



Characterization of lignin-derived products from various lignocellulosics as treated by semi-flow hot-compressed water

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Abstract

To elucidate the decomposition behaviors of lignin from different taxonomic groups, five different lignocellulosics were treated with hot-compressed water (230 °C/10 MPa/15 min) to fractionate lignins into water-soluble portions, precipitates, and insoluble residues. The lignin-derived products in each fraction were characterized and compared. The delignification of monocotyledons [nipa palm (*Nypa fruticans*) frond, rice (*Oryza sativa*) straw, and corn (*Zea mays*) cob] was more extensive than that achieved for Japanese cedar (*Cryptomeria japonica*, gymnosperm) and Japanese beech (*Fagus crenata*, dicotyledon angiosperm). The water-soluble portions contained lignin monomers like coniferyl alcohol and phenolic acids, while the precipitates contained higher molecular weight lignin with high content of ether-type linkages. Lignin in the insoluble residues was rich in condensed-type structures. In all five lignocellulosics, ether-type linkages were preferentially cleaved, while condensed-type lignin showed resistance to hot-compressed water. In the monocotyledons, lignin–carbohydrate complexes were cleaved and gave lignins that had higher molecular weights than those eluted from the woods. These differences would facilitate the delignification in monocotyledons. Such information provides useful information for efficient utilization of various lignocellulosics.

Keywords Lignin · Hot-compressed water · Gymnosperms · Angiosperms · Monocotyledons

Introduction

With the depletion of fossil fuels and the environmental issues of global warming, the utilization of lignocellulosics as renewable resources has been examined from the perspective of biofuel and biochemical production. The cell walls of lignocellulosics consist mainly of cellulose, hemicelluloses, and lignin, and these components enforce a sophisticated structure that provides resistance to water and organic solvents.

A variety of different decomposition methods have been explored for conversion of lignocellulosics into value-added chemicals. Hydrothermal treatments achieve a high level of hydrolysis of polysaccharides, and can even dissolve hydrophobic lignin because of the highly ionic products and low solubility parameter of water [1, 2]. Thus, various

hydrothermal treatments of lignocellulosics have been explored [3, 4]. Among these treatments, hot-compressed water treatment is conducted under relatively mild conditions, but its potential for lignocellulosic decomposition remains high [5]. Hot-compressed water treatment can be categorized into batch, flow, and semi-flow treatments. Of these, the semi-flow system can minimize the further degradation of decomposed products because of rapid removal of the degraded products from the reaction vessel [6, 7].

Capitalizing on these attributes, our research group developed a semi-flow hot-compressed water treatment that achieved high saccharide yields from cellulose and hemicelluloses [8–10]. The obtained saccharides can be utilized as a feedstock for biofuels and chemicals [11, 12]. In contrast, the lignin, which is the other main cell wall component, can also be decomposed in hot-compressed water treatment [8, 9]. To utilize either polysaccharide- or lignin-derived products, it is quite important to understand the decomposition behaviors of lignin in hot-compressed water treatment. While the decomposition behaviors of lignin from woody biomass by hydrothermal treatment such as steam explosion [13–15], supercritical treatment [16–18], and hot water treatment

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[19–21] have been extensively studied, the decomposition behavior of lignin in semi-flow hot-compressed water treatment has not yet been examined well. The decomposition behaviors of woods such as Japanese cedar and Japanese beech by hot-compressed water treatment were examined through the characterization of their lignin-derived products [22, 23].

Besides woody lignocellulosics, nonwoody lignocellulosics from monocotyledon angiosperms such as palm, rice, and corn may serve as potential biomass resources [24]. Among these lignocellulosics, there are several differences in the lignin structures, including the lignin component unit structures and the association with phenolic acids [25]. Accordingly, their decomposition behaviors are expected to be different. In the present study, five lignocellulosics, which are categorized into different taxonomical groups, were treated with semi-flow hot-compressed water (230 °C/10 MPa). Herein, the lignin decomposition behaviors of the five lignocellulosics are compared based on the characterization of the lignin-derived products.

Materials and methods

Samples and chemicals

The lignocellulosic sources were sapwood of Japanese cedar (*Cryptomeria japonica*; gymnosperms), sapwood of Japanese beech (*Fagus crenata*; dicotyledon angiosperm), and nipa palm (*Nypa fruticans*) frond, rice (*Oryza sativa*) straw, and corn (*Zea mays*) cob (monocotyledon angiosperms). The flour of each lignocellulosic (passing 18-mesh screen) was extracted with acetone using a Soxhlet apparatus and dried at 105 °C for 24 h before the experiments in a similar manner to the previous studies [8, 9]. All chemicals used in this study were of reagent grade without any purification.

Characterization of lignocellulosics

The chemical composition of each lignocellulosic was evaluated using the method of Rabemanolontsoa et al. [26].

The content of phenolic acids, which are chemically attached to lignin in monocotyledons, was determined by alkali extraction with 0.5 M NaOH aqueous solution [27]. The extracted portion was acidified and extracted with ethyl acetate. The ethyl acetate-soluble portion was then dehydrated and evaporated under vacuum. The obtained products were trimethylsilylated with *N,O*-bis(trimethylsilyl)tri-fluoroacetamide and analyzed by gas chromatography–mass spectrometry (GC–MS) using a GCMS-QP2010 Ultra (Shimadzu, Kyoto, Japan) with a CP Sil 8CB (Agilent, Santa Clara, CA, USA) capillary column. The temperature program was 1.0 min at 40 °C, ramp at 5.0 °C/min to 300 °C,

and 8.0 min at 300 °C. Helium carrier gas was used at a flow rate of 1.5 mL/min. The injector and detector temperatures were both 230 °C.

For the analysis of lignin structure, alkaline nitrobenzene oxidation was performed according to the reported method [28] and the total yields of vanillin, syringaldehyde, and *p*-hydroxybenzaldehyde were determined by GC using a GC 2014 instrument (Shimadzu). Vanillin can be produced from ferulic acid, and *p*-hydroxybenzaldehyde can be derived from *p*-coumaric acid and 4-hydroxybenzoic acid. Thus, the yields of phenolic acid-derived vanillin and *p*-hydroxybenzaldehyde were subtracted from the original yields of the alkaline nitrobenzene oxidation products to roughly estimate the actual yields of products from the core lignin.

Hot-compressed water treatment

Approximately 0.5 g of oven-dried extractive-free lignocellulosic was placed in a reaction vessel for semi-flow hot-compressed water treatment, as described in a previous report [29]. The treatment condition was 230 °C/10 MPa for 15 min, in which most of the hemicellulose and part of the lignin can be decomposed [29].

After the treatment, the residue remained in the reaction vessel was dried at 105 °C for 12 h to determine its oven-dried weight. The obtained hot-compressed water-soluble portion was allowed to stand for 12 h under ambient conditions to allow precipitates to form. The mixture was then filtered through a Millipore membrane filter (pore size 0.45 µm). The water-soluble portion (filtrate) was then extracted with ethyl acetate to give the lignin-derived products.

Analysis of lignin-derived products

The lignin-derived products were obtained as three fractions: water-soluble portion, precipitates, and insoluble residue. The content of lignin-derived products in each fraction was examined as the combined yields of Klason lignin and acid-soluble lignin [30]. The molecular weight distributions of lignin-derived products in the water-soluble portion and precipitates were evaluated by gel permeation chromatography (GPC), which was performed with an LC-10A chromatograph (Shimadzu) as described in a previous report [23]. Before GPC analysis, the precipitates were acetylated to be solubilized in tetrahydrofuran [31]. The precipitates and insoluble residues were also analyzed by alkaline nitrobenzene oxidation as described above. Monomeric lignin-derived compounds in the water-soluble portion were determined and quantified by GC–MS analysis, which was performed with the same equipment and same methods as described above.

Results and discussion

Characterization of lignocellulosics

Figure 1 shows the chemical compositions of the five different lignocellulosics used in this study. For Japanese cedar, a gymnosperm softwood, the most abundant component was cellulose, followed by lignin (32.0 wt%) and hemicellulose. The minor components included protein, extractives, and ash. For Japanese beech, a dicotyledon angiosperm hardwood, the chemical composition was relatively similar to that of Japanese cedar, but the lignin content was relatively lower. In contrast, for nipa frond, rice straw, and corn cob, all monocotyledon angiosperms, the lignin content was lower at around 18–20 wt%, with the protein and ash contents being much higher than Japanese cedar and Japanese beech.

The contents of phenolic acids, which are attached to lignin in monocotyledons, are given in Table 1. For Japanese cedar and Japanese beech, phenolic acids were not detected.

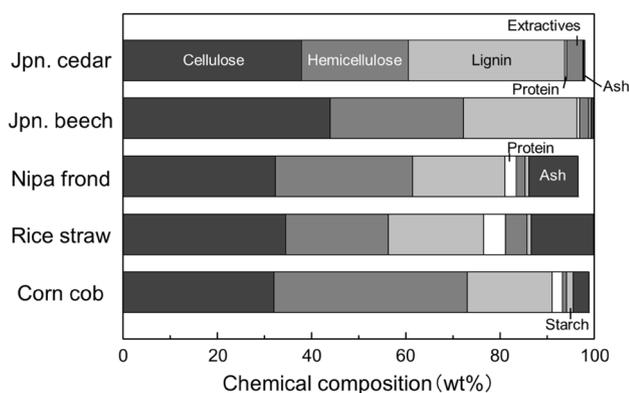


Fig. 1 Chemical composition of lignocellulosics as determined by the method of Rabemanolntsoa and Saka [13]

In contrast, ferulic acid, *p*-coumaric acid, and *p*-hydroxybenzoic acid were detected in the monocotyledons. Ferulic acid connects hemicelluloses with lignin to form a lignin–carbohydrate complex (LCC) in the cell wall of monocotyledons [32]. Corn cob contained the highest concentration of ferulic acid (0.77 wt% on lignocellulosic basis; 4.3 wt% on lignin basis). For *p*-coumaric acid, which is reported to be mainly associated with lignin in monocotyledons [33], corn cob also had the highest content (1.5 wt% lignocellulosic basis; 8.3 wt% on lignin basis). Nipa palm frond contained the most *p*-hydroxybenzoic acid (0.78 wt% on lignocellulosic basis; 4.0 wt% on lignin basis). *p*-Hydroxybenzoic acid is reported to be associated with lignin in palm species [34].

To further examine the lignin component units in these lignocellulosics, alkaline nitrobenzene oxidation was conducted. This process generates vanillin (VA) from guaiacyl (G) lignin, syringaldehyde (SA) from syringyl (S) lignin, and *p*-hydroxybenzaldehyde (HA) from *p*-hydroxyphenyl (H) lignin. The molar ratios of decomposed products to VA are shown in Table 1.

For Japanese cedar, VA was the main product with a small amount of HA, indicating that Japanese cedar lignin is mainly composed of G lignin with a small amount of H lignin. Japanese beech, however, contained both VA and SA, and the ratio of SA to VA was high. For the three monocotyledons, all gave VA, SA, and HA. The nipa frond gave a high ratio of SA to VA and a relatively low ratio of HA to VA. In contrast, rice straw and corn cob had high ratios of HA to VA. These HA/VA ratios relatively higher than typical H lignin ratio of monocotyledons reported in the other literatures using thioacidolysis or NMR [35–37]. In case of wheat straw, it was reported that a part of G and S lignin can be converted into H lignin by demethoxylation under high temperature of alkaline nitrobenzene oxidation [38, 39]. The nitrobenzene oxidation condition used in this study was not

Table 1 Lignin content, phenolic acid content, and alkaline nitrobenzene oxidation yields from five different lignocellulosics

Lignocellulosic	Lignin content (wt%)	Phenolic acids (wt%)			Nitrobenzene oxidation yields ^a (molar ratio to vanillin)		
		Ferulic acid	<i>p</i> -Coumaric acid	<i>p</i> -Hydroxybenzoic acid	VA	SA	HA
Jpn. cedar	32.0	–	–	–	1	0	0.05
Jpn. beech	24.0	–	–	–	1	2.19	0
Nipa frond	19.6	0.13	0.03	0.78	1	2.15	0.10
Rice straw	20.2	0.55	0.79	–	1	0.67	0.41
Corn cob	18.0	0.77	1.50	–	1	0.80	0.79

VA vanillin, SA syringaldehyde, HA *p*-hydroxybenzaldehyde

^aLignin in monocotyledons contained cinnamic acids such as ferulic and *p*-coumaric acids, and they can be converted into VA and HA, respectively. Thus, the cinnamic acids-derived oxidation yields were subtracted from the original oxidation yields to obtain lignin-derived oxidation yields. The amount of ferulic acid-derived VA (mg) is assumed by $0.317 \times$ ferulic acid content in biomass (mg), and the amount of *p*-coumaric acid-derived HA is assumed by $0.321 \times$ *p*-coumaric acid content in biomass (mg)

so severe one, but it might result in the relatively higher HA/VA ratios.

Yields of lignin-derived products in each fraction

Figure 2 shows the yields of the water-soluble portion, the precipitates, and the insoluble residue for the five different lignocellulosics as treated by semi-flow hot-compressed water. After treatment, the hot-compressed water-soluble portion was stood for 12 h to separate the water-soluble portion and the precipitates. For Japanese cedar and Japanese beech, the yields of insoluble residues were relatively high and the yields of hot-compressed water-soluble portion (water-soluble portion + precipitates) were low. In contrast, around 70 wt% of each of the monocotyledons was decomposed and solubilized into hot-compressed water. The precipitates were 5–10 wt% on lignocellulosic basis for all five of the lignocellulosic materials.

Figure 3 shows the yields of lignin-derived products in water-soluble portions, precipitates, and insoluble residues from the five lignocellulosics after treatment with hot-compressed water. For Japanese cedar, 43 wt% of lignin was decomposed and eluted in hot-compressed water with more than half of the lignin remaining as insoluble residue. In contrast, for Japanese beech, more than half of the lignin was decomposed and eluted in hot-compressed water, indicating that the delignification was more effective in Japanese beech than in Japanese cedar. However, at around 80 wt%, the delignification of the monocotyledon lignocellulosics was significantly higher than the delignification of the woods (Japanese cedar and Japanese beech). Among the hot-compressed water-soluble lignin, 20–30 wt% of lignin was retrieved as precipitated lignin.

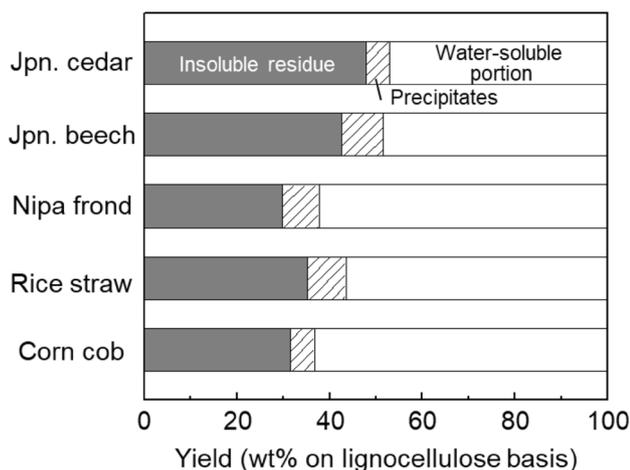


Fig. 2 Yields of water-soluble portions, precipitates, and insoluble residues from five different lignocellulosics as treated by semi-flow hot-compressed water

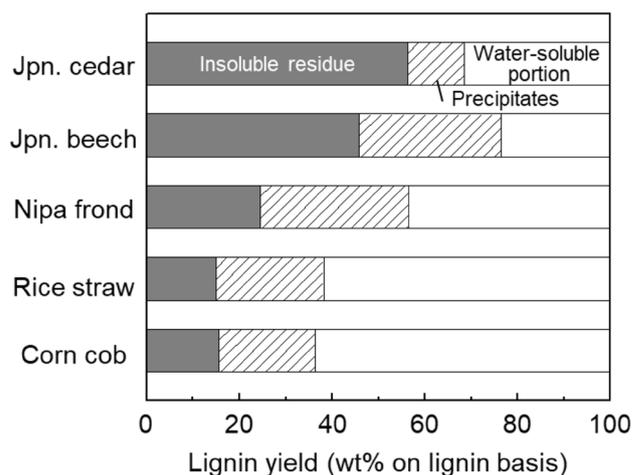


Fig. 3 Yields of lignin in water-soluble portions, precipitates, and insoluble residues from five different lignocellulosics as treated by semi-flow hot-compressed water

Structural analysis of lignin-derived products

Figure 4 shows GPC chromatograms of the water-soluble portions and precipitates from the five lignocellulosics. The molecular weight distributions of the lignin-derived products were evaluated by comparison of elution times with those of polystyrene standards (molecular weight: 162, 580, 1270, and 5000).

The lignin-derived products in water-soluble portions contained monomeric and oligomeric products up

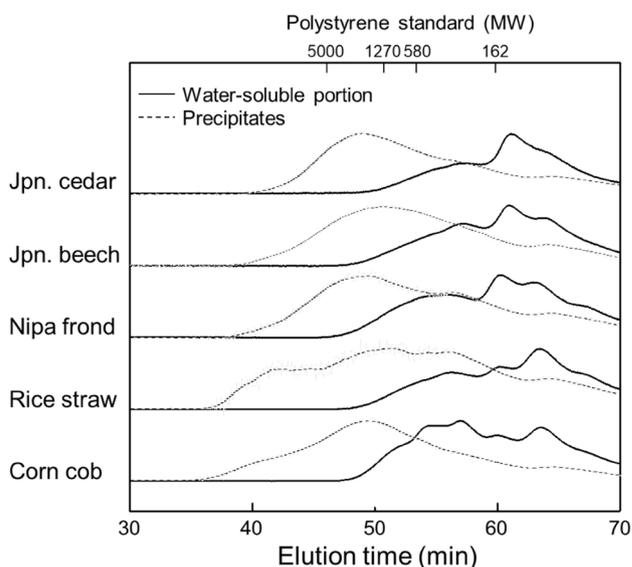


Fig. 4 Gel permeation chromatograms of lignin-derived products in water-soluble portions and precipitates from five different lignocellulosics as treated by hot-compressed water. Detection achieved by measurement of eluent absorbance at 280 nm

to molecular weights of about 1000–2000. The molecular weights of the lignins in the precipitates were higher than those in water-soluble portions for all lignocellulosics. Among the five different lignocellulosics, the lignin-derived products in the precipitates from monocotyledons started to elute about 2–3 min earlier than those from the woods, indicating that the lignin-derived products from the monocotyledons were of higher molecular weight than those from the woods.

The relative proportions of ether linkages in the lignin-derived products were compared in terms of products of the alkaline nitrobenzene oxidation (Fig. 5). For Japanese cedar, the yield of VA from lignin in the precipitate was higher than that from the original wood flour. This indicates that the lignin in the precipitates contains relatively more ether linkages. For the insoluble residue, the yield of VA was relatively low, meaning that the lignin in the insoluble residue is rich in condensed-type lignin. For Japanese beech, both VA and SA were obtained. The total yields from precipitates

were quite high and those from insoluble residue were low, in a trend similar to that shown by Japanese cedar. The SA/VA ratio was 2.6 for the precipitates, 2.2 for the original wood flour, and 1.3 for the insoluble residue, indicating that the precipitated lignin is rich in S lignin, and the residual lignin is rich in G lignin. For the monocotyledons, VA, SA, and HA were obtained from all fractions. The total yields from precipitates were similar to those from the original wood flour, while the total yields from insoluble residues were lower, as in the woods. Regarding the relative proportions of VA, SA, and HA, the main product from the precipitates was SA, and the yields of VA and HA were relatively low. In contrast, the proportions of VA and HA were relatively high in the insoluble residues.

For all the lignocellulosics, the lignin-derived products in the precipitates maintained ether-type linkages, whereas those in the insoluble residues were rich in condensed-type lignin. Except for Japanese cedar, being composed of only G lignin, the lignin component units in the precipitates

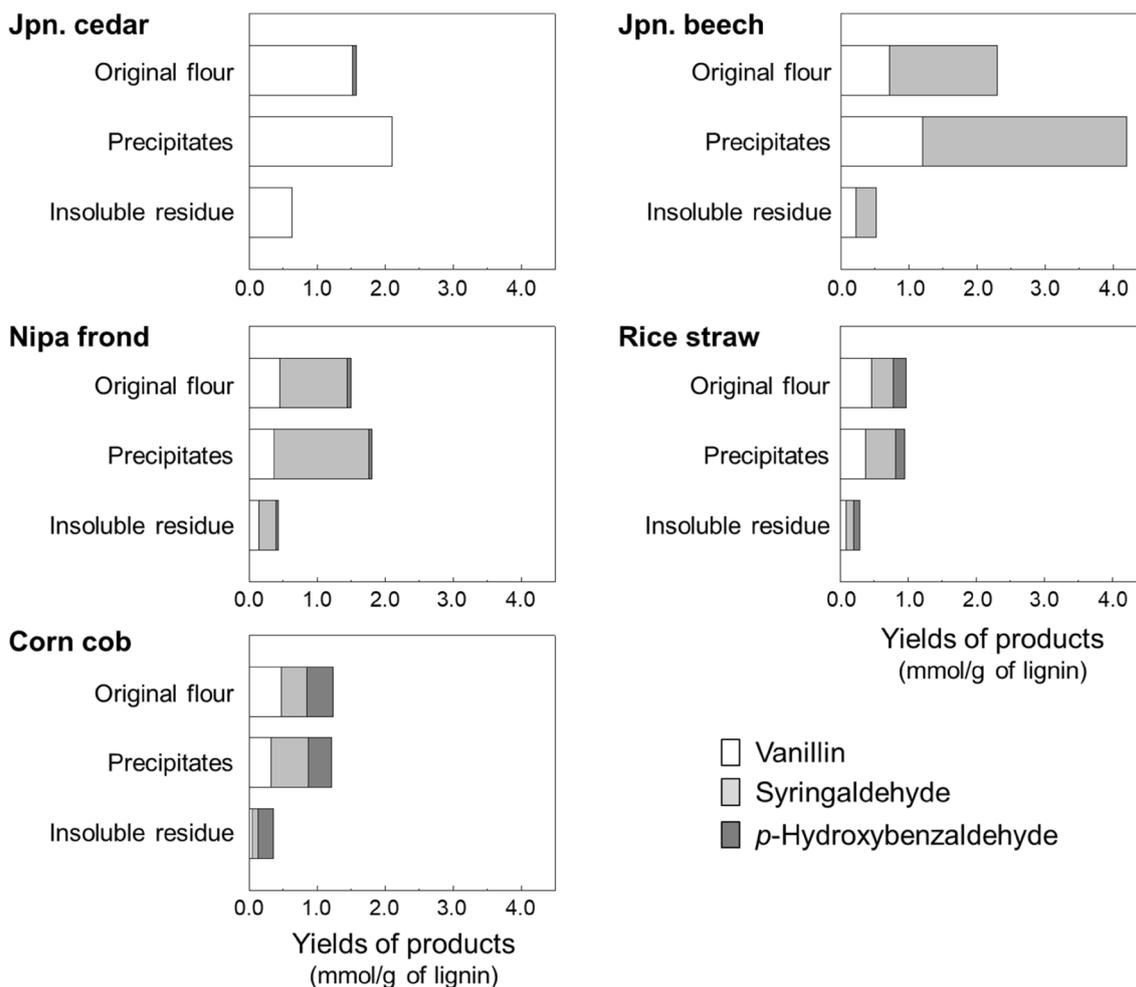


Fig. 5 Yields of alkaline nitrobenzene oxidation products from original flour, precipitates, and insoluble residues from five different lignocellulosics as treated by hot-compressed water

contained more methoxyl groups than those in the insoluble residues.

Lignin-derived products in water-soluble portions

The GPC profiles suggested that the lignin-derived products in the water-soluble portions contained monomeric compounds with substantially low molecular weights (Fig. 4). GC–MS analysis identified these monomers and the yields of the main products are summarized in Table 2. These compounds were identified and quantified by comparing the retention times and the mass fragmentation patterns with those of model compounds. Coniferyl alcohol, sinapyl alcohol, and *p*-coumaryl alcohol, which are the monomer precursors of lignification, were the main products among the monomers detected by GC–MS. The identified monomers corresponded well to the lignin component units detected by alkaline nitrobenzene oxidation (Table 1). For the woods, vanillin and syringaldehyde were also obtained, while these aldehydes were not detected from monocotyledons. However, the yields of lignin-derived monomers were quite low, and most of the lignin-derived products were dimeric or oligomeric compounds.

Phenolic acids were found in the water-soluble portions from the monocotyledons. Given that they were not obtained from Japanese cedar or Japanese beech, the phenolic acids would be derived from the phenolic acids attached to lignin, rather than from the core lignin. *p*-Coumaric acid and *p*-hydroxybenzoic acid were obtained with quite high yields.

With respect to the *p*-coumaric acid in corn cob, the previous analysis of corn stover by two-dimensional nuclear magnetic resonance spectroscopy found that *p*-coumaric acid is attached to lignin at the γ -position of the propane side chain [33]. Although the detailed structural arrangement of *p*-coumaric acid in corn cob has not been clarified, the high yield of *p*-coumaric acid suggests that it is attached to a lignin end position where it can be readily cleaved.

For oil palm (*Elaeis guineensis*) frond, *p*-hydroxybenzoic acid is reported to be attached to lignin at the γ -position of its side chain [34]. Therefore, in the case of nipa palm frond,

it is quite possible that *p*-hydroxybenzoic acid is attached at the end position of lignin.

Ferulic acid was also generated from monocotyledons. The majority of ferulic acid is incorporated through ester or ether linkages to lignin and hemicellulose [40, 41] to form lignin–carbohydrate complexes (LCCs).

Lignin-derived products in precipitates

For all five of the lignocellulosic materials, the lignin-derived products in the precipitates had relatively high molecular weights, and analysis by alkaline nitrobenzene oxidation suggested that their structures were rich in ether linkages. The precipitated lignin from Japanese beech showed structures similar to milled wood lignin with structural analyses such as FT-IR and 2D NMR analyses [29]. Thus, the precipitated lignin seems to maintain the original lignin structure [29]. Yamauchi et al. [22] speculated that some micropores are produced in the cell wall because of the decomposition of hemicellulose and the cleavage of lignin ether linkages in the initial stages of hot-compressed water treatment. The resultant micropores then facilitate the elution of lignin clusters, in which ether-type linkages are maintained to some extent [22]. Hydrophobic lignin can be eluted in hot-compressed water due to the lower than normal dielectric constant of hot-compressed water, whereas some large molecules cluster as precipitates after the hot-compressed water returns to normal water under ambient conditions. Similar phenomena would have occurred in the monocotyledons, because the lignin-derived products in the precipitates from monocotyledons had molecular weights that were relatively higher than those from woods.

Ferulic acid contents of precipitates were quite low, being less than 0.1 wt% on precipitates basis. This indicates that the LCC structures in the cell walls would be mostly cleaved during the hot-compressed water treatment. Under this condition, both hemicelluloses and lignin in the cell wall would be decomposed and eluted out from the cell wall into hot-compressed water [8, 9]. To verify this behavior, further experiments on their LCCs should be performed.

Table 2 Monomeric compounds in water-soluble portions from five different lignocellulosics as treated by semi-flow hot-compressed water

Lignocellulosic	Lignin-derived monomers (wt%)				Phenolic acids (wt%)		
	Vanillin + syringaldehyde	Coniferyl alcohol	Sinapyl alcohol	<i>p</i> -Coumaryl alcohol	Ferulic acid	<i>p</i> -Coumaric acid	<i>p</i> -Hydroxybenzoic acid
Jpn. cedar	0.06	0.81	–	–	–	–	–
Jpn. beech	0.20	0.20	0.80	–	0	–	–
Nipa frond	–	0.13	0.26	0	0.01	0.05	0.40
Rice straw	–	0.19	0.01	0.010	0.06	0.18	–
Corn cob	–	0.05	0.14	0.003	0.02	0.52	–

Lignin-derived products in insoluble residues

About half of the lignin in Japanese cedar and Japanese beech and about 20 wt% of the lignin in the monocotyledons were recovered as the insoluble residue. For all five lignocellulosic materials, the lignin-derived products were rich in condensed-type lignin. Given that ether-type linkages are easily cleaved by hot-compressed water treatment, and condensed-type linkages are difficult to cleave [42], it is not surprising that the insoluble residues were rich in condensed-type linkages. Furthermore, from the alkaline nitrobenzene oxidation analysis, for all lignocellulosics, the lignin in the insoluble residues contained fewer methoxyl groups than the lignin in the precipitates.

Considering that the phenylpropane units with lower methoxyl content are more likely to form condensed-type linkages at the 3 or 5 positions of the aromatic ring, these results are reasonable. Thus, in terms of the component units, H lignin is considered to be the most durable structure. However, corn cob and the rice straw contain high proportions of H lignin (Table 1), and showed the extensive delignification compared with other lignocellulosics. This indicates that the decomposition of lignin is affected not only by the lignin component units, but also by other lignin structures like LCCs as described above.

Conclusions

Various lignocellulosics, belonging to different taxonomic groups, were treated with hot-compressed water (230 °C/10 MPa/15 min), and the obtained lignin-derived products in each fraction were characterized. Delignification of the monocotyledons (nipa palm frond, rice straw, and corn cob) was more extensive than that from the woods of Japanese cedar and Japanese beech. For all lignocellulosics, the lignins of insoluble residues were rich in condensed linkages, indicating that the decomposition of lignin occurred through cleavage of ether linkages. The residual lignin contained fewer methoxyl groups in phenylpropane units than the lignin in the precipitates. Considering that the lignin with lower methoxyl content can readily have condensed-type linkages at the 3 and/or 5 positions of the aromatic ring, low methoxyl content suggests resistance to hot-compressed water, which increases the yield of insoluble residue. It is interesting, however, that corn cob and rice straw, which have a high proportion of H lignin, showed extensive delignification compared with other lignocellulosics. For the lignin-derived products from monocotyledons, the LCCs were cleaved and lignin with molecular weights higher than that from the woods was eluted into hot-compressed water. These differences would have facilitated the delignification from monocotyledons. Based on these lines of evidence, it

was found that there are several differences in the delignification and decomposition behaviors of lignin between the woods (gymnosperm softwood and angiosperm dicotyledonous hardwood) and monocotyledons as treated by hot-compressed water. These results provide useful information for the efficient utilization of various lignocellulosics.

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interests associated with this manuscript.

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