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## Characterization of the lignin-derived products from wood as treated in supercritical water

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**Abstract** Sugi (*Cryptomeria japonica* D. Don) and buna (*Fagus crenata* Blume) woods were treated with supercritical water (>374°C, >22.1 MPa) and fractionated into a water-soluble portion and a water-insoluble residue. The latter was washed with methanol to be fractionated further into a methanol-soluble portion and a methanol-insoluble residue. Whereas the carbohydrate-derived products were in the water-soluble portion, most of the lignin-derived products were found in the methanol-soluble portion and methanol-insoluble residue. The lignin-derived products in the methanol-soluble portion were shown to have more phenolic hydroxyl groups than lignin in original wood. The alkaline nitrobenzene oxidation analyses, however, exhibited much less oxidation product in the methanol-soluble portion and methanol-insoluble residue. These lines of evidence suggest that the ether linkages of lignin are preferentially cleaved during supercritical water treatment. To simulate the reaction of lignin, a study with lignin model compounds was performed;  $\beta$ -O-4-type lignin model compounds were found to be cleaved, whereas biphenyl-type compounds were highly stable during supercritical water treatment. These results clearly indicated that the lignin-derived products, mainly consisting of condensed-type linkages of lignin due to the preferential degradation of the ether linkages of lignin, occurred during supercritical water treatment.

**Key words** Chemical conversion · Supercritical water · Lignin ·  $\beta$ -O-4 linkage · Condensed-type linkage

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### Introduction

The potential for using the woody biomass as a source of useful chemicals and fuels has been a subject of renewed interest in response to depletion of fossil resources. Systematic use of the wood components such as cellulose, hemicelluloses, and lignin has been suggested to be promising by Goldstein.<sup>1</sup> To be realized for this scenario, various approaches to biomass conversion have been applied, such as pyrolysis,<sup>2</sup> acid hydrolysis,<sup>3,4</sup> and enzymatic hydrolysis.<sup>5</sup> In addition to these approaches, supercritical fluid technology has recently received increasing attention.

In our laboratory, treatments in supercritical water<sup>6,7</sup> (>374°C, >22.1 MPa) and supercritical methanol<sup>8,9</sup> (>239°C, >8.1 MPa) have been applied to its biomass conversion. Saka and Ueno reported that various celluloses were effectively converted to glucose and its derivatives using their batch-type supercritical water biomass conversion system.<sup>6</sup> They also reported that the main components of wood, such as carbohydrates (cellulose and hemicelluloses) and lignin, could be separated into water-soluble and methanol-soluble portions, respectively, after supercritical water treatment.<sup>7</sup> The water-soluble portion, which mainly consists of glucose, xylose, and their derivatives, may have potential as a source of ethanol fermentation. However, the methanol-soluble portion, which consists mainly of lignin-derived products, must be studied in detail regarding its potential for efficient utilization. In this study lignin-derived products in the methanol-soluble portion and methanol-insoluble residues from wood after supercritical water treatment were evaluated to provide clues as to their efficient utilization.

### Materials and methods

#### Samples and chemicals

Sugi (*Cryptomeria japonica* D. Don) and buna (*Fagus crenata* Blume) wood flours (80 mesh pass) were used for

supercritical water treatment. High-performance liquid chromatography (HPLC) grade distilled water was used for the supercritical water treatment; other chemicals, such as methanol, nitrobenzene, potassium hydroxide, and sodium periodate, were reagent grade and were used without purification. As lignin model compounds depicted in Fig. 1, phenolic and nonphenolic  $\beta$ -O-4 types [guaiacylglycerol- $\beta$ -guaiacyl ether (**1**) and veratrylglycerol- $\beta$ -guaiacyl ether (**2**)] and phenolic and nonphenolic biphenyl types [2,2'-dihydroxy-3,3'-dimethoxy-5,5'-dimethyl biphenyl (**3**) and 2,2',3,3'-tetramethoxy-5,5'-dimethyl biphenyl (**4**)] were prepared according to the methods by Nakatsubo et al.<sup>10</sup> and Kratzl and Vierhapper,<sup>11</sup> respectively.

### Supercritical water treatment and fractionation of the treated samples

The supercritical water biomass conversion system used in this study was revised to be a batch-type reaction vessel made of Inconel-625.<sup>6,7</sup> This system can cover a range up to 280 MPa pressure and up to 500°C temperature. Water (5 ml) was fed with 150 mg of wood flour to this reaction vessel, and then it was quickly heated by immersing it in the tin bath preheated at 500°C. The reaction vessel was immersed in waterbath to stop the reaction. During this treatment the temperature and pressure in the reaction vessel was monitored by a thermocouple and pressure gauge attached to the reaction vessel, respectively.

After the supercritical water treatment, the water-soluble portion was retrieved by filtration, and the water-insoluble portion was treated with 10 ml of methanol for 10 min by sonication and separated by filtration to a methanol-soluble portion and a methanol-insoluble residue. Subsequently, these fractions of the methanol-soluble portion and the methanol-insoluble residue were determined quantitatively after complete evaporation of methanol. The water-soluble portion was then estimated by subtracting the methanol-soluble portion and methanol-insoluble residue from the original wood (150 mg).

To simulate the reaction of lignin, the lignin model compounds were treated with supercritical water in the same manner, except that the smaller reaction vessel with 1.0 ml volume was used.

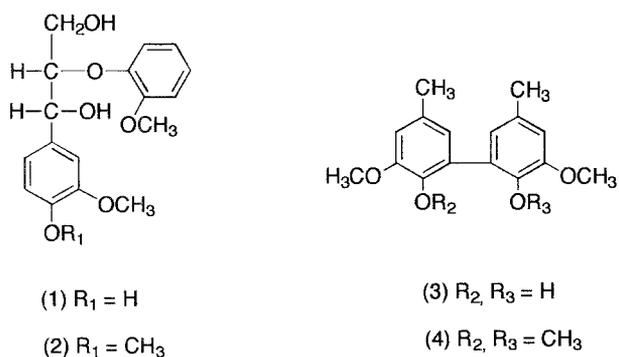


Fig. 1. Structure of lignin model compounds

### Analytical methods

For the methanol-soluble portion and the methanol-insoluble residue, the number of the phenolic hydroxyl groups on 100 phenylpropane ( $C_6-C_3$ ) units were determined by the  $\Delta\epsilon_i$  method<sup>12</sup> and the periodate oxidation method,<sup>13</sup> respectively. In addition, alkaline nitrobenzene oxidation followed by gas chromatographic analysis (Shimadzu GC-14B) was undertaken for these fractions according to the described methods<sup>14</sup> to obtain the total yield of oxidation products of vanillin, vanillic acid, syringaldehyde, and syringic acid. For the lignin content, a sum of Klason lignin plus acid soluble lignin was determined<sup>15</sup> for original wood and water-insoluble portions of sugi and buna. The molecular weight distributions of samples were estimated by gel permeation chromatography on the HPLC system (Shimadzu, LC-10A) with Shodex KF-801 and KF-802.5 columns (Showa Denko, Tokyo, Japan) and tetrahydrofuran as an eluent at a flow rate of 1.0 ml/min. A differential refractometer was used as detector.

In the lignin model compound experiment, the reaction mixture was analyzed using HPLC (Shimadzu LC-10A) with a column (ODS-STR11, C18) and the ultraviolet detector (Shimadzu, SPD-10A) set at 280 nm. The technique of linear gradient elution with distilled water and methanol ( $H_2O/CH_3OH$  8:2 to 0:10 v/v) was applied at a flow rate of 0.8 ml/min.

## Results

### Supercritical water treatment and fractionation of the treated samples

Figure 2 shows the changes of temperature and pressure in the reaction vessel immersed in the tin bath preheated at 500°C. One treatment in subcritical water and two treatments in supercritical water were done changing the immersion time of the reaction vessel in the tin bath. The treatment time in subcritical water and supercritical water was defined as a residence time over 250°C and 374°C (critical temperature of water,  $T_c$ ), respectively.

The results are summarized in Table 1. The ionic products of water ( $K_w$ ) in these treatments are in a range of  $10^{-12}$  to  $10^{-10} \text{ mol}^2/\text{l}^2$ , which is about three to four orders of magnitude larger than that under ordinary conditions.<sup>16</sup> Therefore, supercritical water can act as an acidic catalyst. The dielectric constant of water in these treatments is, on the other hand, in a range between 15 and 20, which is close to that of acetone under ordinary conditions.<sup>17</sup> Because water in an ordinary condition has a dielectric constant of about 80, the properties of water must be drastically changed in the supercritical state.

The methanol-soluble portion in this study is water-insoluble and oily. This is because the methanol-soluble portion is originally soluble in supercritical water. As already mentioned, the dielectric constant of the supercritical water at a given condition is in a range between 15 and 20,

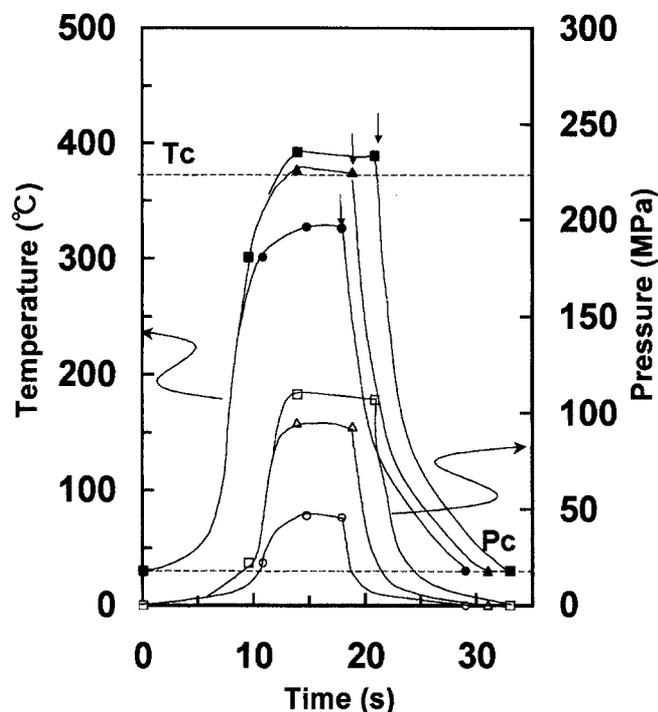
so the hydrophobic substances can be solvated with supercritical water. These substances, however, become insoluble and oily in water, as it has returned to ordinary conditions from the supercritical state. Most of such sub-

stances are soluble in methanol, so water-insoluble and oily substances can be soluble in methanol and collected as the methanol-soluble portion. Therefore, a sum of the water-soluble and methanol-soluble portions can be defined as being supercritical water-soluble.

Liquefaction of sugi and buna woods was carried out in supercritical water for 5 s and 8 s and in subcritical water for 10 s. Table 2 shows the yields of fractionated portions of wood after these treatments. It is apparent that the yield of the water-soluble portion is higher than that treated in supercritical water. In addition, it is higher in buna than sugi wood. It is known that the water-soluble portion mainly consists of the carbohydrate-derived products.<sup>7</sup> Therefore, supercritical water treatment is more efficient for decomposing the carbohydrate than subcritical water treatment.

It is evident in Table 2 that the yield of the methanol-soluble portion is higher again in supercritical water. In addition, the values in parentheses show that the lignin content in the fraction is higher in supercritical water. Therefore, the methanol-soluble portion must consist mainly of lignin-derived products, particularly with supercritical water treatment. It is interesting that the lignin content of the methanol-insoluble residue increases with the extent of the liquefaction.

Total lignin content of the methanol-soluble portion and the methanol-insoluble residue is also shown in Table 2 as that of the water-insoluble portion. Interestingly, these figures are close to the lignin contents of the original wood (33.6% in sugi, 26.3% in buna). These results indicate that because of the hydrophobic nature of lignin the lignin-derived products are mainly fractionated into the methanol-soluble portion and the methanol-insoluble residue. This finding is in good agreement with our previous results in



**Fig. 2.** Changes in temperature and pressure in the reaction vessel immersed in a tin bath preheated at 500°C. Short arrows indicate the points at which the reaction vessels were immersed in the waterbath. Filled and open symbols show temperature and pressure, respectively. *T<sub>c</sub>*, critical temperature; *P<sub>c</sub>*, critical pressure

**Table 1.** Treatment conditions and properties of subcritical and supercritical water

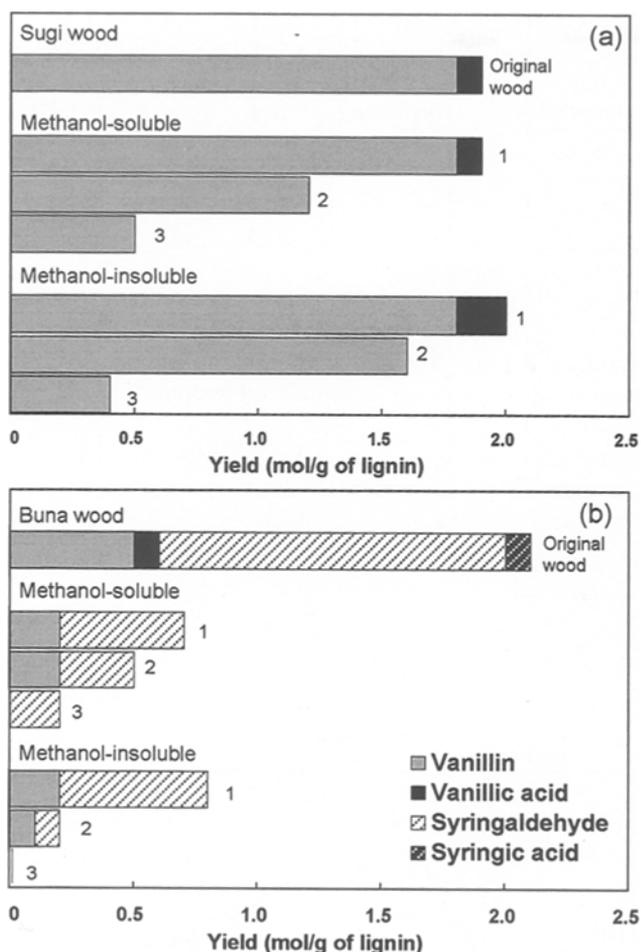
Run no.	Condition			Density (g/cm <sup>3</sup> )	Ionic product <sup>a</sup> -log <i>K<sub>w</sub></i> (mol <sup>2</sup> /l <sup>2</sup> )	Dielectric constant <sup>a</sup>
	Treatment	Maximal temperature (°C)	Maximal pressure (MPa)			
1	Subcritical, 10 s	330	50	0.735	11.2	15–20
2	Supercritical, 5 s	380	100	0.724	10.8	15–20
3	Supercritical, 8 s	400	115	0.724	10.7	15–20

<sup>a</sup> Ionic product and dielectric constant were calculated according to Holzapfel<sup>16</sup> and Franck,<sup>17</sup> respectively

**Table 2.** Yields of fractionated portions of sugi and buna woods as treated in subcritical and supercritical water

Treatment	Yield (%)				Lignin content of water-insoluble portion
	Water-soluble	Water-insoluble			
		Methanol-soluble	Methanol-insoluble		
Sugi wood (lignin content 33.6%)					
Subcritical, 10 s	20.5	6.1 (68.7)	73.4 (35.1)	–	30.0
Supercritical, 5 s	40.2	15.6 (84.6)	44.2 (38.8)	–	30.3
Supercritical, 8 s	66.1	25.6 (83.5)	8.3 (94.6)	–	29.2
Buna wood (lignin content 26.3%)					
Subcritical, 10 s	49.8	11.9 (90.6)	38.3 (18.6)	–	19.0
Supercritical, 5 s	71.2	19.8 (93.9)	9.0 (37.4)	–	22.0
Supercritical, 8 s	73.7	17.8 (95.6)	8.5 (89.8)	–	24.6

Values in the parentheses refer to the lignin content, a sum of Klason lignin plus acid-soluble lignin, in the fractionated portion



**Fig. 3.** Yields of alkaline nitrobenzene oxidation products for wood, methanol-soluble portion, and methanol-insoluble residue in **a** sugi and **b** buna. 1, 2, and 3 refer to the run numbers in Table 1

which milled wood lignin was mainly converted to a methanol-soluble portion and a methanol-insoluble residue after supercritical water treatment.<sup>7</sup>

#### Characterization of lignin-derived products

Table 3 shows the results of phenolic hydroxyl group determinations in wood and fractionated portions treated in sub-

critical and supercritical water. The number of the phenolic hydroxyl groups of lignin in sugi and buna woods were determined to be 16.7 and 14.1 per 100 units of phenylpropane ( $C_6-C_3$ ) residues, respectively. However, the methanol-soluble portions and methanol-insoluble residues were found to have more phenolic hydroxyl groups than wood. This result suggests that the ether linkages of lignin would be effectively cleaved by supercritical water, with phenolic hydroxyl groups formed by its splitting.

Figure 3 shows the yields of alkaline nitrobenzene oxidation products for wood, its methanol-soluble portion, and its methanol-insoluble residue. The total yields of oxidation products for sugi and buna woods were found to be 1.9 and 2.2 mol/g of lignin, respectively. However, the yields of oxidation products of the methanol-soluble portion and the methanol-insoluble residue were lower, and they decreased with longer treatment in supercritical water. Because alkaline nitrobenzene oxidation products would mainly be derived from ether linkages of lignin, it is a measure of the noncondensed types of the lignin linkages present. Therefore, this result suggests that the methanol-soluble portion and methanol-insoluble residue treated in supercritical water consist mainly of the condensed-type linkages of lignin.

#### Lignin model compounds treated in supercritical water

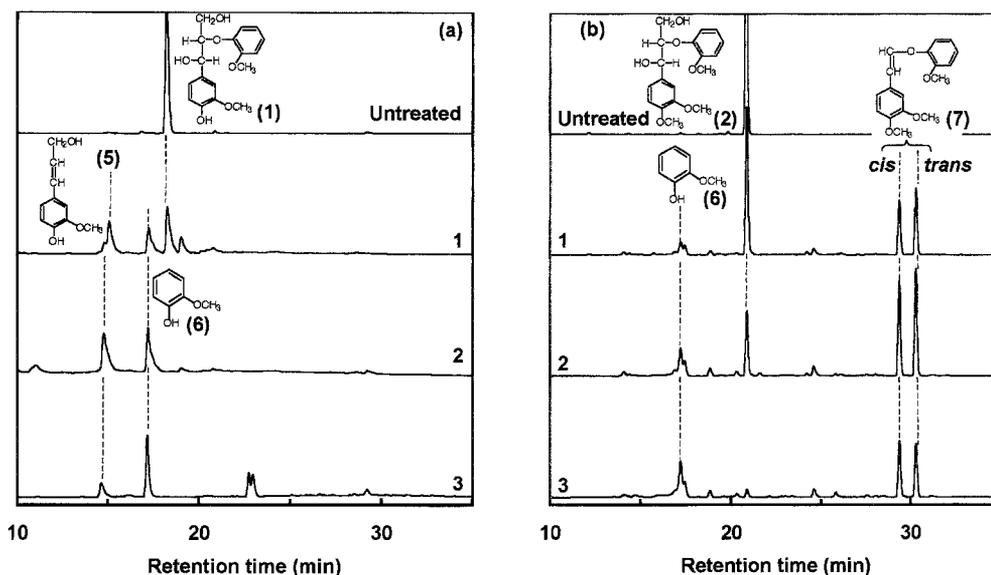
To simulate the reaction of lignin in supercritical water, dimeric  $\beta-O-4$  and biphenyl type lignin model compounds were treated with supercritical water in the same manner. Figure 4 shows HPLC chromatograms of the reaction mixture obtained from the phenolic and nonphenolic  $\beta-O-4$  types of lignin model compounds, guaiacylglycerol- $\beta$ -guaiacyl ether (**1**) and veratrylglycerol- $\beta$ -guaiacyl ether (**2**), respectively. When the phenolic  $\beta-O-4$  compounds were treated with subcritical water for 10s, about 80% of the compound disappeared, and coniferyl alcohol (**5**) and guaiacol (**6**) (50 mol%) were produced. In the case of supercritical water treatment, however, the model compound completely disappeared, being converted to guaiacol and other unknown products.

As to the nonphenolic  $\beta-O-4$  model compound, 80% and 100% of the model compound disappeared by supercritical water treatment for 5s and 8s, respectively; and *cis* and *trans* isomers of vinyl ether-type compounds

**Table 3.** Determination of phenolic hydroxyl groups in wood and fractionated portions as treated in subcritical and supercritical water

Treatment	No. of phenolic hydroxyl groups in $C_6-C_3$ units		
	Untreated wood	Methanol-soluble	Methanol-insoluble
Sugi wood	16.7	–	–
Subcritical, 10s	–	24.9	35.2
Supercritical, 5s	–	26.3	26.4
Supercritical, 8s	–	31.3	19.4
Buna wood	14.1	–	–
Subcritical, 10s	–	38.6	30.6
Supercritical, 5s	–	42.0	14.1
Supercritical, 8s	–	38.6	16.5

**Fig. 4.** High-performance liquid chromatography (HPLC) chromatograms obtained from **a** phenolic and **b** nonphenolic  $\beta$ -*O*-4-type lignin model compounds treated in subcritical and supercritical water. 1, 2, and 3 refer to the run numbers in Table 1



[2-(2'-methoxyphenyl)-1-(3',4'-dimethoxyphenyl)-ethene (7)] were found with guaiacol.

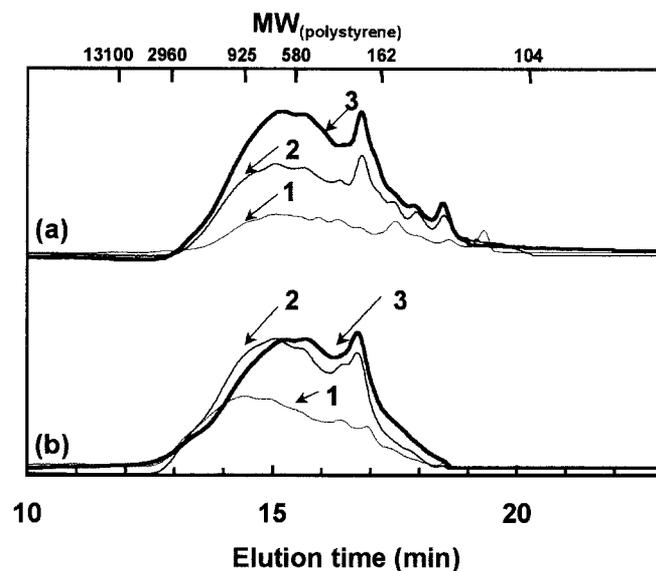
On the other hand, more than 90% of the phenolic and nonphenolic type biphenyl (5-5 linkages) model compounds 2,2'-dihydroxy-3,3'-dimethoxy-5,5'-dimethyl biphenyl (3) and 2,2',3,3'-tetramethoxy-5,5'-dimethyl biphenyl (4) were recovered under these treatment conditions.

Although more quantitative and detailed experiments are necessary to propose the cleavage mechanisms of the lignin linkages, these results are sufficient to demonstrate that the  $\beta$ -*O*-4 linkages of lignin are easily cleaved under these supercritical treatment conditions, whereas the 5-5 linkages of lignin are rather stable.

## Discussion

As shown in Table 2, both sugi and buna woods were effectively converted to a water-soluble portion, methanol-soluble portion, and methanol-insoluble residue by supercritical water treatment. Comparing the methanol-insoluble sugi residue with that from buna, sugi was less likely to be liquefied than buna, and the lignin content of methanol-insoluble sugi residue was higher than that from buna. This difference would be attributed to a structural difference of lignin because sugi and buna consist of guaiacyl-type and guaiacyl-syringyl-type lignin, respectively.

The alkaline nitrobenzene oxidation products from buna wood treated in supercritical water had a relatively lower yield than those from sugi under the same conditions (Fig. 3). This result provides evidence that the guaiacyl-syringyl lignin in buna wood is more susceptible to being degraded than the guaiacyl lignin in sugi wood. Probably liquefaction of carbohydrates contributes to the degree of liquefaction



**Fig. 5.** Gel permeation chromatography (GPC) chromatograms of methanol-soluble portions from **a** sugi and **b** buna treated in subcritical and supercritical water. 1, 2, and 3 refer to the run numbers in Table 1

of lignin because the wood cell wall is a complex of these natural polymers.

Although Thornton and Savage reported that hydroxylation of phenol occurred in supercritical water,<sup>18</sup> it was ascertained in this study that guaiacol and 2,6-dimethoxyphenol were highly stable under supercritical conditions of water. Therefore, the increase in the number of phenolic hydroxyl groups would be mainly brought about by cleavage of ether linkages of lignin.

Alkaline nitrobenzene oxidation analysis can be, on the other hand, used as a measure of the ether linkages of lignin. Thus, a decrease in its yield suggests an increase in

the condensed-type linkages of lignin in the fraction. Therefore, the results in Fig. 3 indicate that both the methanol-soluble portion and the methanol-insoluble residue, particularly from the supercritical treatments, are rich in the condensed types of lignin linkages. For possibility of a condensation reaction<sup>19,20</sup> occurred in the aromatic nuclei, forming carbon-carbon bonds, the longer treatment in the supercritical water did not result in an increase in higher-molecular-weight substances, as shown in Fig. 5. In addition, a concentration of the wood-derived products to water is low to be only 3.0% after supercritical treatment. These facts suggest that the methanol-soluble portion is not repolymerized during supercritical water treatment. In addition, the role of the solvent so abundantly present may be to build a cage of molecules, known as the cage effect of the solvent, around the supercritical water-soluble products, thus protecting them from secondary repolymerization.<sup>21,22</sup> Therefore, both the methanol-soluble portion and the methanol-insoluble residue are rich in the condensed-type linkages originally present in native lignin.

## Conclusions

Lignin-derived products after supercritical water treatment appeared mainly in the methanol-soluble portion and the methanol-insoluble residue. These fractions were found to consist of condensed-type linkages of lignin due to the preferential degradation of ether linkages that occurred in supercritical water. Further study on the obtained lignin-derived products may provide a clue as to its efficient utilization not only for bioenergy but also for value-added useful chemicals.

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