yield of Fractionated Portions from Japanese Beech and Its Cell Wall
Components as Treated in Supercritical Water (380°C, 100 MPa, 5 s)

Sample	Yield (%) ^a		
	Water soluble	Water insoluble	
		Methanol soluble	Methanol insoluble
Beech	80.1 (73.0)	18.3 (16.6)	1.6 (10.4)
Cellulose	86.3	10.5	3.2
Xylan	93.7	5.3	1.0
MWL	30.0	37.8	32.2

^aValues in parentheses were calculated from the data for cellulose, xylan, and MWL using the content of 50.0, 23.4, and 26.5%, respectively, for cellulose, hemicellulose, and lignin in beech.

The separation of organic acids by HPLC was not enough to identify their composition. Thus, the CE analysis was also conducted, which found that these organic acids were mainly formic acid, glycolic acid, acetic acid, and lactic acid. In addition, a trace amount of pyruvic acid was detected from Japanese beech, cellulose, and xylan but not MWL.

Table 2 shows the yield of products in the water-soluble portion from Japanese beech as treated in supercritical water. For the Japanese beech, the total yields of dehydrated products, fragment products, and organic acids were 8.7, 7.0, and 17.3%, respectively. The values shown in parentheses, calculated by the same manner as described earlier, were 4.3, 4.3, and 11.8%, respectively, and tended to be lower than the actual yield from Japanese beech. The calculated value of organic acid (11.8%) was the total of 5.6, 4.4, and 1.8% from cellulose, xylan, and MWL, respectively. Therefore, the contribution of cellulose, hemicellulose, and lignin to the production of organic acids could be expected to be 47.5% (i.e. $[5.6/11.8 \times 100]$), 37.3% (i.e. $[4.4/11.8 \times 100]$) and 15.2% (i.e., $[1.8/11.8 \times 100]$), respectively. This calculation indicates that decomposition of lignin contributed little to the production of organic acid. In addition, a significant difference was noticed in the yield of acetic acid between actual Japanese beech (9.2%) and the calculated value (2.7%). The reason for this is discussed subsequently.

Some researchers have also attempted to produce organic acids from biomass resources such as glucose, cellulose, and waste fish entrails by subcritical water and supercritical water treatments (27). Calvo and Vallejo (28) conducted supercritical water treatment of cellulose at the conditions of 400°C, 27.6 MPa, 0.24 g/cm³ and 5 min and reported that the production of organic acids was typically <15% of the total organic compounds by supercritical water treatment without any catalyst present. Our result in

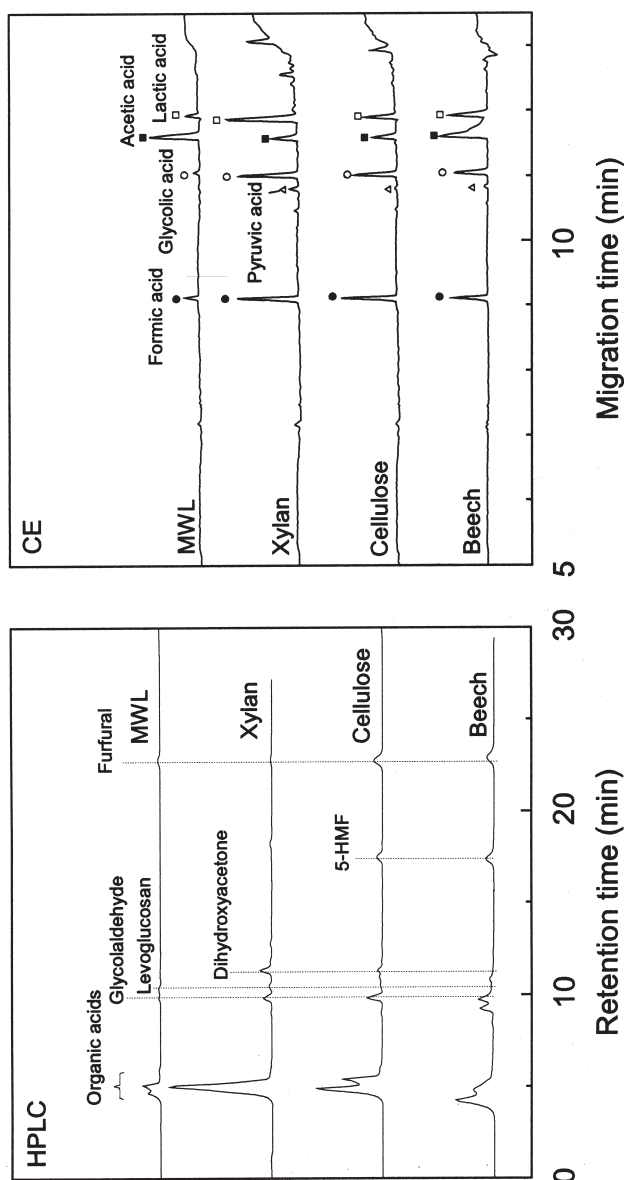


Fig. 2. HPLC chromatograms and CE electropherograms of water-soluble portion from Japanese beech and its cell wall components as treated in supercritical water (380°C, 100 MPa, 5 s).

Table 2
Yield of Products in Water-Soluble Portions from Japanese Beech and Its Components as Treated in Supercritical Water
(380°C, 100 MPa, 5 s)

Sample	Yield (%) ^a														
	Dehydrated				Fragmented			Organic acid							
	Levo glucosan	5-HMF	Furfural	Subtotal	Dihydroxy acetone	Glycol aldehyde	Subtotal	Formic acid	Acetic acid	Glycolic acid	Lactic acid	Pyruvic acid	Subtotal	Others ^b	Total
Beech	0.6 (0.8)	4.4 (1.3)	3.7 (2.2)	8.7 (4.3)	1.4 (1.7)	5.6 (2.6)	7.0 (4.3)	1.8 (2.4)	9.2 (2.7)	3.0 (3.6)	3.0 (2.8)	0.3 (0.3)	17.3 (11.8)	47.1 (52.6)	80.1 (73.0)
Cellulose	0.7	2.4	3.8	6.9	1.6	3.8	5.4	2.8	1.9	4.1	2.2	0.3	11.3	62.7	86.3
Xylan	0.9	0.6	0.4	1.9	3.9	2.5	6.4	3.6	2.2	6.3	6.1	0.7	18.9	66.5	93.7
MWL	0.8	0.0	0.8	1.6	0.0	0.5	0.5	0.6	4.6	0.4	1.0	0.0	6.6	21.3	30.0

^aValues in parentheses were calculated from the data for cellulose, xylan, and MWL using the content of 50.0, 23.4, and 26.5%, respectively, for cellulose, hemicellulose, and lignin in beech.

^bThe value was estimated by subtracting the yield of all identified products from the total.

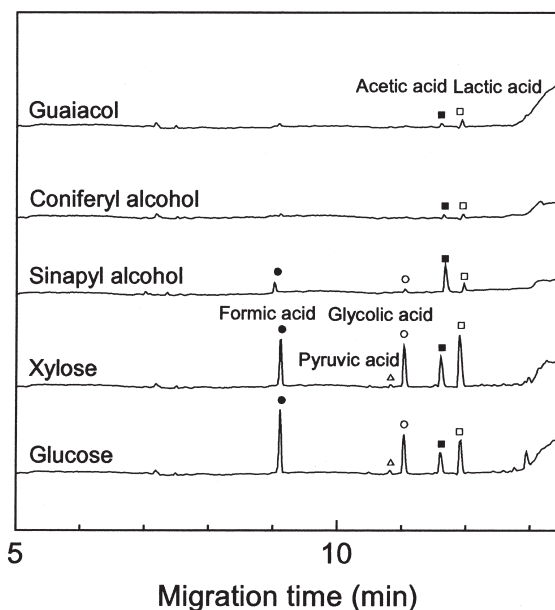


Fig. 3. CE electropherograms of water-soluble portions from glucose, xylose, sinapyl alcohol, coniferyl alcohol, and guaiacol as treated in supercritical water (380°C, 100 MPa, 5 s).

the present study was similar if slightly higher than the limit of their report except for MWL. Our treatment density (380°C, 100 MPa, 0.72 g/cm³) is higher than their conditions (400°C, 27.6 MPa, 0.27 g/cm³) and can provide higher ionic products (H⁺, OH⁻) of water (29,30). Our slightly higher result might be owing to the higher ionic products of water and is expected to improve if the treatment condition can be optimized.

To evaluate the production of organic acids from Japanese beech in supercritical water, simple model compounds—glucose, xylose, sinapyl alcohol, coniferyl alcohol, and guaiacol—were treated in the same manner. The compounds were used as a model compound of carbohydrate (cellulose and hemicellulose) and lignin, respectively. Figure 3 shows CE electropherograms of water-soluble portions from glucose, xylose, sinapyl alcohol, coniferyl alcohol, and guaiacol as treated in supercritical water. It is evident that glucose and xylose were decomposed to formic acid, acetic acid, glycolic acid, lactic acid, and pyruvic acid. On the other hand, sinapyl alcohol, coniferyl alcohol, and guaiacol were decomposed inefficiently to acetic acid and lactic acid.

Table 3 shows the yield of organic acids from model compounds constructing wood cell wall components as treated in supercritical water. For glucose and xylose, total yields of organic acids were >13%. On the other hand, sinapyl alcohol, coniferyl alcohol, and guaiacol were barely

Table 3
Yield of Organic Acids from Model Compounds Constructing Wood Cell Wall Components as Treated in Supercritical Water (380°C, 100 MPa, 5 s)

Sample	Yield (%)					Total
	Formic acid	Acetic acid	Glycolic acid	Lactic acid	Pyruvic acid	
Glucose	3.4	1.7	3.4	2.3	0.3	13.9
Xylose	2.8	2.3	3.6	3.9	0.3	13.3
Coniferyl alcohol	0.2	0.8	0.1	0.2	0.0	0.7
Sinapyl alcohol	0.2	0.8	0.1	0.2	0.0	1.3
Guaiacol	0.1	0.3	0.0	0.3	0.0	0.8

Table 4
Yield of Organic Acids in Water-Soluble Portions from Japanese Beech, Its Cell Wall Components, and Lignin Model Compounds as Treated in Supercritical Water (380°C, 100 MPa, 4 min)

Sample	Yield (%)					Total
	Formic acid	Acetic acid	Glycolic acid	Lactic acid	Pyruvic acid	
Beech	0.2	9.6	4.2	0.2	0.2	14.4
Cellulose	0.7	3.1	3.4	0.4	0.3	7.9
Xylan	0.2	6.3	4.8	2.9	0.2	14.4
MWL	0.3	9.5	3.9	0.2	0.3	14.2
Coniferyl alcohol	0.2	4.5	0.3	1.0	0.0	6.0
Sinapyl alcohol	0.2	4.1	1.4	0.2	0.2	6.1
Guaiacol	0.0	0.3	0.0	0.4	0.0	0.7

decomposed to organic acids, with a total yield of 1.3%. This result also indicated that organic acids were mainly derived from cellulose and hemicellulose but not from lignin.

In our previous study, it was revealed that aromatic nuclei in lignin-derived products were fairly stable in supercritical water (400°C, 115 MPa) for 8 s (12). This evidence further supports the result that lignin did not contribute to the production of organic acids during supercritical water treatment of lignocellulosics. On the other hand, Thornton and Savage (31) reported that phenol can be decomposed to organic acid, such as formic acid, glyoxylic acid, and oxalic acid, through a cleavage of aromatic moiety in supercritical water with a reaction time >10 s using a flow-type system. Their report raises the possibility that lignin may also be decomposed to organic acids under appropriate supercritical water treatment.

Table 4 presents the yield of organic acids in water-soluble portions from Japanese beech, its cell wall components, and lignin model compounds

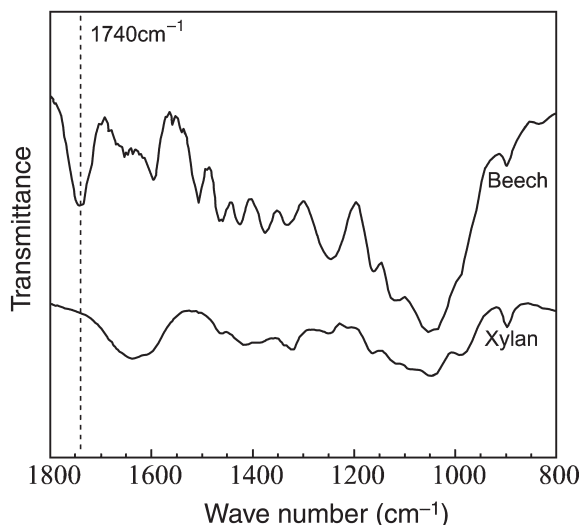


Fig. 4. FT-IR spectra of Japanese beech xylan.

using prolonged supercritical water treatment (380°C, 100 MPa, 4 min). The total yield of organic acids from Japanese beech was 14.4%, which was slightly lower than that of the 5-s treatment (Table 2). The total yields of organic acids from cellulose and xylan were also lower, suggesting that the organic acids produced by the 5-s treatment were decomposed to some other products. On the other hand, the total yield of organic acids from MWL was 14.2%, higher than that of the 5-s treatment. In addition, coniferyl alcohol and sinapyl alcohol were also decomposed to total organic acid yields of 6.1 and 6.0%, respectively. However, guaiacol was barely decomposed to organic acid. These lines of evidence suggest that the production of organic acid from lignin is owing to decomposition of the propyl chain of the phenyl propane unit but not the aromatic moiety in lignin. The organic acids seem to be produced from both carbohydrates (cellulose and hemicellulose) and lignin. It is evident that an optimum condition for producing organic acids is different between carbohydrates (cellulose and hemicellulose) and lignin.

The yields of acetic acids from glucose and xylose, as well as cellulose and xylan, were relatively less than those from Japanese beech (Table 3). To investigate the reason for this, we studied the FT-IR spectra of Japanese beech and xylan used in our study. As shown in Fig. 4, the peak at 1740 cm^{-1} is attributed to unconjugated ketone and carbonyl groups and represents the acetyl group of hemicellulose in Japanese beech (32,33). On the other hand, the same peak could not be observed in xylan, suggesting that this isolated xylan had been deacetylated through the isolation process. Therefore, such a production of acetic acid from Japanese beech could have resulted from the acetyl group originally existing in hemicellulose molecules.

Acknowledgments

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