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1 **Comparative study on topochemistry of delignification from**
2 **Japanese cedar and Japanese beech by hydrothermal**
3 **treatment**

4

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14 **The type of article**

15 Original article

16

17 **Abstract**

18

19 The difference in decomposition behavior of lignin from Japanese cedar (*Cryptomeria*
20 *japonica*) as softwood and Japanese beech (*Fagus crenata*) as hardwood treated by
21 hot-compressed water was investigated. The obtained residual lignin was then evaluated
22 by the alkaline nitrobenzene oxidation analysis, the relative proportion of ether type
23 linkages had decreased for both species as the hot-compressed water treatment was
24 prolonged. Furthermore, the microscopic observation of the residual lignin indicated
25 that lignin in tracheid cell walls was removed to a greater extent than that in middle
26 lamella in Japanese cedar. Similarly in Japanese beech, lignin in fiber cell walls was
27 removed more extensively, with middle lamella lignin remaining after two-step
28 treatment. Such observation was supported by a result obtained from the methoxyl
29 content analysis per C₉ unit of the residual lignin. Based on these lines of evidence,
30 lignin in middle lamella must be rich in condensed type, and these differences between
31 softwood and hardwood can be attributed to the intrinsic characteristics of the original
32 lignin and its topochemistry in woods.

33

34 **Keywords**

35 Japanese cedar, Japanese beech, Hydrothermal treatment, Topochemistry,

36 Delignification

37 **Introduction**

38

39 Woody biomass has gained attention as an alternative resource to fossil fuel due to its
40 abundance and noncompetition with foodstuff. In order to utilize the woody biomass as
41 biofuels and chemicals, various chemical and physical degradation methods have been
42 examined. Among them, hydrothermal treatments such as steam explosion, subcritical
43 and supercritical water, and hot-compressed water treatments are expected as a
44 promising technology due to its catalyst-free and environmentally benign characteristics.
45 As a feature of hydrothermal treatment, an increase in temperature of water at a high
46 pressure results in a decrease in its dielectric constant and an increase in its ionic
47 product [1,2].

48 Compared with supercritical water, hot-compressed water is milder in its
49 condition for hydrothermal reaction, thus it has gained considerable attention as a
50 promising decomposition medium for lignocellulosics [2]. Among hot-compressed
51 water treatment systems, semi-flow type can prevent obtained products from excessive
52 decomposition due to the appropriate residence time [3,4]. Phaiboonsilpa et al. [5] and

53 Lu et al. [6] treated Japanese cedar (*Cryptomeria japonica*) and Japanese beech (*Fagus*
54 *crenata*) with semi-flow hot-compressed water (1st stage: 230 °C/10 MPa/15 min, 2nd
55 stage: 270 °C/10 MPa/15 min), and the hemicelluloses and cellulose were decomposed
56 in the 1st and 2nd stages, respectively. Lignin has been decomposed and eluted in
57 hot-compressed water at both stages [7,8].

58 These polymer components such as cellulose, hemicelluloses and lignin are not
59 uniformly distributed within wood cells, and their concentrations are different from one
60 morphological region to another. Therefore, it would be quite essential to understand the
61 topochemistry of wood to discuss the decomposition behavior of its chemical
62 constituents.

63 Therefore, the delignification behaviors by pulping such as kraft [9-10], sulphite
64 [11-14], soda methods [9] have been studied. In addition, some researchers studied the
65 topochemistry of wood with hydrothermal treatment as pretreatment media [15-17].
66 However, delignification behavior of wood by hydrothermal treatment has not been
67 discussed yet. Thus, the aim of this study is to elucidate the topochemical differences in
68 delignification between softwoods and hardwoods as treated by hot-compressed water.

69

70

71 **Materials and methods**

72

73 Hot-compressed water treatment

74

75 Extractive-free wood flour of Japanese cedar (*Cryptomeria japonica*) and Japanese
76 beech (*Fagus crenata*) were prepared according to the previous reports [5,6]. All
77 chemicals used in this study were of reagent grade without purification.

78 Hot-compressed water treatment was conducted with two-step (1st stage: 230
79 °C/10 MPa/15 min and 2nd stage: 270 °C/10 MPa/15 min) as shown in Fig. 1. Each step
80 of the treatment includes time up for 10 and 5 min for the 1st and 2nd stages,
81 respectively, followed by time at for 15 min. The pressure was increased up to 10 MPa
82 by pressure pump, and controlled with a back-pressure regulator. A half gram of
83 oven-dried extractive-free wood flour was placed in the reaction vessel, for which the
84 two-step semi-flow hot-compressed water treatment was carried out as described in

85 previous papers [5,6].

86

87 Analytical methods

88

89 The lignin content of insoluble residue was examined as the combined yields of Klason
90 lignin and acid-soluble lignin [18]. For acid-soluble portion, the amounts of various
91 monosaccharides were determined by high-performance anion-exchange
92 chromatography. Its cellulose and hemicellulose contents were then estimated based on
93 the amounts of monosaccharides [5].

94 In order to evaluate chemical characteristics of residual lignin, alkaline
95 nitrobenzene oxidation was conducted and the total yields of vanillin and
96 syringaldehyde were analyzed with gas chromatography [19].

97 The obtained insoluble residues of the 1st and 2nd stage treatments were
98 gradually dehydrated with ethanol-water mixtures with increasing ethanol concentration
99 up to 99.5 %. After the ethanol was replaced by propylene oxide, the residues were
100 embedded in epoxy resin. Ultrathin sections of 0.5 μm for ultraviolet (UV) microscopy

and 80 nm for transmission electron microscopy (TEM) were prepared from embedded samples with a diamond knife mounted on ultramicrotome (Leica Reichert Supernova Microtome: Leica Reichert). For UV microscopy, the obtained sections were placed on quartz slides, mounted with glycerin, and then covered with quartz cover glass. They were, then, examined under MSP-800 (Carl Zeiss) at a wavelength of 280 nm with a wavelength width of ± 10 nm. For TEM observation, lignin was selectively stained with 1 % aqueous solution of KMnO_4 and the stained sections, mounted on copper grids, were observed by JEM-1400 (JEOL) at an acceleration voltage of 100 keV.

Methoxyl content of residual lignin was analyzed according to general method [20]. After insoluble residues were treated with 57 % of hydroiodic acid, the yield of obtained methyl iodide was determined with gas chromatography to study the average methoxyl content of the residual lignin.

Results and discussion

117 The hot-compressed water treatment was conducted under constant pressure (10
118 MPa) with two-step treatment consisting of the 1st stage (230 °C/10 MPa) and 2nd stage
119 (270 °C/10 MPa) as shown in Fig. 1. The 1st stage consists of 10 min time up towards
120 the maximum temperature 230 °C for 15 min, while the 2nd stage consists of 5 min time
121 up towards the maximum temperature 270 °C for 15 min.

122 Table 1 shows the yield of the obtained insoluble residues from Japanese cedar
123 and Japanese beech treated by hot-compressed water as mentioned above. The insoluble
124 residues at the end of the time up and time at for both stages were collected and
125 examined. For both species, more than half of the initial wood was decomposed and
126 removed with hot-compressed water at the end of the 1st stage, and at the end of the 2nd
127 stage, 13 % of Japanese cedar and 7 % of Japanese beech remained as insoluble residue.

128 The chemical composition of the insoluble residues is also shown in Table 1. It
129 seems apparent that the insoluble residues consist of lignin together with cellulose and
130 hemicelluloses without any chars. For both species, hemicelluloses were decomposed
131 completely, while cellulose was partly remained as insoluble residues after two step
132 treatment. On the other hand, the lignin concentration increased as the hot-compressed

water treatment was prolonged, for both species. Consequently, insoluble residues at the end of the 2nd stage were mostly composed of lignin to be 0.87 g/g in Japanese cedar and 0.77 g/g in Japanese beech, indicating that lignin has much higher resistance to hot-compressed water than other cell wall components.

The delignification of insoluble residue is also shown in Table 1. It is apparent that the delignification progressed as the hot-compressed water treatment was prolonged, while around one-third of lignin in Japanese cedar and one-fourth of lignin in Japanese beech still remained as insoluble residues. Thus, lignin of Japanese cedar has higher resistance to hot-compressed water than that of Japanese beech.

Linkage between the phenylpropane units is one of the significant factors in the decomposition behavior of lignin. Lignin has two types of linkages which are ether type linkages such as β -O-4, and condensed type linkages such as 5-5'. Ehara et al. treated dimeric model compounds of β -O-4 and 5-5' types with the subcritical water (330 °C/50 MPa/10 sec), and ether linkages were readily cleaved, while condensed type linkages had resistance to subcritical water [21].

Based on this information, alkaline nitrobenzene oxidation analysis was

149 conducted for the insoluble residues in order to compare the relative proportion of ether
150 linkages of the residual lignin. The obtained yields of the products from insoluble
151 residue of Japanese cedar and Japanese beech are shown in Fig. 2. Vanillin derived from
152 guaiacyl (G) lignin and syringaldehyde from syringyl (S) lignin were mainly obtained
153 as decomposed products. Just comparison, the yields of obtained products from the
154 untreated wood are also shown. For both species, the yields of products decreased as
155 hot-compressed water treatment was prolonged. The obtained products would mainly be
156 derived from ether type linkages of lignin. Considering on the previous experiment of
157 lignin model compounds, this result would indicate that the relative proportion of ether
158 linkages in the residual lignin was decreased by hot-compressed water treatment.
159 Consequently, lignin in the final residues would be rich in condensed type lignin and/or
160 unknown structures produced in the hot-compressed water treatment. This result would
161 be owing to the fact that hot-compressed water cleaves only ether linkages of lignin but
162 not condensed type linkages, as observed in a study on the lignin model compounds
163 [21].

164 In order to observe the distribution of lignin in the insoluble residues, UV

165 microscopic observations were performed as shown in Fig. 3. Among the main cell wall
166 components, only lignin can absorb UV light due to its aromatic structure. Thus, the
167 darker area in UV micrographs shows the higher concentration of lignin. The
168 destruction of a part of the cell wall would be caused by the physical burden during the
169 preparation of wood flour. In case of Japanese cedar, the tracheid cell wall structure has
170 been maintained until the end of the 1st stage. However, at the end of the 2nd stage,
171 tracheid cell walls were extensively compressed and their structure altered greatly. A
172 similar tendency has been observed in Japanese beech. The structure of fiber and vessel
173 cell walls has been maintained until the end of 1st stage, whereas at the end of the 2nd
174 stage, most of the cell walls disappeared, with only middle lamella portions remaining.
175 The dotted square portion was enlarged to show the remaining middle lamella portion
176 consisting mainly of the lignin.

177 Such an observation can be made more clearly in the high magnified TEM
178 images as shown in Figs. 4 and 5, in which the middle lamella at cell corner region
179 remained as insoluble residue with some residual lignin fraction from the cell walls, all
180 of which are stained with KMnO_4 specific for lignin. However, in case of Japanese

181 cedar (Fig. 4), lignin in tracheid cell walls partly remained. From these lines of
182 evidence, lignin in tracheid cell wall of Japanese cedar had higher resistance to
183 hot-compressed water than fiber cell wall of Japanese beech.

184 Lignin is composed of up to three different phenylpropane (C₉) units, which are
185 guaiacyl (G), syringyl (S) and *p*-hydroxyphenyl (H) lignins with, respectively, one, two
186 and no methoxyl groups per aromatic ring. The distribution of these phenylpropane
187 units is different from one morphological region to another. For example, in case of
188 softwood tracheid, Whiting and Goring elucidated that lignin in the cell wall is mainly
189 composed of G lignin, whereas that in middle lamella, especially at cell corner, is
190 composed of H and G lignins according to the methoxyl content analysis [22]. On the
191 other hand, hardwood lignin is composed of G and S lignins, distributed differently
192 from one morphological region to another. Saka and Goring examined the distribution
193 of lignin in white birch (*Betula papyrifera*) with UV-EDXA analysis and elucidated that
194 G:S ratio of lignin in fiber S₂ layer, which occupied most of the cell wall, is 12:88,
195 while G:S ratio of the cell corner middle lamella is 88:12 [23]. Thus, lignin in middle
196 lamella has lower methoxyl content per C₉ unit than that in cell wall for both softwood

197 tracheid and hardwood fiber.

198 The methoxyl content of residual lignin was, therefore, analyzed and shown in
199 Fig. 6 as lignin-derived wt%. In order to make the discussion understandable clearly, the
200 methoxyl content per phenylpropane unit is also shown in Fig. 6. Sarkanen and Hergert
201 [24] indicated that normal hardwood lignins are composed of G and S lignins in varying
202 ratio with the methoxyl content per phenylpropane units in a range 1.2 to 1.5. Japanese
203 beech used in this study was 0.21 % ($\doteq$ 1.35/C₉ unit) in its content as in Fig. 6. On the
204 other hand, normal softwood lignins are mainly composed of G lignin with a trace
205 amount of H lignin, as observed to be 0.16 % ($\doteq$ 0.96/C₉ unit) for Japanese cedar. If
206 both lignins in cell wall and middle lamella were uniformly removed at the same rate,
207 the average methoxyl content per C₉ unit should be the same during hot-compressed
208 water treatment. However, the methoxyl content per C₉ unit was decreasing for both
209 species as the delignification was progressed. According to the analysis of the
210 lignin-derived products in water-soluble portion, the methoxyl residue was not removed
211 from aromatic ring by hot-compressed water treatment [8]. Thus, this result in Fig. 6
212 indicates that the relative proportion of middle lamella lignin has increased, compared

213 with cell wall lignin, as observed in Fig. 3 through Fig. 5 for insoluble residues.

214 The phenylpropane units with lower methoxyl content have higher possibility to
215 form condensed type linkages at 3 or 5 position of aromatic ring. Based on these lines of
216 evidence, lignin in middle lamella must be rich in condensed types, thus remaining after
217 two-step treatment. Similarly, lignin in tracheid cell wall of Japanese cedar would have
218 higher condensed types than lignin in fiber cell wall of Japanese beech. Thus, lignin in
219 Japanese cedar had higher resistance to hot-compressed water than that in Japanese
220 beech as shown in Table 1 and Fig. 4.

221 A part of lignin in the insoluble residue might be intermolecularly condensed
222 during hot-compressed water treatment. If the condensed type linkages in insoluble
223 residue are the result of condensation during the treatment, condensation would be
224 occurred not only in the middle lamella but also in the cell wall. However, as described
225 already, the delignification behavior was different from each other. Thus, the
226 distribution of the remaining lignin must be reflected from that in the original
227 condensed type lignin itself. Moreover, the semi-flow type of the hot-compressed water
228 treatment may minimize its possibility due to prompt removal of the delignified

229 products.

230

231

232 **Concluding remarks**

233

234 The difference in delignification from Japanese cedar and Japanese beech as treated by

235 hot-compressed water was investigated from a topochemical viewpoint. Due to the

236 preferential cleavage of ether linkages of lignin, lignin in cell walls of the tracheid and

237 fiber was preferentially decomposed and removed in hot-compressed water.

238 Consequently, lignin in middle lamella remained as insoluble residue after treatment.

239 Although some alternation of the lignin through condensation might result during

240 hot-compressed water treatment and obscure the data, overall the results obtained would

241 suggest that middle lamella lignin is rich in condensed type. For cell wall lignin, on the

242 other hand, Japanese cedar tracheids would have relatively higher condensed type lignin

243 than Japanese beech fibers. These results, thus, indicate that the decomposition

244 behaviors of softwood and hardwood differ due to their intrinsic characteristics of lignin.

245 Such information must elucidate the importance of topochemistry in the decomposition

246 behaviors of lignin.

247

248

249 **Acknowledgments**

250

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254

255

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323

Table 1 The yield of residue, lignin concentration, and delignification from Japanese cedar and Japanese beech as treated by two-step hot-compressed water

Wood	Treatment	Yield of residue (wood-based wt%)	Chemical composition (g / g of residue)			Delignification (lignin-based wt%)		
			Cellulose	Hemicelluloses	Lignin*			
Japanese cedar	Untreated	—	100	0.49	0.19	0.32 (= 0.31 + 0.009)	0	
	1st stage	Time up	10 min	71	0.57	0.11	0.32 (= 0.32 + 0.002)	30
		Time at	15 min	48	0.59	0.03	0.37 (= 0.37 + 0.002)	44
	2nd stage	Time up	5 min	32	0.53	0	0.47 (= 0.47 + 0.002)	52
		Time at	15 min	13	0.12	0	0.87 (= 0.87 + 0.004)	65
	Japanese beech	Untreated	—	100	0.46	0.28	0.26 (= 0.22 + 0.034)	0
1st stage		Time up	10 min	64	0.56	0.15	0.29 (= 0.26 + 0.029)	29
		Time at	15 min	46	0.70	0.01	0.29 (= 0.28 + 0.005)	49
2nd stage		Time up	5 min	28	0.71	0	0.29 (= 0.28 + 0.005)	60
		Time at	15 min	7	0.23	0	0.77 (= 0.77 + 0.006)	77

* The numbers in parenthesis indicate, respectively, the Klason lignin and acid-soluble lignin

325

326 Fig. 1 The treatment temperature of hot-compressed water for 1st stage (230°C/10 MPa)

327 and 2nd stage (270°C/10 MPa)

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332 Fig. 2 The yield of alkaline nitrobenzene oxidation products for insoluble residue of

333 Japanese cedar and Japanese beech at the end of the 1st and 2nd stages of the two-step

334 hot-compressed water treatment

335

336

Fig. 3 UV micrographs taken at 280 nm of insoluble residues at the end of 1st and 2nd stages from Japanese cedar (upper) and Japanese beech (bottom)

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339

340

341 Fig. 4 KMnO_4 -stained TEM micrographs of insoluble residue for Japanese cedar.

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346 Fig. 5 KMnO_4 -stained TEM micrograph of insoluble residue for Japanese beech.

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349 Fig. 6 Methoxyl group per C₉ unit of residual lignin for Japanese cedar and Japanese

350 beech.

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352