1	Characterization of lignin-derived products from Japanese
2	cedar as treated by semi-flow hot-compressed water
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14	
15	Abstract
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17	In order to elucidate the decomposition behavior of lignin from Japanese cedar
18	(Cryptomeria japonica) as treated by two-step semi-flow hot-compressed water (1st
19	stage: 230 °C/10 MPa/15 min, 2 nd stage: 270 °C/10 MPa/15 min), water-soluble portion,

20	precipitate and water-insoluble residue obtained by hot-compressed water treatment
21	were separated and characterized. Consequently, the water-soluble portion was found to
22	contain lignin-derived monomeric compounds such as coniferyl alcohol and coniferyl
23	aldehyde and β -1, β -5 and 5-5' linked dimeric compounds. These lignin-derived
24	compounds maintained methoxyl and phenolic hydroxyl groups in aromatic rings. The
25	water-soluble portion also contained lignin-derived oligomeric compounds up to
26	heptamers. In contrast, the precipitate was found out to consist of higher molecular
27	weight lignin with high ether type linkages. The water-insoluble residue, however,
28	consisted mostly of lignin with high condensed type linkages. Based on these lines of
29	evidence, condensed type lignin must be resistant to hot-compressed water, remained as
30	water-insoluble residue after two-step treatment. Such information provides a clue as to
31	efficient utilization of lignin-derived products.

33 Keywords Japanese cedar, Hot-compressed water, Lignin-derived products,
34 condensed type lignin

35 Introduction

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37 The wood cell wall consists mainly of cellulose, hemicelluloses and lignin, and these 38 compounds are composed intricately in an artful manner to enforce the wood cell wall, 39 having a resistance to water and most organic solvents. Among the natural polymers, lignin is the second most abundant macromolecule with 24-33 % in softwoods and 40 41 19-28 % in hardwoods [1]. Since lignin may become a resource of energy and valuable 42 aromatic chemicals due to its abundance, various degradation methods have been 43 studied for its utilization. Among them, hydrothermal treatments such as steam 44 explosion, subcritical and supercritical water and hot-compressed water are catalyst-free 45 and environmentally benign. An increase in temperature of water at a high pressure 46 results in a decrease in its dielectric constant and an increase in its ionic product [2,3]. 47 Moreover, decreased dielectric constant of water enhances solubility of some 48 hydrophobic compounds into subcritical and supercritical water. 49 Tanahashi et al. examined the degradation mechanism of lignin by steam explosion

50 (230 °C/2.9 MPa/16 min) with dehydrogenated polymer of coniferyl alcohol and β -*O*-4 51 linkage model compounds [4]. Ehara et al. [5] and Takada et al. [6] treated Japanese 52 beech (*Fagus crenata*) and Japanese cedar (*Cryptomeria japonica*) with supercritical 53 water (380-400 °C/100-115 MPa/5-8 sec). Consequently obtained lignin-derived 54 compounds were fractionated as methanol-soluble portion but water-insoluble oily products were composed of monomeric aromatic and condensed type dimeric 55 56 compounds. Yokoyama et al. treated organosolv lignin with supercritical water (350-420 57 °C/10-40 MPa/60 min), and the resultant yields of oil and char were dependant on the 58 treatment condition [7]. Saisu et al. [8] and Okuda et al. [9] also treated organosolv lignin with supercritical water (400 °C/pressure not given/10-64 min) and obtained 59 60 reactive compounds such as formaldehyde and lignin-derived monomeric compounds 61 such as guaiacol, catechol and phenol were obtained due to hydrolysis and the 62 subsequent dealkylation. They explained that low molecular weight compounds were 63 reactive such that they reacted with residual lignin to become high molecular weight residue. 64

Phenol and aromatic nuclear are known to be stable to supercritical water treatment through some experiments with various monomeric lignin model compounds [10-15]. The resistance of lignin to supercritical water differs, dependent on the types of its linkages, and ether linkages in lignin are readily cleaved by protic solvent such as supercritical water and methanol, while condensed type of linkages and aromatic ring are difficult to be cleaved [16,17].

Recently, hot-compressed water, a milder condition for hydrothermal reaction with
high ionic products, has gained considerable attention as a promising decomposition

73	medium for lignocelluloses [3], and the hot-compressed water treatment system could
74	be simply categorized into batch, flow, and semi-flow (percolation) types. Since the
75	decomposition reaction becomes dominant in batch and flow type treatments, some
76	products are excessively decomposed, whereas semi-flow type treatment can prevent
77	products from excessive decomposition due to the short residence time [18,19]. Lignin
78	also can be depolymerized and decomposed by semi-flow hot-compressed water [20].
79	Lu et al. treated Japanese beech (Fagus crenata) with two-step semi-flow
80	hot-compressed water (1 st stage: 230 °C/10 MPa/15 min, 2 nd stage: 270 °C/10 MPa/15
81	min) and the hemicelluloses and cellulose were selectively decomposed [21]. They also
82	elucidated that lignin was decomposed in both stages and the lignin-derived monomeric
83	products such as coniferyl alcohol and sinapyl alcohol were obtained in water-soluble
84	portion [21]. Yamauchi et al. showed that hot-compressed water can cleave only ether
85	linkages of lignin through the structural analysis of lignin-derived products [22].
86	Additionally, these lignin-derived products could be converted into acetic acid and
87	phenolic compounds via microorganism, thus, these are expected to become valuable
88	chemicals [23].

Phaiboonsilpa et al. treated Japanese cedar (*Cryptomeria japonica*) and obtained lignin-derived products with hot-compressed water [24]. However, detailed analysis of the lignin-derived products obtained from Japanese cedar has not yet been conducted.

92	Yamauchi et al. have studied the decomposition behavior of lignin in Japanese beech as
93	described above [22], while lignin content and its structure in Japanese cedar are
94	different from Japanese beech, and their decomposition behaviors of lignin should also
95	differ. Thus, the aim of this study is to analyze the lignin-derived products in order to
96	elucidate the decomposition behavior of lignin from Japanese cedar as treated by
97	hot-compressed water.
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100	Materials and methods
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102	Wood sample and chemicals
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104	Wood flour of Japanese cedar (Cryptomeria japonica) passing through an 18-mesh
105	screen was extracted with acetone using Soxhlet apparatus, and dried at 105 $^{\circ}$ C for 24 h
106	before experiment. All chemicals used in this study were of reagent grade without
107	purification.
108	
109	Two-step semi-flow hot-compressed water treatment
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111	Approximately 0.5 g of oven-dried extractive-free wood flour was placed in the reaction
112	vessel for which the two-step semi-flow hot-compressed water treatment was performed
113	as described in a previous paper [24]. Two-step treatment conditions were 230 $^{\circ}C/10$
114	MPa/15 min for the 1 st stage and 270 °C/10 MPa/15 min for the 2 nd stage. After
115	treatment, hot-compressed water-insoluble residue left over in the reaction vessel was
116	separated from hot-compressed water-soluble portion and dried at 105 °C for 12 h to
117	measure its oven-dried weight. The separated hot-compressed water-soluble portion was
118	kept standing for 12 h under ambient condition. During this period, it was separated to
119	be water-soluble portion and precipitate which were retrieved by filtration with
120	Millipore (pore size: 0.45 μ m). For the water-soluble portion, the lignin-derived
121	compounds were obtained by extraction with ethyl acetate.
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123	Analytical methods
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125	The content of lignin-derived products in each fraction was examined as the combined
126	yields of Klason lignin and acid-soluble lignin [25]. For the acid-soluble portion

127 obtained, the amounts of various mono-saccharides were determined by high

128 performance anion-exchange chromatography. Its cellulose and hemicellulose contents

129 were then estimated based on the amounts of glucose and other mono-saccharides,

130	respectively [26]. Molecular weight distribution was evaluated by gel permeation	
131	chromatography (GPC) analysis, which was performed with LC-10A (Shimadzu, Kyoto,	
132	Japan) under the following conditions: column, Shodex KF-801 + KF-802 + KF-802.5	
133	+ KF-803 (Showa Denko, Tokyo, Japan); eluent, tetrahydrofuran; flow-rate, 0.6	
134	mL/min; column temperature, 50 °C; detector, Ultraviolet light at 280 nm. For	
135	comparison, polystyrene standards (Molecular weight (MW): 162, 580, 1270, 2960,	
136	5000) were used as standard.	
137	In order to evaluate chemical characteristics of lignin-derived products, alkaline	
138	nitrobenzene oxidation was performed and gas chromatographic analysis was	
139	undertaken to obtain the total yield of vanillin [27]. However, since water-soluble	
140	portion contained some monomeric products like coniferyl alcohol, the vanillin yield	
141	from oligomeric products was evaluated from the total yield of vanillin minus vanillin	
142	from monomeric products, according to the following equation (1):	
143 144	Yield of vanillin from oligomeric lignin-derived products (mmol/g)	
145	= $\frac{\text{Total yield of vanillin (mmol)} - \text{Yield of vanillin from monomeric lignin} - \text{derived products (mmol)}}{\text{Lignin} - \text{derived products (g)} - \text{Monomeric lignin} - \text{derived products (g)}}$	
146	Since monomeric compounds are mostly composed of conferyl alcohol, coniferyl	
147	aldehyde, vanillin and isoeugenol, other monomeric products were ignored in this	
148	calculation.	

149	Monomeric and dimeric compounds of lignin-derived products in water-soluble
150	portion were analyzed by gas chromatography - mass spectrometry (GC-MS), which
151	was performed with Hitachi M7000s and M9000 series. The capillary column was a
152	DB-5MS. The temperature program was 1.0 min at 40 °C, 5.0 °C/min to 300 °C, and 8.0
153	min at 300 °C. Helium carrier gas was used at a flow-rate of 1.5 mL/min. Injector and
154	detector temperatures were both 230 °C.
155	High molecular weight compounds of lignin-derived products in water-soluble
156	portion were analyzed by matrix-assisted laser desorption ionization-time of flight /
157	mass spectrometry (MALDI-TOF/MS), which was performed with AXIMA
158	Performance (Shimadzu, Kyoto, Japan). Nitrogen gas was used as the laser source. The
159	analysis was performed in positive-ion, linear mode and 2,5-dihydroxybenzaldehyde
160	was used as matrix.
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163	Results and discussion
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165	Lignin-derived products
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167	The temperature change of hot-compressed water treatment for the 1^{st} stage (230 °C/10

168	MPa/15 min) and 2 nd stage (270 °C/10 MPa/15 min) is shown in Fig. 1. The yield of
169	water-insoluble residue and its chemical composition are also shown. When
170	extractive-free Japanese cedar is treated by hot-compressed water, hemicelluloses and a
171	part of cellulose are decomposed and eluted as hot-compressed water-soluble portion at
172	the 1 st stage, and then in the 2 nd stage, the rest of cellulose is mostly decomposed and
173	eluted. It seems likely that cellulose decomposed in the 1 st stage might have lower
174	crystallinity compared with remaining cellulose decomposed in the 2 nd stage. In contrast,
175	lignin is decomposed at both stages recovered as lignin-derived products in all fractions.
176	However, some of lignin has much higher resistance to hot-compressed water as
177	compared with cellulose and hemicelluloses. The hot-compressed water-soluble portion
178	eluted is later partially precipitated under ambient conditions.
179	The yields of water-soluble portion, precipitate, and water-insoluble residue in both
180	stages are summarized in Table 1. In addition, the yield of the lignin-derived products
181	and the lignin concentration in each fraction are also shown. In the 1^{st} stage, 52.1 (=
182	47.1 + 5.0) % of Japanese cedar was eluted in hot-compressed water, and 5.0 % was
183	obtained as precipitate. Consequently, 47.1 % was obtained in water-soluble portion and
184	47.9 % remained as water-insoluble residue. After the 2 nd stage treatment of the
185	water-insoluble residue (47.9 %), 35.1 (= $29.8 + 5.3$) % was eluted in hot-compressed
186	water, and then 5.3 % and 29.8 % were obtained as precipitate and water-soluble portion,

187 respectively. As a result, 12.8 % of Japanese cedar remained as water-insoluble residue 188 after two-step treatment. This value is, in fact, much higher than that of Japanese beech 189 only to be 4.4 % [22]. The observed difference in lignin between the two woods would 190 be due to the fact that Japanese cedar has less ether linkages than Japanese beech.

The yields of lignin-derived products in each fraction are shown in extractive-free wood-based wt %. For the 32.0 % lignin content of Japanese cedar, 14.0 (= 10.1 +3.9) % was eluted in hot-compressed water at the 1st stage, with 10.1 % and 3.9 % being from water-soluble portion and precipitate, respectively. In the 2nd stage, 6.8 % (=5.0 +1.8) was resulted with 5.0 % and 1.8 % as water-soluble portion and precipitate, respectively. On the other hand, 11.2 %, about one third of the initial lignin content, still remained as water-insoluble residue after two-step treatment.

The lignin concentration was 0.214 g/g and 0.168 g/g for water-soluble portion in 198 both 1st and 2nd stages, respectively. For precipitate, the lignin concentration in the 1st 199 stage was 0.780 g/g, whereas that in the 2^{nd} stage was much lower to be 0.340 g/g. This 200 201 difference could indicate that high molecular weight cellulose-derived products, eluted in the 2nd stage, aggregated and precipitated with lignin-derived products under ambient 202 203 condition [28]. A similar tendency was observed in the case of Japanese beech [22]. The 204 lignin concentration of the water-insoluble residue increased as the hot-compressed water treatment was prolonged, and the final residue consisted mostly of lignin (0.875 205

206 g/g), indicating that the residual lignin has much higher resistance to hot-compressed207 water than other chemical components.

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209 Structural analysis of lignin-derived products

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211 Fig. 2 demonstrates GPC chromatograms of water-soluble portion and precipitate of 212 both stages, detected at 280 nm in wavelength. In order to evaluate the molecular weight 213 distribution of their lignin-derived products, the elution time of polystyrene standards (MW: 162, 580, 1270, 2960, 5000) were displayed. The higher molecular weight 214 215 standards show the shorter elution time in GPC chromatograms. The chromatogram of water-soluble portion in the 2nd stage has an intensive peak at around 63 min, which 216 217 indicates 5-hydroxymethylfurfural derived from polysaccharides. For the water-soluble portion, the lignin-derived products in the 1st stage contain 218 oligomeric products up to MW of 1270, equivalent to heptamer of coniferyl alcohol, 219 whereas the lignin-derived products in the 2^{nd} stage contain smaller than those in the 1^{st} 220 221 stage. In contrast, the molecular weight of the precipitate lignin is higher than the 222 lignin-derived products in water-soluble portion for both stages.

In order to compare the relative proportion of ether linkages of lignin-derived products, alkaline nitrobenzene oxidation was conducted, and the obtained yield of its 225 products products from the lignin-derived is shown in Fig.3. Since *p*-hydroxybenzaldehyde derived from *p*-hydroxyphenylpropane (H) lignin was a trace 226 227 amount, the yield of vanillin derived from guaiacyl (G) lignin was only shown. For 228 comparison, the yield of vanillin from the untreated Japanese cedar is also shown. Since 229 water-soluble portion contains some monomeric products like coniferyl alcohol as 230 already described, the vanillin yield from oligometric products was obtained by 231 subtracting vanillin yield from monomeric products from the total yield of vanillin. For the water-soluble portion, the yields of vanillin from the 1^{st} and 2^{nd} stages are 232 0.64 and 0.38 mmol/g of original lignin, respectively, both of which are smaller than the 233 vanillin derived from untreated wood (1.53 mmol/g). This suggests that the 234 235 lignin-derived products of water-soluble portion contain a lower ratio of ether type lignin than untreated wood lignin. On the other hand, the yields of vanillin from 236 precipitate lignin in both stages were comparable to that from untreated wood. This 237 could indicate that precipitate lignin maintains ether linkages. For water-insoluble 238 residue, the yield of vanillin from the 1^{st} stage is 0.63 mmol/g, whereas that from 2^{nd} 239 240 stage 0.17 mmol/g, decreasing in its yield as the hot-compressed water treatment progressed. Consequently, lignin in the water-insoluble residue, especially for 2nd stage, 241 242 is rich in condensed type lignin.

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244 Lignin-derived products in water-soluble portion

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In order to identify lignin-derived low molecular weight compounds in water-soluble portion, GC-MS analysis was performed. GC-MS spectrum of lignin-derived products in water-soluble portion is shown in Fig. 4. The peaks in the short retention time before 35 min are lignin-derived monomeric compounds, while those in the longer retention time after 35 min are dimeric compounds.

251 According to the comparison with lignin monomeric model compounds, 17 kinds of 252 lignin-derived compounds such as vanillin (No. 5), isoeugenol (No. 6), coniferyl 253 aldehyde (No. 17), and coniferyl alcohol (No. 18) are identified as shown in Table 2. 254 Some lignin-derived products were identified from their mass spectra by comparing 255 their retention times with those of authentic compounds as in the literature [6,29,30]. 256 Consequently, these identified compounds contained guaiacol skeletons, indicating that 257 the methoxyl group has not been removed from aromatic ring by hot-compressed water 258 treatment. These compounds also contain phenolic hydroxyl group. Most of this 259 hydroxyl group could be produced due to the cleavage of ether linkages by 260 hot-compressed water treatment. Among these identified compounds, trans-coniferyl 261 alcohol is obtained as the main product. It would mean that the lignin-derived products in water-soluble portion largely maintain the side-chain structure of lignin. A few of 262

ketone compounds, which are characteristic products from acidolysis of lignin, are
observed in water-soluble portion. Thus, acidolysis reaction can be ignored in the
hot-compressed water treatment.

266 Takada et al. obtained the lignin-derived compounds from Japanese cedar 267 (Cryptomeria japonica) by supercritical water treatment (380 °C/100 MPa/8 sec) as methanol-soluble portion, and analyzed the lignin-derived monomeric and dimeric 268 269 compounds with GC-MS [6]. The lignin-derived monomeric products in 270 methanol-soluble portion contained guaiacol skeletons, indicating that the methoxyl 271 group had not been removed from aromatic ring by supercritical water treatment. In addition, vanillin was mainly obtained without recovered coniferyl alcohol. Thus, the 272 273 side-chain structure in lignin was more altered by supercritical water treatment 274 compared with hot-compressed water treatment. This suggests that hot-compressed 275 water treatment used in this study can suppress the decomposition of side-chain in 276 lignin more effectively than supercritical water treatment.

Table 3 demonstrates mass fragments and retention time of lignin-derived dimeric compounds. Among these compounds, Nos. 20, 26, and 31 in Fig. 5 are identified from their mass spectra by comparing their retention times with those of authentic compounds as in the literature and the number of hydroxyl groups, estimated from the increase of molecular weight after trimethylsilyl derivatization [6]. These identified compounds are condensed type, containing methoxyl and phenolic hydroxyl groups as observed in the monomeric compounds in Table 2, whereas Nos. 20 and 26 in Fig. 5 did not maintain the structure of phenylpropane side-chain. Generally, in hydrothermal treatment, formaldehyde produced from lignocellulose forms diarylmethane structure [31]. However, diarylmethane compound could not be found in GC-MS analysis. This could be because semi-flow type treatment minimizes possible formation due to prompt removal of the decomposed products.

289 In order to identify lignin-derived oligomeric compounds, MALDI-TOF/MS 290 analysis was performed. The obtained MALDI-TOF/MS spectra of the lignin-derived products of the 1^{st} and 2^{nd} stages are shown in Fig. 6. The spectra of the 1^{st} stage contain 291 292 several molecular weight intervals (MWIs) of 178 and 196. According to the experiment 293 with lignin model compounds, β - β or β -5 linkage results in the MWI of 178 due to the 294 radical mechanism of coniferyl alcohol (MW: 180.20), whereas β -O-4 linkage results in 295 the MWI of 196 due to the addition of hydroxyl group [32]. Besides, some other 296 linkages such as 5-5', 4-O-5 and α -O-4 linkages can result in the MWI of 178 as well. 297 Namely, the MWIs of 178 and 196 obtained from MALDI-TOF/MS spectra of the lignin-derived products in the 1st stage should be derived from coniferyl alcohol, 298 299 suggesting that the oligomeric lignin-derived products up to heptamers contain both ether and condensed type linkages. In contrast, the spectrum of 2nd stage does not 300

301	contain the MWIs of 178 or 196, indicating that the phenylpropane side-chain could be
302	altered by hot-compressed water. The oligomeric lignin-derived products consisted up
303	to tetramer. These results really agree with those from GPC analysis.
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305	Lignin-derived products in precipitate
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307	The lignin-derived products in precipitate in both stages are composed of the higher
308	molecular weight products than water-soluble portion, and their structure was rich in
309	ether linkage according to the yield of vanillin by alkaline nitrobenzene oxidation (Fig.
310	3). These results suggest that some micropores were produced in the cell wall due to the
311	decomposition of hemicelluloses and the cleavage of ether linkages in lignin in the 1 st
312	stage, and the micropores would have facilitated the elution of the lignin cluster, which
313	maintains ether linkages [22]. Hydrophobic lignin could be eluted in hot-compressed
314	water due to the low dielectric constant of hot-compressed water, whereas some large
315	molecules cluster precipitated after hot-compressed water returned to the ordinary water
316	under ambient condition. In the 2 nd stage, cellulose- and lignin-derived oligomeric
317	compounds were also eluted in hot-compressed water.
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320 Lignin-derived products in water-insoluble residue

322	Approximately one third of original lignin still remained as water-insoluble residue after
323	two-step treatment, and the water-insoluble residue is 0.875 g/g in lignin concentration
324	as in Table 1. The ratio of condensed type linkages was observed to increase as the
325	hot-compressed water treatment was prolonged. Consequently, lignin in the
326	water-insoluble residue after two-step treatment was rich in condensed type due to the
327	cleavage of ether type linkages and possible recondensation reaction.

328 Concluding remarks

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330 Decomposition behavior of lignin in Japanese cedar as treated by two-step semi-flow
331 hot-compressed water was discussed through the analyses of the lignin-derived products
332 fractionated during the treatment.

333 For the 1st stage (230 °C/10 MPa/15 min), in which hemicelluloses and a part of 334 cellulose were decomposed, approximately half of lignin was decomposed and eluted in hot-compressed water. Due to the decomposition of hemicelluloses and the cleavage of 335 336 ether linkages in lignin, some micropores would be occurred in the cell wall, which 337 would have facilitated the elution of the lignin cluster together with 338 hemicelluloses-derived products. The hydrophobic lignin could be, thus, eluted in 339 hot-compressed water due to the low dielectric constant of hot-compressed water. After 340 its elution, cluster of some large molecules were precipitated under ambient condition. Since the precipitated lignin contained ether linkages, it would be considered to 341 342 maintain the structure of original lignin. On the other hand, lignin-derived products in 343 water-soluble portion were low molecular weight up to heptamers, and identified 344 monomeric compounds maintained methoxyl and phenolic hydroxyl groups such as 345 coniferyl alcohol, coniferyl aldehyde and vanillin.

For the 2nd stage (270 °C/10 MPa/15 min), in which the rest of cellulose was mostly

348	molecules of lignin clusters would be eluted and then precipitated under ambient
349	condition.
350	The rest of the remaining lignin to be one third of the original one had resistance to
351	hot-compressed water and remained as water-insoluble residue, which was mostly
352	composed of lignin. It is interesting that the remaining lignin rich in condensed type as
353	compared with untreated wood can be expected to be utilized as materials resistant to
354	any external attacks.
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356	
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358	
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decomposed, a part of lignin was decomposed and eluted. As in the 1st stage, some large

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Stage	Fraction	Yield of fractionated product (wood-based wt%)	Yield of lignin-derived product (wood-based wt%)	Lignin concentration* (g/g)
	Water-soluble portion	47.1	10.1	0.214
1 st stage	Precipitate	5.0	3.9	0.780
	Water-insoluble residue	47.9	18.0	0.376
	Water-soluble portion	29.8	5.0	0.168
2 nd stage	Precipitate	5.3	1.8	0.340
	Water-insoluble residue	12.8	11.2	0.875
	Total	100	32.0	-

519	* Lignin	concentration	was obtaine	d by the y	yield of li	gnin-derived	l product	divided b	by that	of
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520 fractionated product.

Table 2. Lignin-derived monomeric products in water-soluble portion and their retention

times, structures, and MS fragments for GC-MS analysis.

No.	Product	Retention Time (min)	Structure* ^a	MS fragments (m/z)
1	Guaiacol	11.6	G	124, 109, 89, 81
2	Methylguaiacol	14.9	G-C	138, 123, 95, 77
3	Vinylguaiacol* ^b	18.0	G-C=C	150, 136, 107, 78
4	Eugenol	19.1	G-C-C=C	164, 149, 134, 104, 77
5	Vanillin	20.3	G-CHO	152, 123, 109, 77
6	trans-Isoeugenol	21.5	G-C=C-C	164, 150, 104
7	Homovanillin	21.7	G-C-СНО	166, 138, 123
8	Unknown	22.2		162, 148, 120, 92
9	Acetovanillone	22.4	G-CO-C	166, 152
10	Guaiacylacetone*c	23.4	G-C-CO-C	180, 137
11	Unknown	24.1		178, 150, 90, 78
12	2-Methoxy-4-(prop-1-en-3-one)phenol* ^d	24.8	G-CO-C-C	178, 151
13	Homovanillic acid	26.1	G-C-COOH	182, 152
14	3-4-Hydroxy-3-methoxyphenyl-1-propanol	26.2	G-C-C-C-OH	182, 138
15	cis-Coniferyl alcohol	26.7	G-C=C-C-OH	180, 163, 138, 124, 78
16	Hydroferulic acid	27.8	G-C-C-COOH	196, 137
17	trans-Coniferyl aldehyde	28.2	G-C=C-CHO	178, 164, 149
18	trans-Coniferyl alcohol	28.4	G-C=C-C-OH	180, 164, 138
19	Ferulic acid	28.8	G-C=C-COOH	194, 167, 138
* ^a : G	: guaiacyl (2-methoxylphenyl)	^{*b} : Product identifie	d according to literature 6	

*^c: Product identified according to literature 29 *^d: Product identified according to literature 30

529 Table 3. Lignin-derived dimeric products in water-soluble portion and their retention

	No.	Retention Time (min)	Product	MS fragments (m/z)
_	20	38.7	Stilbene type (β-1)	272, 240, 226, 212, 198, 180, 170, 152, 138, 77, 66
	21	41.4		281, 274, 260, 230, 214
	22	42.3		278, 275, 245, 160, 153, 124, 109, 78, 66, 52
	23	43.2		290, 152, 139, 124, 108, 94, 78, 66
	24	43.7		429, 377, 356, 301, 273, 160, 153, 124, 96, 78, 66, 53
	25	43.9		311, 300, 287, 272, 256, 240, 161, 152, 95, 78, 67
	26	44.3	Phenylcoumaran type (β -5)	272, 241, 225, 213, 212 199, 169
	27	45.0		303, 273, 153, 124, 108, 82, 78, 66, 53
	28	45.7		358, 313, 298, 282, 253, 210, 181, 162, 79, 52
	29	46.9		357, 341, 313, 286, 269, 208, 175, 160, 152, 137, 78
	30	47.7		344, 326, 312, 207, 139, 121, 93, 78, 66
	31	48.8	Biphenyl type (5-5')	326, 312, 298, 266, 190, 177, 162, 139, 124, 104, 92, 78
	32	49.0		314, 298, 252, 239, 178, 138, 95, 78
_	33	51.1		327, 324, 312, 135, 130, 78

530 times and MS fragments for GC-MS analysis.

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