

**DECOMPOSITION BEHAVIORS OF LIGNIN
IN HYDROTHERMAL TREATMENT OF LIGNOCELLULOSICS**

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Chapter 1

Introduction

1.1 Biomass resources for energy

The economic growth has improved our living standard, and the energy consumption in the world has remarkably increased from 9 billion tonnes in 1990 to 12.9 billion tonnes in 2008, in crude oil equivalent. Furthermore, the energy consumption in the world is estimated to be 19.6 billion tonnes by 2035 due to the excessive energy consumption in the developing countries such as China and India (International Energy Agency, 2011). Recently, many countries depend on the fossil fuels such as oil, natural gas and coal as the main energy resources (International Energy Agency, 2015a, 2015b), and their depletion has become an urgent issue. In fact, the reserve-production ratio of oil, natural gas and coal were estimated to be 52.5, 54.1 and 110 years, respectively, in 2014 (British Petroleum, 2015).

On the other hand, in accordance with the increase in the energy consumption, environmental issues such as the destruction of tropical rain forests, detrimental effects from acid rain, spread of desertification and depletion of ozone layer have increased in magnitude and gained international attention. Among them, global warming is one of the most important issues which has caused various abnormal climates all over the world. If the average temperature rises at the present increase rate, the rise in average global temperature is estimated to be 1.8~4.0 °C in 100 years (Intergovernmental Panel on Climate Change, 2007). Moreover, the increase in global temperature has caused a series of problems such as the expansion of Malaria distribution, rise in sea level, and ecological damages all over the world (Bright, 2000).

Global warming is caused by the increase in the greenhouse gases (GHGs) emissions such as carbon dioxide and methane. In fact, the concentration of carbon

dioxide in the atmosphere has risen rapidly in the past 100 years (Parry et al., 2007), so it is an urgent global problem to reduce their emissions. Thus, nuclear and renewable energies are expected to serve as the alternatives to fossil fuels energy. However, in March 2011, Fukushima Daiichi nuclear disaster occurred due to the great east Japan earthquake and the following tsunami accident. This accident has highlighted safety concerns about nuclear power plant and increased expectations for renewable energy.

Renewable energy includes wind, hydro, solar, geothermal and biomass energy. The biggest advantage is that it can be used semi-permanently as an energy resource. Among the renewable energy, biomass energy is positively promoted to be utilized as a new energy resource. In Japan, for example, biomass energy was added into the new energy according to the law reform of the new energy law (Advisory committee for natural resources and energy, 2001). It is also reported that biomass is promoted to be used as energy which contribute to the regional activation in strategic energy plan (Agency for natural resources and energy, 2014).

Biomass is a kind of biological material derived from living, or recently living organisms. Namely, not only animals, plants and microorganisms but also architectural wood, scrap wood, wet garbage and livestock excretion are defined as biomass resource (Saka, 2001). When producing energy by burning biomass, the concentration of carbon dioxide in the atmosphere would not be increased since the gas emitted from combustion of biomass will be absorbed and fixed by plants through photosynthesis. Therefore, biomass is expected to be an environmentally friendly energy resource.

Plant biomass accounts for a large portion of biomass resources. It is presumed that the total amount of plant in the world and the total amount of biomass produced by photosynthesis are 840 billion tonnes and 80 billion tonnes, respectively, in carbon equivalent (Saka, 2001). Biomass is a promising energy resource since the annual energy consumption in the world is only approximately 10 billion tonnes (Saka, 2001).

1.2 Lignocellulosic biomass

Lignocellulosic biomass is a natural complex of carbohydrate (cellulose and hemicelluloses) and lignin. Unlike agricultural feedstock such as sugarcane, cassava and corn, the use of lignocellulosic biomass does not affect the production of foodstuffs because of its inedibility. In order to utilize the lignocellulosics efficiently, it is quite essential to understand its evolution because there are some correlations between the taxonomy and chemical composition. Fig. 1-1 shows the simplified taxonomy of vascular plants (Shimaji, 1982). Recently, because of the development of phylogenetic analysis based on the DNA sequence, new classifications were reported for pteridophytes (Christenhusz et al., 2011), gymnosperms (Soltis et al., 2011), and angiosperms (APG, 2016). However, since these classifications are not well generalized in the field of wood science, this dissertation refers to the conventional taxonomy according to the Engler system.

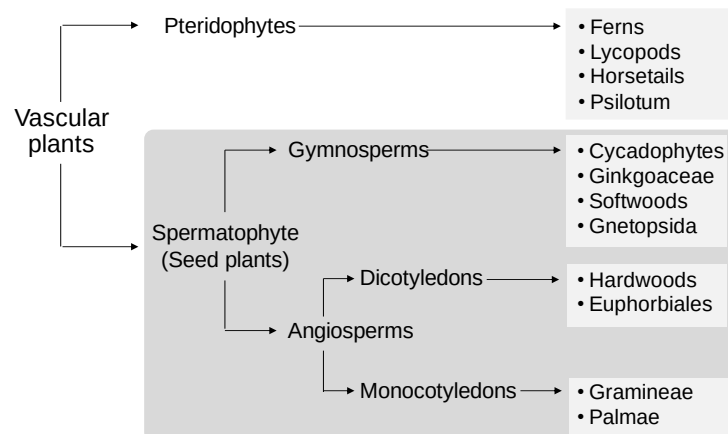


Fig. 1-1 Taxonomy of vascular plants (Shimaji, 1982)

The vascular plants are categorized into pteridophyte, gymnosperms and angiosperms. However, for the pteridophyte such as ferns and lycopods, the secondary

growth does not occur due to the poor development of vascular cambium. Gymnosperms include cycadophyta, ginkgoales and softwoods such as cedar and cypress, and secondary growth takes place in all of them. As a more evolved species than gymnosperms, angiosperms is comprised of about 2.5 hundred thousand species all over the world (Thorne, 1992). Generally, angiosperms can be classified into dicotyledonous and monocotyledonous plants. Furthermore, dicotyledonous plants include hardwoods such as beech, birch and poplar, and the hardwoods have much more species than softwoods. On the other hand, monocotyledonous plants, which contain about half million species, are considered as one of the most evolved groups in the plant kingdom (Thorne, 1992). Most of the monocotyledonous plants are herbaceous plants such as gramineae. Among these vascular plants, softwoods and hardwoods are called as the woody biomass.

1.3 Structure of cell wall in lignocellulosics

During the past 350~400 million years, various structural and physiological changes have occurred as vascular plants evolved to adapt to terrestrial environments such as gravity and ultraviolet light. Therefore, the cell wall structures of lignocellulosics are different according to the taxonomy. As an example, the structure of wood trunk is illustrated in Fig. 1-2.

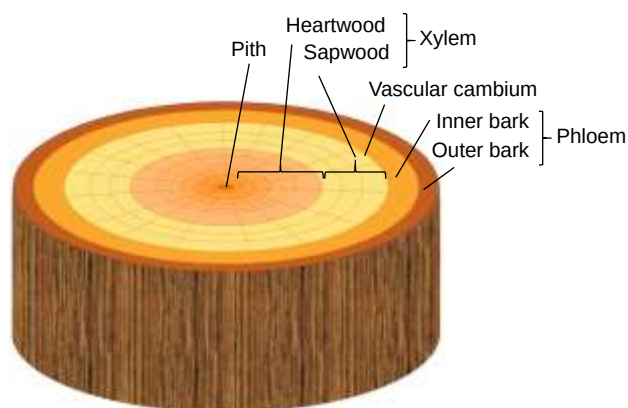


Fig. 1-2 Structure of wood trunk

The wood trunk is composed of the xylem (heartwood and sapwood), vascular cambium and phloem (inner and outer barks) as shown in Fig. 1-2. The formation of the cell walls is initiated in the vascular cambium located between xylem and phloem. The trunk has three important functions, which are the mechanical support, pathway of water and storage of nutrient element (Furuno et al., 1994). In general, the component cell and tissues are divided into axial elements and radial elements, and these elements are different between softwoods and hardwoods.

The structure of the cell in softwoods and hardwoods as one of the gymnosperms and as one of the dicotyledonous angiosperms are described below.

1.3.1 Softwoods

The cells in softwoods are composed of tracheid and axial parenchyma cell as the axial components, and the ray tracheid and ray parenchyma cell as the radial cells. The epithelial cell exists in both axial and radial cells. Among these cells, tracheid and ray parenchyma exist in all softwoods, but the existence of other cells depend on the species. As shown in Table 1-1, the most part of xylem tissues are occupied by the tracheid in various softwoods (Sano, 2003). The tracheid is the main component cell and it has important functions of pathway of both water and mechanical support.

Table 1-1 Proportion of main cell types in various softwoods (Sano, 2003)

Japanese name	Species name	Cell type (v/v %)			
		Tracheid	Axial parenchyma cell	Ray parenchyma cell	Axial resin canal
Akamatsu	<i>Pinus densiflora</i>	95.9	-	3.4	0.7
Karamatsu	<i>Larix kaempferi</i>	95.1	-	4.6	0.3
Sugi	<i>Cryptomeria japonica</i>	97.2	0.8	2.0	-
Togasawara	<i>Pseudotsuga japonica</i>	94.7	0.1	4.7	0.5
Hinoki	<i>Chamaecyparis obtusa</i>	97.1	0.6	2.3	-

1.3.2 Hardwoods

As compared with softwoods, hardwoods are more advanced plants and composed of more kinds of cell elements. The various combinations of cells can generate the diversity of hardwoods tissues. The function of each cell in hardwoods is specialized. For example, the vessel is the main pathway of water, while the fiber contributes to the mechanical support of wood trunk (Furuno et al., 1994).

Hardwoods xylem is made up of the vessel elements, fiber tracheid, wood fiber and axial parenchyma cell as the axial direction elements, and the ray parenchyma cell as the radial direction elements. The shape and array of hardwoods cell vary from one species to another. The proportion of cell types in various hardwoods is shown in Table 1-2. Although the proportion of main cell types differs based on the species, fiber is the main element in hardwoods xylem (Sano, 2003).

Table 1-2 Proportion of main cell types in various hardwoods (Sano, 2003)

Japanese name	Species name	Cell type (v/v %)			
		Vessel	Fiber ^{a)}	Axial parenchyma cell	Ray parenchyma cell
Aodamo	<i>Fraxinus lanuginosa</i> f. <i>serrata</i>	4.7	83.2	6.2	5.9
Katsura	<i>Cercidiphyllum japonicum</i>	51.9	39.5	0.6	8.0
Kusunoki	<i>Cinnamomum camphora</i>	12.2	66.9	12.5	8.4
Buna	<i>Fagus crenata</i>	41.2	32.1	9.2	17.5
Mizunara	<i>Quercus crispula</i>	12.6	65.6	6.8	15.0

a) Fiber: fiber tracheid + libroform wood fiber

1.3.3 Ultrastructure of the cell wall

The thin cell wall, formed on the surface layer of cell during the occurrence and growth of cell, is termed as primary wall. Most of the cell walls in the woody biomass are layered

and made of both a primary wall and a secondary wall.

Generally, the secondary wall of tracheid and fiber is made up of three distinct layers (S_1 , S_2 , S_3) according to the differences in the orientation of the cellulose microfibrils as shown in Fig. 1-3. In some species, peculiar layer occurs inside of the secondary wall. As shown in Fig. 1-3, for most softwood tracheid and a part of hardwood tracheid and fiber, warty layer (W) can be observed.

Plant tissue is an assembly of a number of cells and the adjacent cells are associated with middle lamella (ML), which mainly comprises lignin, and makes the bond between each cell much stronger. In particular, the ML at cell corner region (ML_{CC}) shows a high lignin concentration (Saka et al. 1982a).

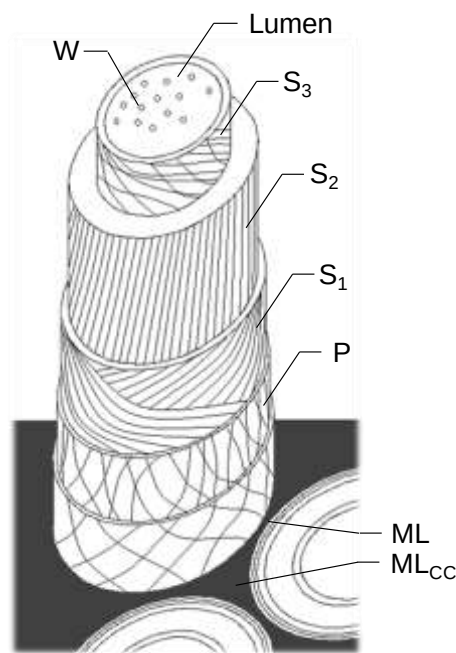


Fig. 1-3 Cell wall structure of tracheid in softwoods

W: thin warty layer, S_1 , S_2 , S_3 : secondary wall, P: primary wall,

ML: middle lamella, ML_{CC} : middle lamella at cell corner

1.4 Cell wall components of lignocellulosics

In the cell wall of woody biomass, cellulose, hemicelluloses and lignin are composed intricately and three-dimensionally. The chemical structures of these components are different from one species to another. The features of these chemical components are briefly described below.

1.4.1 Cellulose

Cellulose, which accounts for 40~70 wt% of dried plants, is the most abundant organic substance in the world. It is a polysaccharide based on D-glucose and its glucopyranose units are linked together by the formation of β -1,4-glucosidic bonds to form linear glucan chain molecules, as demonstrated in Fig. 1-4. As the feature of this structure, the molecular chain of cellulose has the interface of both hydrophilic and hydrophobic natures because the hydrogen atom associated with carbon atom orders in vertical direction to glucose ring and the hydroxyl group orders in horizontal direction. The properties are closely related with the crystalline structure of cellulose. According to the order of several molecular chains, the hydrogen bonds are formed not only in intra-molecule but also in inter-molecule. Thus, cellulose has a strong crystalline structure and the degree of polymerization of natural cellulose is estimated to be between 5,000 and 15,000 (Gralén and the Svedberg, 1943).

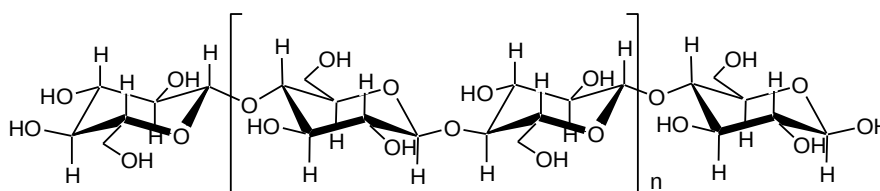


Fig. 1-4 Chemical structure of cellulose

1.4.2 Hemicelluloses

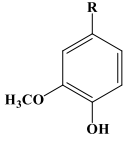
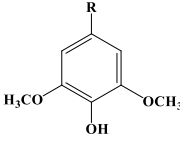
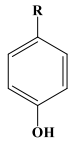
Hemicelluloses are defined as non-cellulosic and amorphous polysaccharides which can be extracted with aqueous alkali. The biological role of hemicelluloses is significant because they strengthen the cell wall by interacting with cellulose and lignin. Hemicelluloses include xyloglucans, xylans, mannans and glucomannans (Scheller and Ulvskov, 2010). Among them, xylan and glucomannan are two kinds of the main hemicelluloses components in the secondary wall of woody biomass.

There are some structural differences of hemicelluloses between gymnosperms and angiosperms. For example, the hemicelluloses of gymnosperms are mainly composed of glucomannan, while hemicelluloses of angiosperms are mainly of glucuronoxytan for both monocotyledonous and dicotyledonous plants (Scheller and Ulvskov, 2010). In addition, some hemicelluloses are often acetylated, and the acetylated polysaccharide of gymnosperms and angiosperms are glucomannan and glucuronoxytan, respectively (Scheller and Ulvskov, 2010).

1.4.3 Lignin

Lignin is defined as an amorphous polyphenolic macromolecule arising from an enzyme-mediated dehydrogenative polymerization of phenylpropanoid monomers, which are coniferyl alcohol, sinapyl alcohol and *p*-coumaryl alcohol (Dence and Lin, 1992). Lignin contributes to the stiffness under humid condition and resistance to corrosion due to the hydrophobic features (Dence and Lin, 1992). In lignocellulosic body, lignin prevents the swelling of xylem cell wall and diffusion of water from the transporting tube. Furthermore, lignin protects lignocellulosics body from severe external conditions such as the gravity, weather and decay fungus. The tension strength is mainly contributed by cellulose, whereas the compression strength under humid condition is contributed by lignin (Sakai, 1990).

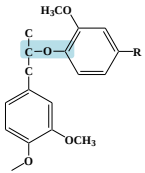
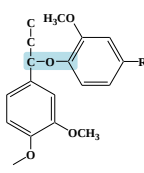
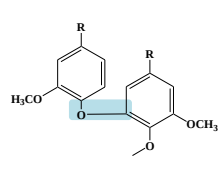
Table 1-3 Aromatic structure of lignin and the composition in gymnosperms, dicotyledonous angiosperms, and monocotyledonous angiosperms
(R: side-chain structure)

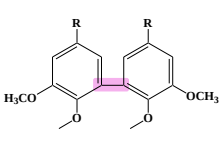
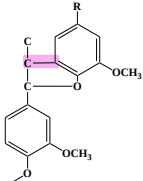
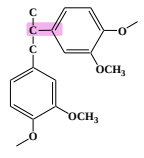
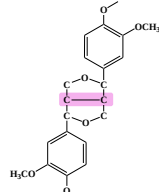
			
	Guaiacyl (G) unit	Syringyl (S) unit	<i>p</i> -hydroxyphenyl (H) unit
Softwoods (Gymnosperms)	○	—	△ (5%)
Hardwoods (Dicotyledonous angiosperms)	○	○	—
Herbaceous plant (Monocotyledonous angiosperms)	○	○	○

There are still many unclear points about lignin due to the difficulty of isolating it from lignocellulosics. Therefore, lignin has been studied from various points of view such as the topochemical observations, chemical degradations and analytical methods. Lignin is composed of guaiacyl (G), syringyl (S) and *p*-hydroxyphenyl (H) units as shown in Table 1-3. The lignin component unit differs depending on the taxonomy. Lignin of gymnosperms, dicotyledons and monocotyledons in angiosperms are composed of G lignin, S + G lignins, and S + G + H lignins, respectively.

These phenylpropane units are polymerized with various linkages, and the number of these linkages per 100 C₉ units in milled wood lignin (MWL) of spruce as softwoods and beech as hardwoods are demonstrated in Table 1-4 (Lai and Sarkanen, 1972; Nimz, 1974). The MWL is widely considered to be a good representative of the original lignin due to its mild condition to be prepared. As shown in Table 1-4, among various linkages, β -arylether (β -O-4) linkage is the most abundant for both species. These linkages can be classified into condensed type (carbon-carbon linkage) such as biphenyl (5-5') and phenylcoumaran (β -5) and non-condensed type (ether linkage) such as β -O-4 and α -O-4.

Table 1-4 Various linkages in lignin and the number of their linkages per 100 C₉ units in spruce and beech MWL (Lai and Sarkanen, 1972; Nimz, 1974)
(R: side-chain structure)

Non-condensed type (Ether linkage)				
		β -arylether (β -O-4)	α -arylether (α -O-4)	Biphenyl ether (4-O-5)
Number of linkages per 100 C ₉ units	Spruce MWL	45	8	5
	Beech MWL	65	—	1.5

Condensed type (Carbon-carbon linkage)					
		Biphenyl (5-5')	Phenylcoumaran (β -5)	Diarylpropane (β -1)	Pinoleginal (β - β)
Number of linkages per 100 C ₉ units	Spruce MWL	25	10	15	17
	Beech MWL	2.3	6	15	5.5

It is significant to clarify the difference between each kind of linkages because the reactivity and decomposition behavior of lignin by various treatments may vary extensively depending on the composition of linkages.

As shown in Fig. 1-5, Freudenberg proposed the structure model of spruce lignin in consideration of various information such as elemental analysis, functional group analyses, the number of each linkage, spectrographic analyses and chemical structure analyses (Freudenberg, 1965).

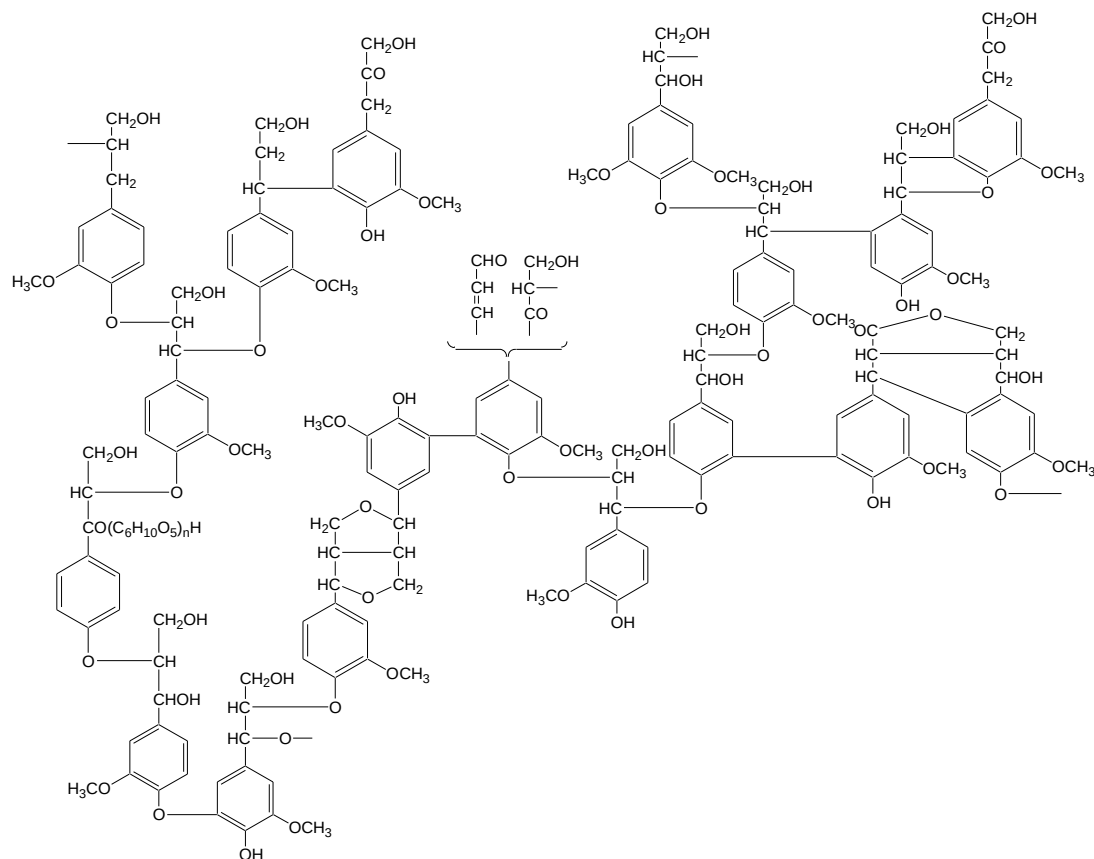


Fig. 1-5 Structure model of softwood lignin (Freudenberg, 1965)

1.5 Distribution of lignin in the cell wall

Lignin is not uniformly distributed within the cell wall of lignocellulosics. The methods to study the distribution of lignin in the lignocellulosic cell wall can be divided into two distinctive approaches. First, wood samples are fractionated into each layer portion by chemical or physical treatment and then the fractionated portions are chemically analyzed. Second, the distribution of lignin is observed by microscopic technologies in a non-destructive way. As an example of the former approach, Whiting et al. obtained the middle

lamella lignin portion from fine ground wood flour by the centrifugation with dioxane-carbontetrachloride (Whiting et al., 1981). Conversely, as an example of the latter approach, ultraviolet (UV) microscopy can be used to elucidate the lignin distribution in each cell of woody biomass. Lange analyzed the lignin content in the middle lamella and secondary wall of spruce tracheid by using UV photomicrographs (Lange, 1954). Since then, many researchers had developed this technology. Subsequently, Goring and his co-workers established the technique of UV microspectrometry for investigating the quantitative distribution of lignin (Fergus and Goring, 1970a, 1970b; Fergus et al., 1969; Procter et al., 1967; Saka and Goring, 1988; Saka et al., 1982b; Whiting and Goring, 1982; Wood and Goring, 1971). Saka et al. quantified the detailed lignin distribution in the middle lamella and cell wall of brominated wood using scanning electron microscopy with an energy dispersive X-ray analyzer (SEM-EDXA). In addition, the ratio of G and S lignins in each morphological region was evaluated by the combination of SEM-EDXA and UV spectrophotometry (Saka and Goring, 1988; Saka and Thomas, 1982a; Saka et al., 1982a, 1982b, 1978). The lignin distribution in each morphological region with autoradiography technology was examined by Terashima et al. (Terashima and Fukushima, 1988; Terashima et al., 1986). In this method, after radioisotope-labeled compounds were injected into the wood body, the distribution of radioisotope-labeled compounds in metabolized wood was analyzed. Through this technology, the process of lignification in woody biomass was identified (Terashima and Fukushima, 1988).

The distribution of lignin in softwoods as gymnosperms and hardwoods as dicotyledonous angiosperms are described below.

1.5.1 Softwoods

The distribution of lignin in loblolly pine (*Pinus taeda*) tracheid as determined by bromination with SEM-EDXA is organized in Table 1-5 (Saka and Thomas, 1982b). In softwoods, the lignin concentration (g/g) of the compound middle lamella (CML; middle

lamella + two primary walls) is higher than 50 %, whereas in the secondary wall, it is only approximately 20 %. However, since the secondary wall represents a much greater volume than the middle lamella, most of the lignin in wood is distributed in the secondary wall. Thus, it is quite important to distinguish lignin concentration (g/g) and lignin content (%). The middle lamella (ML), especially cell corner region (ML_{CC}), is more highly lignified than other morphological regions (Fergus et al., 1969; Fukazawa and Imagawa, 1981; Wood and Goring, 1971). Softwoods lignin is composed of G lignin and a slight amount of H lignin, however their distributions are not uniform. Whiting et al. quantified the methoxyl content of fractionated morphological regions, and elucidated that the cell corner region contained about 40 % of H lignin (Whiting and Goring, 1982). For the black pine (*Pinus nigra*), the CML also contains H lignin based on the results from the autoradiography analysis (Terashima et al., 1986). Condensed type G lignin is distributed in the ML compared to the secondary wall (Donaldson, 2001; Terashima et al., 1986). On the other hand, little information on the lignification and distribution of lignin in ray parenchyma cells was reported.

Table 1-5 Lignin distribution in loblolly pine tracheid as determined by bromination technique with SEM-EDXA (Saka and Thomas, 1982b)

Wood	Morphological differentiation	Fractional volume (%)	Lignin (% of total)	Lignin concentration (g/g)
Earlywood	S ₁	13	11.8	0.25
	S ₂	60	43.7	0.20
	S ₃	9	9.2	0.28
	ML	12	21.4	0.49
	ML _{CC}	6	14.0	0.64
Latewood	S ₁	6	6.2	0.23
	S ₂	80	64.2	0.18
	S ₃	5	5.6	0.25
	ML	6	13.6	0.51
	ML _{CC}	3	10.4	0.78

1.5.2 Hardwoods

Hardwoods lignin contains a mixture of G and S lignins, and the G : S ratio is different between species. The methoxyl content per phenylpropanoid unit in hardwoods lignin is typically in the range between 1.2 and 1.5 (Lai and Sarkanen, 1972). The G : S ratio is also different from one morphological region to another. Fergus and Goring analyzed UV-absorption spectra of the vessel secondary wall, fiber secondary wall and cell corner of fiber in white birch (*Betula papyrifera*), where wavelength at the maximum absorbance in each region are 279 nm, 270 nm, and 275 nm, respectively (Fergus and Goring, 1970b). According to the experiment with model compounds, the wavelength at maximum absorbance of G and S lignins are 280 nm and 270 nm, respectively. Therefore, the vessel secondary wall is composed of G lignin, the fiber secondary wall is of S lignin and the cell corner of fiber is of both G and S lignins (Fergus and Goring, 1970b).

Saka et al. analyzed white birch (*Betula papyrifera*) lignin with UV-EDXA and elucidated that the G : S ratio of vessel secondary wall, fiber secondary wall, and parenchyma cell wall is 88:12, 12:88, and 49:51, respectively (Saka and Goring, 1988). With the same method, it was found that more than 80 % of the lignin is G lignin in the ML_{CC}. However, these values are possibly different according to the species, location within a growth ring, and tree habitat (Wu et al., 1992).

Table 1-6 shows that the lignin concentration of white birch (*Betula papyrifera*) by UV-EDXA and UV microscopy. The lignin distribution in each morphological region determined by these methods are in good agreement with each other (Fergus and Goring, 1970a; Saka and Goring, 1988). From the results of UV-EDXA method, lignin is not uniformly distributed between S₁, S₂, S₃ layers in both fiber and vessel. The lignin concentration of the ML, especially in the cell corner (ML_{CC}), is higher than those of other morphological regions.

Cho et al. investigated the chemical characteristics of middle lamella lignin isolated from birch (*Betula ermanii*) and the lignin concentration of the isolated middle lamella was 0.44 g/g with lower S/G ratio and lower methoxyl contents compared with the

secondary wall (Cho et al., 1980).

Table 1-6 The distribution of lignin in white birch as determined by UV-EDXA and UV microscopy (Fergus and Goring, 1970a; Saka and Goring, 1988)

Element	Morphological differentiation	Fractional volume (%)	Lignin concentration (g/g)	
			UV-EDXA	UV microscopy ^{a)}
Fiber	S ₁	11.4	0.14	—
	S ₂	58.5	0.14	0.16
	S ₃	3.5	0.12	—
	ML	5.2	0.36	0.34
	ML _{CC(F/F)} ^{b)}	2.4	0.45	0.72
Vessel	S ₁	1.6	0.26	—
	S ₂	4.3	0.26	0.22
	S ₃	2.3	0.27	—
	ML	0.8	0.40	0.35
	ML _{CC(F/V)} ^{c)}	≈ 0	0.58	—
Ray	S	8.0	0.12	0.22
Parenchyma	ML	2.0	0.38	—
	ML _{CC(F/R)} ^{d)}	≈ 0	0.47	—
	ML _{CC(R/R)} ^{e)}	≈ 0	0.41	—

a) Calculated using xylem lignin content of 0.199 g/g. Results from Fergus and Goring (1970)
b) Fiber/Fiber c) Fiber/ Vessel d) Fiber/Ray e) Ray/Ray

1.6 Decomposition methods of lignin

Research on the decomposition treatment of lignin has been conducted before the structure of lignin was elucidated, and a variety of decomposition methods of lignin such as the alkaline nitrobenzene oxidation and hydrogenolysis have given us some clues to clarify the structure of lignin (Freudenberg et al., 1939; Pepper and Steck, 1963; Wada and Sakakibara, 1969). Most of those methods are still used for the evaluation of lignin structures (Chen, 1992; Sakakibara, 1992).

Recently, various chemical, physical and biological decomposition methods of

lignin have been employed for the research on utilization of lignin as biofuels and valuable chemicals. As the examples, chemical decomposition methods include the pyrolysis (Hosoya et al., 2008; Kawamoto et al., 2007), nucleus exchange reaction (Funaoka and Abe, 1980, 1978), ozonation (Doree and Cunningham, 1913; Matsumoto et al., 1986), acidolysis (Adler et al., 1957), metal catalysis (Zakzeski et al., 2010) and hydrothermal treatment. Among them, the recent study on hydrothermal treatment is introduced in 1.7. As to the physical decomposition method, milling is used to prepare the milled wood lignin (MWL). As an example of biological decomposition methods, microorganism is used to delignify woody biomass (Hata, 1966; Higuchi, 1971). Among the woody-rotting fungus, only white-rot fungus has the delignification activity. *Singobium sp.* SYK-6, which is one of the bacteria, can cleave not only ether type linkages but also the condensed type linkages such as 5-5' linkage (Masai et al., 2007).

1.7 Hydrothermal treatment of lignin

Hydrothermal treatments such as the steam explosion, subcritical and supercritical water, and hot-compressed water treatments, are expected as a promising technology due to their catalyst-free and environmentally benign characteristics.

In 1974, Nimz elucidated the proportions of various linkage types in lignin with the mild hydrolysis of beech (*Fagus silvatica*) by treating the sample with hot water of 100 °C for several weeks (Nimz, 1974). An increase in temperature of water at a constant pressure results in a decrease in its dielectric constant and an increase in its ionic products (Bröll et al., 1999; Savage, 1999). A decrease in dielectric constant of water under subcritical and supercritical conditions helps to enhance the solubility of some hydrophobic compounds into the subcritical and supercritical water. Much research on hydrothermal treatment of lignin have been studied as described below.

Tanahashi et al. investigated the degradation mechanism of lignin by the steam explosion (230 °C/2.9 MPa/16 min) with the dehydrogenated polymer (DHP) of coniferyl

alcohol and β -O-4 linkage model compounds (Tanahashi et al., 1989). Ehara et al. and Takada et al. treated Japanese beech (*Fagus crenata*) and Japanese cedar (*Cryptomeria japonica*) with supercritical water (380~400 °C/100~115 MPa/5~8 sec) (Ehara et al., 2005; Takada et al., 2004). Consequently, after washing the decomposed products with methanol, the obtained lignin-derived compounds were fractionated as the methanol-soluble portion and the methanol-soluble oily products composed of monomeric aromatic and condensed type dimeric compounds. Yokoyama et al. treated the organosolv lignin with supercritical water (350~420 °C/10~40 MPa/60 min), and the resultant yields of oil and char were dependent on the treatment condition (Yokoyama et al., 1998). Furthermore, Saisu et al. and Okuda et al. treated the organosolv lignin with supercritical water (400 °C/pressure not shown/10~64 min) and obtained reactive compounds such as formaldehyde and lignin-derived monomeric compounds such as the guaiacol, catechol and phenol, which were obtained by the hydrolysis and the subsequent dealkylation (Okuda et al., 2004; Saisu et al., 2003). They explained that such low molecular weight compounds were reactive and reacted with the residual lignin to be high molecular weight residue.

Based on some experiments with various monomeric lignin model compounds, it was found that phenol and aromatic nuclei are stable to the supercritical water treatment (Martino and Savage, 1997; Sato et al., 2004; Thornton and Savage, 1992; Townsend et al., 1988; Yokoyama et al., 1996, 1994). The resistance of lignin to the supercritical water is different depending on the types of its linkages. The ether linkages in lignin are readily cleaved by the protic solvent such as supercritical water and methanol, while the condensed type of linkages and the aromatic ring are difficult to be cleaved (Ehara et al., 2002).

Recently, hot-compressed water, a milder condition for hydrothermal reaction with high ionic products, is drawing considerable attention as a promising decomposition medium for lignocellulosics (Ando et al., 2000). Generally, the hot-compressed water treatment system could be simply categorized into batch, flow, and semi-flow (percolation) types. Decomposition reaction becomes dominant in batch and flow types,

which causes the obtained products to be excessively decomposed. However, the semi-flow type can prevent the products from excessive decomposition due to the low residence time (Liu and Wyman, 2003; Mosier et al., 2005; Sakaki et al., 2002). In addition, lignin can also be depolymerized and decomposed by semi-flow hot-compressed water (Tirtowidjojo et al., 1988).

Previously, Lu et al. treated Japanese beech (*Fagus crenata*) with two-step semi-flow hot-compressed water (1st stage: 230 °C/10 MPa/15 min, 2nd stage: 270 °C/10 MPa/15 min) and its hemicelluloses and cellulose were selectively decomposed (Lu et al., 2009). They also found that lignin was decomposed in both stages and the lignin-derived monomeric products such as coniferyl alcohol and sinapyl alcohol were obtained in the water-soluble portion.

1.8 The objectives of this dissertation

For Japanese cedar (*Cryptomeria japonica*) as one of the gymnosperms (Phaiboonsilpa et al., 2010), Japanese beech (*Fagus crenata*) as one of the dicotyledonous angiosperms (Lu et al., 2009), their hemicelluloses and cellulose can be selectively decomposed by two-step semi-flow hot-compressed water treatment (1st stage: 230 °C/10 MPa/15 min, 2nd stage: 270 °C/10 MPa/15 min). For both species, it has been reported that the lignin can be decomposed and eluted in hot-compressed water in both stages. However, the detailed decomposition behaviors of lignin are not clarified yet. Thus, the main objective of this dissertation is to elucidate the decomposition behaviors of lignin in the lignocellulosics by hydrothermal treatment.

In order to elucidate the delignification behaviors, the author attempted in two ways: 1) characterization of the obtained lignin-derived products, 2) discussion of delignification from a topochemical viewpoint.

Firstly, Japanese cedar was treated by two-step semi-flow hot-compressed water, and the hot-compressed water-soluble portion and water-insoluble residue were obtained.

For the hot-compressed water-soluble portion, during standing for 12 h under ambient condition, it was separated to be water-soluble portion and precipitates, which were retrieved by the filtration. In *Chapter 2*, the lignin-derived products in the obtained water-soluble portion, precipitates and insoluble residue were characterized with the analytical instruments and chemical analyses.

Secondly, the author focused on the topochemistry of lignin in the water-insoluble residues after the hydrothermal treatment. As mentioned already, the cell components in softwoods and hardwoods are totally different, thus, the distributions of lignin within the cell wall are also different. Therefore, the topochemistry of delignification from Japanese cedar and Japanese beech (*Fagus crenata*) by hydrothermal treatment were examined and compared. The details are reported in *Chapter 3*.

Thirdly, for the utilization of the lignin-derived products obtained by hydrothermal treatment, the precipitated lignin from Japanese beech was characterized with the analytical instruments and chemical analyses. Its structure was then compared with the milled wood lignin (MWL), which is widely considered to be a good representative of the original lignin. The results were described in *Chapter 4*.

Fourthly, corn (*Zea mays*) cob, which is one of the monocotyledonous angiosperms, was treated by hydrothermal treatment, and the obtained products were determined. Its decomposition behaviors were then compared with woody biomass in *Chapter 5*.

Fifthly, for the further understanding of delignification behaviors, phenol was applied for the subcritical treatment of the woody biomass for its high ability to delignify lignocellulosics. Then, the delignification behaviors of Japanese beech as treated by subcritical water and subcritical phenol were compared from a topochemical viewpoint and reported in *Chapter 6*.

Finally, the decomposition behaviors of lignocellulosics by hydrothermal treatment were summarized. Prospects for the future studies to fulfill this research were further proposed in *Chapter 7* as conclusions.

Chapter 2

Characterization of the Lignin-derived Products from Japanese Cedar by Hydrothermal Treatment

2.1 Introduction

The wood cell wall consists mainly of cellulose, hemicelluloses and lignin, and these compounds are composed intricately in an artful manner to enforce the wood cell wall, having a resistance to water and most organic solvents. Among the natural polymers, lignin is the second most abundant macromolecule with 24~33 wt% in softwoods and 19~28 wt% in hardwoods (Sarkanen and Hergert, 1971). Lignin, with an abundant reserve, is considered as a potential resource of energy and value-added aromatic chemicals. In order to utilize it efficiently, various degradation methods have been studied. Among them, hydrothermal treatment is catalyst-free and environmentally benign as explained in 1.7.

Lu et al. treated Japanese beech (*Fagus crenata*) with two-step semi-flow hot-compressed water (1st stage: 230 °C /10 MPa/15 min, 2nd stage: 270 °C /10 MPa/ 15 min) and the hemicelluloses and cellulose were selectively decomposed (Lu et al., 2009). They elucidated that lignin was decomposed in both stages and that the lignin-derived monomeric products such as coniferyl alcohol and sinapyl alcohol were obtained in water-soluble portion (Lu et al., 2009). Additionally, these lignin-derived products could be converted into acetic acid and phenolic compounds via microorganisms, and thus expected to become valuable chemicals (Nakamura et al., 2011). Phaiboonsilpa et al. treated Japanese cedar (*Cryptomeria japonica*) and obtained lignin-derived products in the hot-compressed water (Phaiboonsilpa et al., 2010). However, detailed analyses of the lignin-derived products obtained from Japanese cedar have not yet been conducted. The decomposition behavior of lignin in Japanese beech has been studied by Yamauchi et al.

(Yamauchi et al., 2013), while lignin content and its structure in Japanese cedar are different from Japanese beech, and their decomposition behaviors of lignin should also differ. Thus, the aim of this chapter is to elucidate the decomposition behavior of lignin from Japanese cedar as treated by hot-compressed water with the analyses of the lignin-derived products

2.2 Materials and methods

2.2.1 Wood samples and chemicals

Wood flour of Japanese cedar (*Cryptomeria japonica*) passing through an 18-mesh screen was extracted with acetone using Soxhlet apparatus and dried at 105 °C for 24 h before experiment. Table 2-1 shows the chemical composition of the extractive-free Japanese cedar, determined by Klason lignin method (Dence, 1992) for lignin, and the obtained acid-soluble portion for hemicellulose and cellulose. Ash content was measure by incinerating the sample in a muffle furnace at 600 °C for 4 h. All chemicals used in this study were of reagent grade without any purification, and purchased from Nacalai Tesque, Inc., Kyoto, Japan.

Table 2-1 Chemical composition of extractive-free Japanese cedar used in this study

Cellulose	Hemicelluloses	Lignin		Ash	Total
		Klason lignin	Acid-soluble		
48.5	18.2	31.0	1.0	0.3	100.0

2.2.2 Two-step semi-flow hot-compressed water treatment

The semi-flow hot-compressed water treatment was performed by the apparatus as shown in Fig. 2-1. Approximately 0.5 g of oven-dried extractive-free wood flour was placed in the reaction vessel. From Water tank-1, deionized water was flowed through the reaction vessel by Pump-1 to pressurize the system to 10 MPa controlled by the back-pressure regulator (B-1). Then, two preheating units (Heater-1 and Heater-2), of which the temperatures could be assigned separately, were controlled to achieve the two-step temperatures, while the Heater-3 equipped at the base of the reaction vessel was manipulated to control the temperatures in the reaction vessel. When the temperatures of the Heater-1, Heater-2 and Heater-3 reached their designated temperatures, the Pump-1 was turned on for the 1st stage of 230 °C with the needle valve N-1 opened and N-2 closed. After the 1st stage, N-1 was closed and then N-2 was opened for the 2nd stage of 270 °C. The thermocouples (T-3 and T-4) demonstrated the temperatures for the inlet and

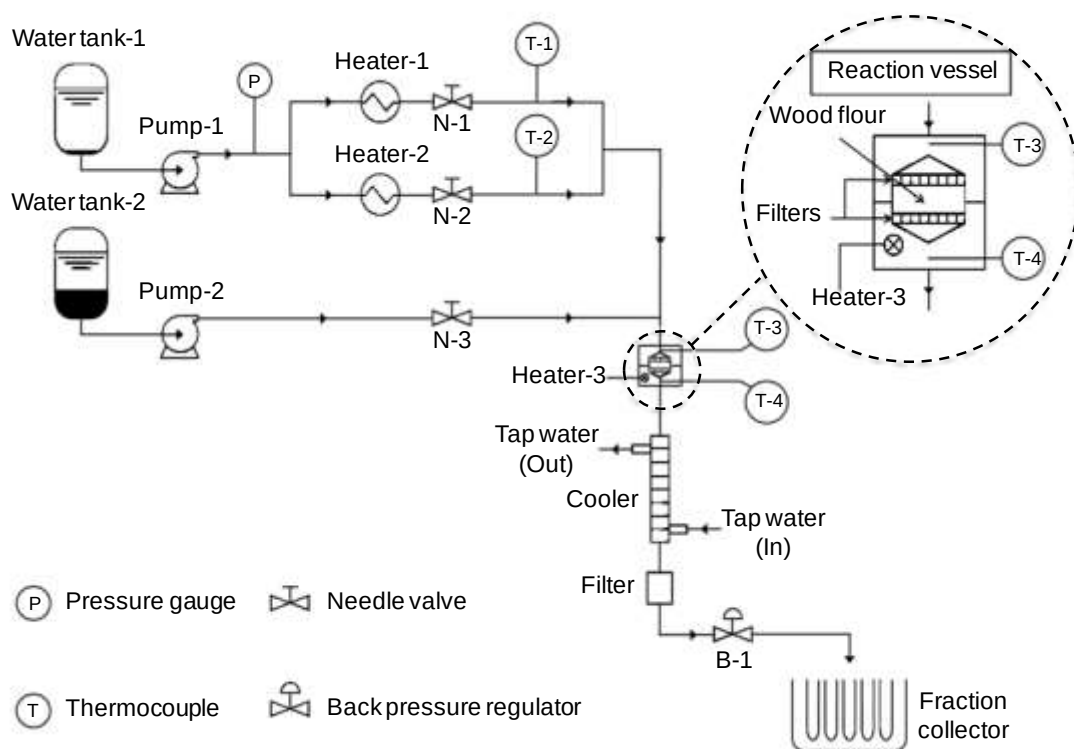


Fig. 2-1 Schematic diagram of semi-flow hot-compressed water system (Lu et al., 2009)

outlet of the reaction vessel during the hot-compressed water treatment. Only hot-compressed water-soluble products could pass through the filter equipped at the base of reaction vessel. After that, hot-compressed water-soluble products were cooled down immediately by the cooling the system to terminate all fractions. Hot-compressed water-soluble portions were collected by the fraction collector. After the two-step treatment, from Water tank-2 the deionized water was flowed through the reaction vessel to quench the reaction.

Fractionation process of Japanese cedar as treated by two-step semi-flow hot-compressed water is shown in Fig. 2-2. Two-step treatment conditions were 230 °C/10 MPa/15 min for the 1st stage and 270 °C/10 MPa/15 min for the 2nd stage. After the treatment, the hot-compressed water-insoluble residue left over in the reaction vessel was separated from hot-compressed water-soluble portion and the residue was dried at 105 °C for 12 h to measure its oven-dried weight.

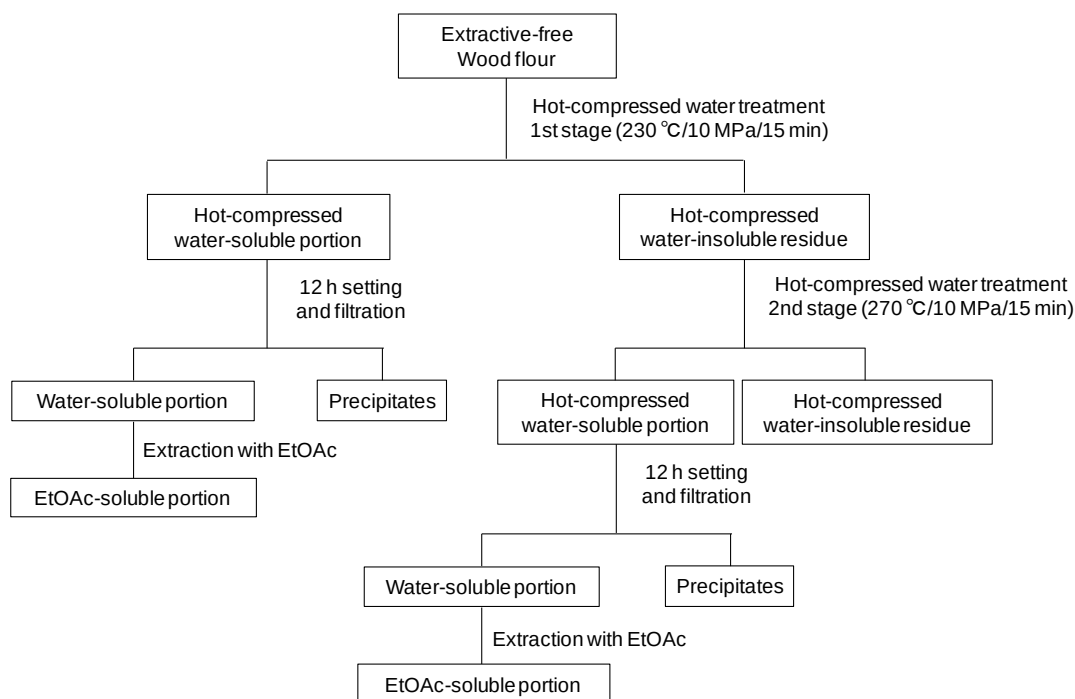


Fig. 2-2 Fractionation process of Japanese cedar as treated by semi-flow two-step hot-compressed water treatment. EtOAc: ethyl acetate

The obtained hot-compressed water-soluble portion was kept standing for 12 h under ambient condition. During this period, it was separated to be water-soluble portion and precipitates, which were retrieved by the filtration with a membrane filter (pore size: 0.45 μm , Merck Millipore Ltd., Massachusetts, United States). For the water-soluble portion, the lignin-derived compounds were obtained by the extraction with ethyl acetate.

2.2.3 Analytical methods

The content of lignin-derived products in each fraction was examined as the combined yields of Klason lignin and acid-soluble lignin (Dence, 1992). For the acid-soluble portion obtained, the amounts of various mono-saccharides were determined by high-performance anion-exchange chromatography (HPAEC: ICS-3000, Thermo Fisher Scientific, Massachusetts, United States) under the following conditions: column, CarboPac PA-1 (4 mm $\times$ 250 mm, Thermo Fisher Scientific, Massachusetts, United States); mobile phase, a gradient-programmed mixture of distilled water, 0.2 M NaOH and 1.0 M sodium acetate; flow-rate, 1.0 mL/min; column temperature, 35 $^{\circ}\text{C}$; detector, electrochemical cell (Thermo Fisher Scientific, Massachusetts, United States). Its cellulose and hemicelluloses contents were then estimated based on the amounts of glucose and other mono-saccharides, respectively (Minami and Saka, 2003). Molecular weight distribution was evaluated by gel permeation chromatography (GPC) analysis, which was performed with LC-10A (Shimadzu Co., Kyoto, Japan) under the following conditions: column, Shodex KF-801 + KF-802 + KF-802.5 + KF-803 (Showa Denko, Tokyo, Japan); mobile phase, tetrahydrofuran; flow-rate, 0.6 mL/min; column temperature, 50 $^{\circ}\text{C}$; detector, Ultraviolet light at 280 nm. For comparison, polystyrene standards (molecular weight (MW): 162, 580, 1270, 2960, 5000) were used as standard.

In order to evaluate the chemical characteristics of lignin-derived products, alkaline nitrobenzene oxidation was performed and gas chromatographic (GC: GC-14B, Shimadzu Co., Kyoto, Japan) analysis was undertaken to obtain the total yield of vanillin

(Chen, 1992). However, since water-soluble portion contained some monomeric products like coniferyl alcohol, the vanillin yield from oligomeric products was evaluated from the total yield of vanillin minus vanillin from monomeric products, according to the following equation,

Yield of vanillin from oligomeric lignin-derived products (mmol/g)

$$= \frac{(\text{Total yield of vanillin} - \text{Yield of vanillin from monomeric lignin-derived products}) (\text{mmol})}{(\text{Total lignin-derived products} - \text{Monomeric lignin-derived products}) (\text{g})}$$

Since the monomeric compounds are mostly composed of coniferyl alcohol, coniferyl aldehyde, vanillin, and isoeugenol, other monomeric products were ignored in this calculation. Monomeric and dimeric compounds of lignin-derived products in water-soluble portion were analyzed by gas chromatography–mass spectrometry (GC–MS), which was performed with Hitachi M7000s and M9000 series (Hitachi Ltd., Hitachi, Japan). The capillary column was a DB-5MS (Agilent Technology, Inc., California, United States). The temperature program was 1.0 min at 40 °C, 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. Injector and detector temperatures were both 230 °C. High molecular weight compounds of lignin-derived products in water-soluble portion were analyzed by matrix-assisted laser desorption ionization-time of flight/mass spectrometry (MALDI-TOF/MS), which was performed with AXIMA Performance (Shimadzu Co., Kyoto, Japan). Nitrogen gas ($\lambda = 337 \text{ nm}$) was used as the laser source. The analysis was performed in positive-ion, linear mode and 2,5-dihydroxybenzaldehyde was used as matrix.

2.3 Results and discussion

2.3.1 Yield of lignin-derived products

The temperature change of the hot-compressed water treatment for the 1st stage (230 °C/10 MPa/15 min) and 2nd stage (270 °C/10 MPa/15 min) is shown in Fig. 2-3. The yield of water-insoluble residue and its chemical composition are also shown. When the extractive-free Japanese cedar is treated by hot-compressed water, hemicelluloses and a part of cellulose are decomposed and eluted as hot-compressed water-soluble portion at the 1st stage, and then in the 2nd stage, the rest of cellulose is mostly decomposed and eluted. It seems likely that cellulose decomposed in the 1st stage might have lower crystallinity compared with remaining cellulose decomposed in the 2nd stage.

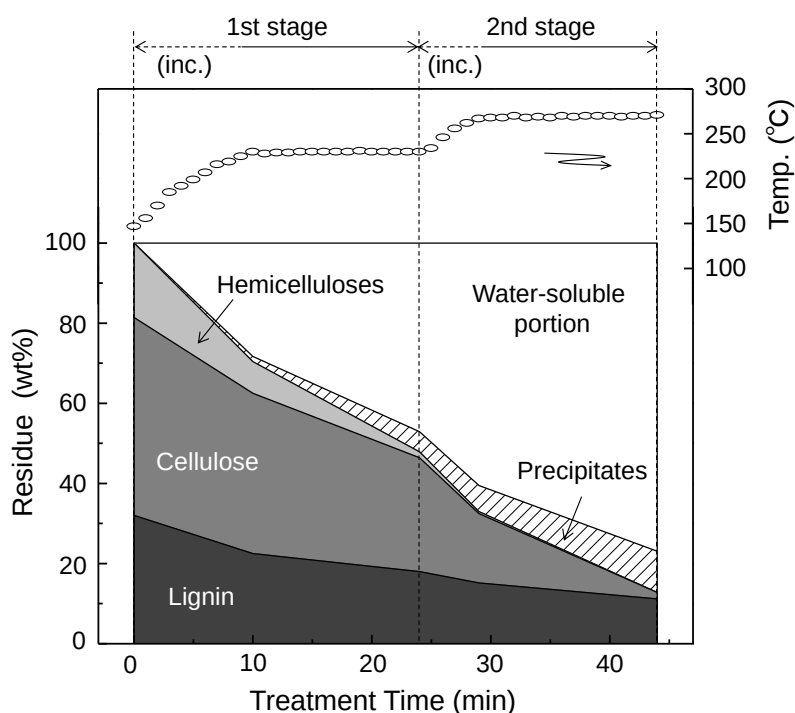


Fig. 2-3 Yield of water-soluble portion, precipitates and water-insoluble residue composed of hemicelluloses, cellulose and lignin as treated by two-step semi-flow hot-compressed water. The temperature change is also shown.

In contrast, lignin is decomposed at both stages recovered as lignin-derived products in all fractions. However, some of lignin has much higher resistance to hot-compressed water as compared with cellulose and hemicelluloses. The hot-compressed water-soluble portion eluted is later precipitated partially under ambient conditions.

The yields of water-soluble portion, precipitates, and water-insoluble residue in both stages are summarized in Table 2-2. In addition, the yields of the lignin-derived products and the lignin concentration in each fraction are also shown. In the 1st stage, 52.1 (= 47.1 + 5.0) % of Japanese cedar was eluted in hot-compressed water, and 5.0 % was obtained as the precipitates. Consequently, 47.1 wt% was obtained in the water-soluble portion and 47.9 wt% remained as the water-insoluble residue. After the 2nd stage treatment of the water-insoluble residue (47.9 wt%), 35.1 (= 29.8 + 5.3) wt% was eluted in hot-compressed water, and then 5.3 and 29.8 wt% were obtained as the precipitates and water-soluble portion, respectively. As a result, 12.8 wt% of Japanese cedar remained as the water-insoluble residue after two-step treatment. This value is, in fact, much higher than that of Japanese beech only to be 4.4 wt% (Yamauchi et al., 2013). The observed difference in the yields of water-insoluble residues between the two woods would be due to the fact that lignin of Japanese cedar has less ether linkages than that of Japanese beech.

The yields of lignin-derived products in each fraction are shown in extractive-free wood-based wt%. For the 32.0 wt% lignin content of Japanese cedar, 14.0 (= 10.1 + 3.9) wt% was eluted in hot-compressed water at the 1st stage, with 10.1 and 3.9 wt% being from the water-soluble portion and precipitates, respectively. In the 2nd stage, 6.8 (= 5.0 + 1.8) wt% was resulted with 5.0 and 1.8 wt% as the water-soluble portion and precipitates, respectively. On the other hand, 11.2 wt%, about one-third of the initial lignin content, still remained as water-insoluble residue after two-step treatment. The lignin concentration was 0.214 and 0.168 g/g for water-soluble portion in both 1st and 2nd stages, respectively. For the precipitates, the lignin concentration in the 1st stage was 0.780 g/g, whereas that in the 2nd stage was much lower to be 0.340 g/g. This difference could indicate that high molecular-weight cellulose-derived products, eluted in the 2nd stage, gradually aggregated and precipitated with lignin-derived products under ambient

condition (Ehara and Saka, 2002). A similar tendency was observed in the case of Japanese beech (Yamauchi et al., 2013). The 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 g/g), indicating that the residual lignin has much higher resistance to hot-compressed water than other chemical components.

Table 2-2 The yields of fractionated product and lignin-derived product, and lignin concentration in water-soluble portion, precipitates and water-insoluble residue in the 1st and 2nd stages, as Japanese cedar treated by two-step semi-flow hot-compressed water

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

a) Lignin concentration was obtained by the yield of lignin-derived products divided by that of products.

2.3.2 Structural analysis of lignin-derived products

Fig. 2-4 demonstrates GPC chromatograms of the water-soluble portion and precipitates of both stages, detected at 280 nm in wavelength. In order to evaluate the molecular weight distribution of their lignin-derived products, the elution times of polystyrene standards (MW: 162, 580, 1270, 2960, 5000) were displayed. The higher molecular weight 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 indicates 5-hydroxymethylfurfural derived from polysaccharides. For the water-soluble portion, the lignin-derived products in the 1st stage contain oligomeric products up to MW of 1270, equivalent to heptamer of coniferyl alcohol, whereas the lignin-derived products in the 2nd stage contain smaller than those in the 1st stage. In contrast, the molecular weight of the lignin in precipitates is higher than the lignin-derived products in water-soluble portion for both stages.

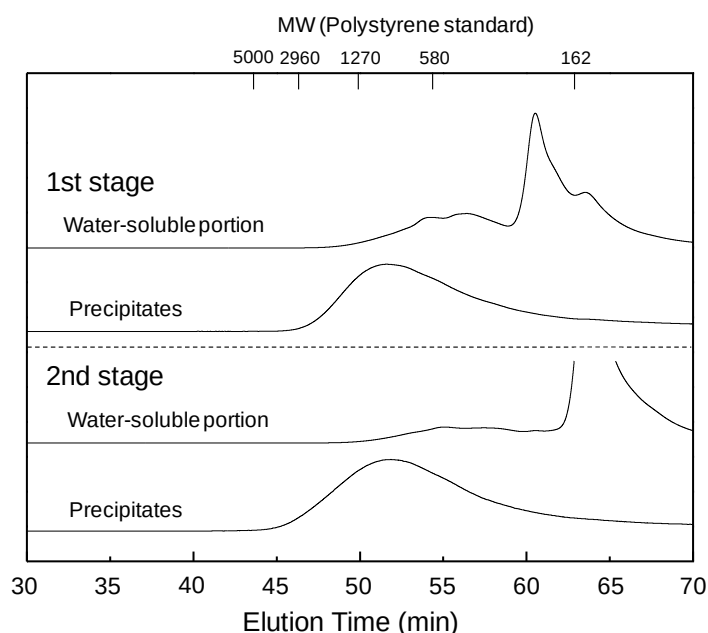


Fig. 2-4 GPC chromatograms of the water-soluble portion and the precipitates in the 1st stage (upper), and those in the 2nd stage (lower)

In order to compare the relative proportion of ether linkages of lignin-derived products, alkaline nitrobenzene oxidation analysis was conducted, and the obtained yield of its products from the lignin-derived products is shown in Fig. 2-5. Since *p*-hydroxybenzaldehyde derived from *p*-hydroxyphenyl (H) lignin was a trace amount, the yield of vanillin derived from guaiacyl (G) lignin was only shown. For comparison, the yield of vanillin from the untreated Japanese cedar is also shown. Since water-soluble portion contains some monomeric products like coniferyl alcohol as already described, the yield of vanillin from oligomeric products was obtained by subtracting vanillin yield from monomeric products from the total yield of vanillin.

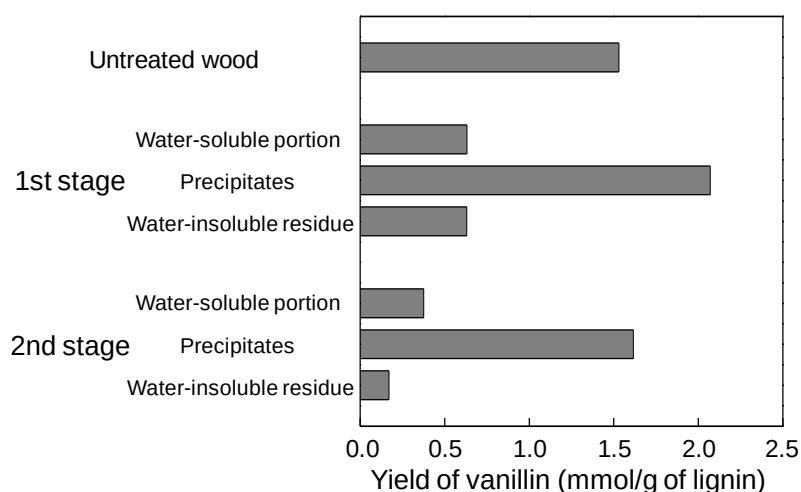


Fig. 2-5 The yield of alkaline nitrobenzene oxidation products for the water-soluble portion, precipitates and water-insoluble residue in the 1st stage and 2nd stage. Just comparison, the yield of the untreated Japanese cedar is included.

For the water-soluble portion, the yields of vanillin from the 1st and 2nd stages are 0.64 and 0.38 mmol/g of original lignin, respectively, both of which are smaller than the vanillin derived from untreated wood (1.53 mmol/g). This suggests that the 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 the lignin in precipitates in both stages were comparable to that from untreated wood. This could indicate that lignin in precipitates maintains ether linkages. For water-insoluble residue, the yield of vanillin from the 1st stage is 0.63 mmol/g, whereas that from 2nd stage is 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, is rich in condensed type lignin.

2.3.3 Lignin-derived products in water-soluble portion

In order to identify the lignin-derived low molecular weight compounds in the water-soluble portion, GC-MS analysis was performed. GC-MS spectrum of the lignin-derived products in the water-soluble portion is shown in Fig. 2-6. The peaks in the short retention

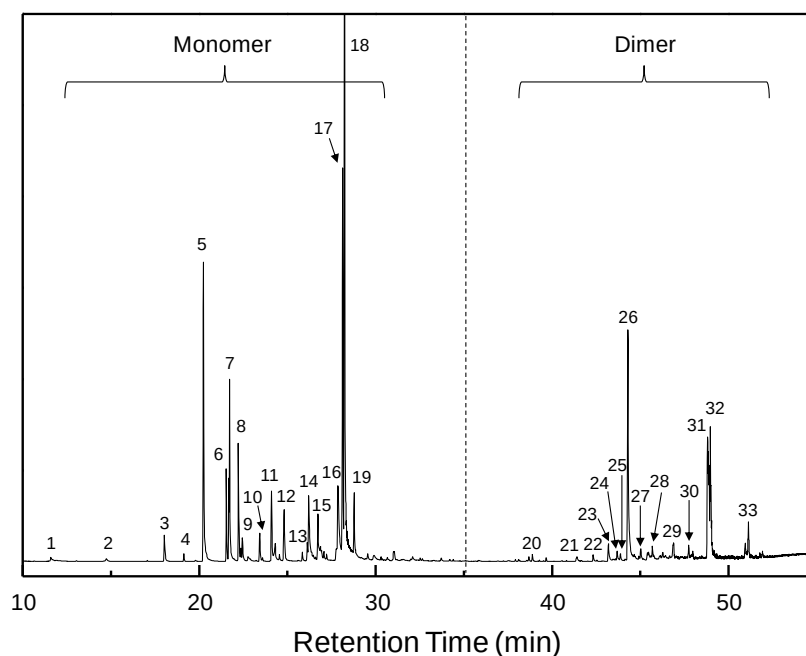


Fig. 2-6 Total ion chromatogram of the water-soluble portion from Japanese cedar as treated by semi-flow hot-compressed water by GC-MS. The numbers correspond to those in Tables 2-3 and 2-4.

time before 35 min are the lignin-derived monomeric compounds, while those in the longer retention time after 35 min are dimeric compounds.

According to the comparison with lignin monomeric model compounds, 17 kinds of lignin-derived compounds such as vanillin (No. 5), isoeugenol (No. 6), coniferyl aldehyde (No. 17), and coniferyl alcohol (No. 18) are identified as shown in Table 2. Some lignin-derived products were identified from their mass spectra by comparing their retention times with those of authentic compounds as in the literature (Meier and Faix, 1992; Takada et al., 2004; Vane, 2003). Consequently, these identified compounds contained guaiacol skeletons, indicating that the methoxyl group has not been removed from aromatic ring by the hot-compressed water treatment. These compounds also contained the phenolic hydroxyl group. Most of this hydroxyl group could be produced due to the cleavage of ether linkages by the hot-compressed water treatment. Among these identified compounds, *trans*-coniferyl alcohol is obtained as the main product. It would mean that the lignin-derived products in the water-soluble portion largely maintained the side-chain structures of lignin. A few of ketone compounds, which are characteristic products from acidolysis of lignin, are observed in the water-soluble portion. Thus, acidolysis reaction can be ignored in the hot-compressed water treatment.

Table 2-3 Lignin-derived monomeric products in the water-soluble portion and their retention times, structures, and MS fragments for GC-MS analysis

No.	Product	Retention time (min)	Structure ^{a)}	MS fragments (<i>m/z</i>)
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	<i>trans</i> -Isoeugenol	21.5	G–C=C–C	164, 150, 104
7	Homovanillin	21.7	G–C–CHO	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	<i>cis</i> -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	<i>trans</i> -Coniferyl aldehyde	28.2	G–C=C–CHO	178, 164, 149
18	<i>trans</i> -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-methoxyphenyl)

^{b)} Product identified according to the literature (Takada et al., 2004)

^{c)} Product identified according to the literature (Meier and Faix, 1992)

^{d)} Product identified according to the literature (Vane, 2003)

Table 2-4 Lignin-derived dimeric products in water-soluble portion and their retention times and MS fragments for GC-MS analysis

No.	Retention time (min)	Product	MS fragments (<i>m/z</i>)
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	Phenylcoumaran type (β -5)	311, 300, 287, 272, 256, 240, 161, 152, 95, 78, 67
26	44.3		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	Biphenyl type (5-5')	344, 326, 312, 207, 139, 121, 93, 78, 66
31	48.8		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

Takada et al. obtained the lignin-derived compounds from Japanese cedar (*Cryptomeria japonica*) by supercritical water treatment (380 °C /100 MPa/8 s) as the methanol-soluble portion and analyzed the lignin-derived monomeric and dimeric compounds with GC–MS analysis (Takada et al., 2004). The lignin-derived monomeric products in the methanol-soluble portion contained guaiacol skeletons, indicating that the methoxyl group had not been removed from aromatic ring by supercritical water treatment. In addition, vanillin was mainly obtained without recovered coniferyl alcohol. Thus, the side-chain structure in lignin was more altered by supercritical water treatment compared with the hot-compressed water treatment. This suggests that the hot-

compressed water treatment used in this study can suppress the decomposition of side-chain in lignin more effectively than supercritical water treatment.

Table 2-4 demonstrates mass fragments and retention time of lignin-derived dimeric compounds. Among these compounds, Nos. 20, 26, and 31 in Fig. 2-7 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 in molecular weight after trimethylsilyl derivatization (Takada et al., 2004). These identified compounds are condensed type, containing methoxyl and phenolic hydroxyl groups as observed in the monomeric compounds in Table 2-3, whereas Nos. 20 and 26 in Fig. 2-7 did not maintain the structure of phenylpropane side-chain. Generally, in hydrothermal treatment, formaldehyde produced from lignocellulosics forms diarylmethane structure (Brosse et al., 2010). 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.

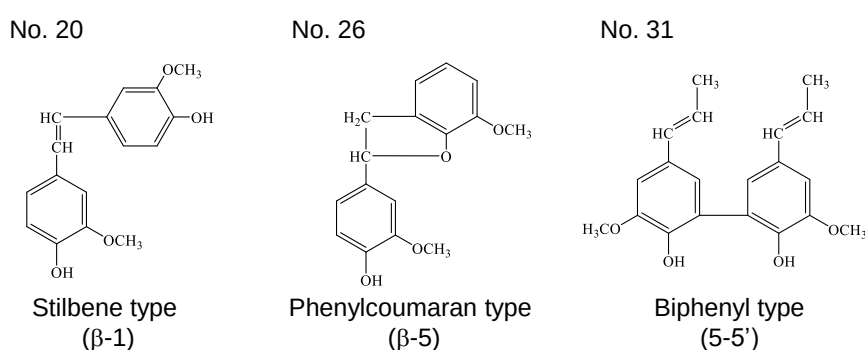


Fig. 2-7 Speculative structures of some lignin-derived dimeric products

In order to identify lignin-derived oligomeric compounds, MALDI-TOF/MS analysis was performed. The obtained MALDI-TOF/MS spectra of the lignin-derived products of the 1st and 2nd stages are shown in Fig. 2-8. The spectra of the 1st stage contain several molecular weight intervals (MWIs) of 178 and 196. According to the experiment with lignin model compounds, β - β or β -5 linkage results in the MWI of 178 due to the radical mechanism of coniferyl alcohol (MW: 180.20), whereas β -O-4 linkage results in the MWI of 196 due to the addition of hydroxyl group (Yoshioka et al., 2011). Besides, some other linkages such as 5-5', 4-O-5, and α -O-4 linkages can result in the MWI of 178 as well. 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, suggesting that the oligomeric lignin-derived products up to heptamers contain both ether type and condensed type linkages. In contrast, the spectrum of 2nd stage does not contain the MWIs of 178 or 196, indicating that the phenylpropane side-chain could be altered by hot-compressed water. The oligomeric lignin-derived products consisted up to tetramer. These results really agree with those from GPC analysis.

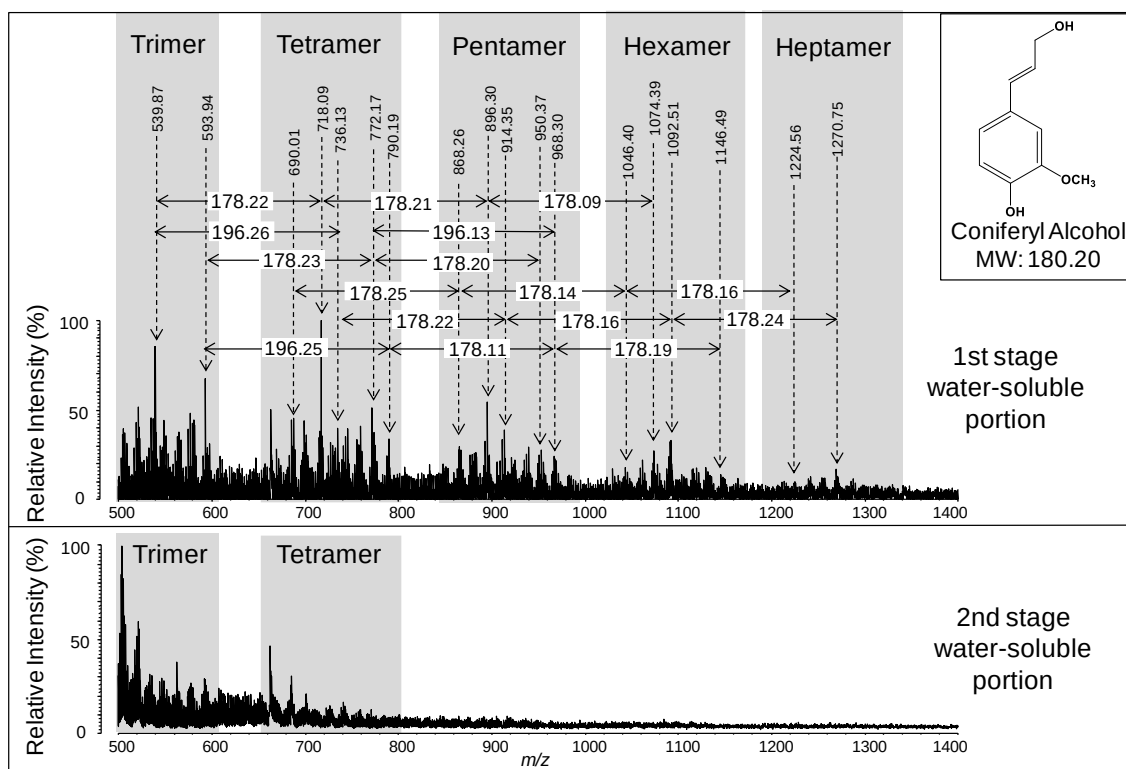


Fig. 2-8 MALDI-TOF/MS spectra of the lignin-derived products in the water-soluble portion for the 1st (upper) and 2nd stages (lower) (m/z : 500-1400). Several molecular weight intervals (MWI) of about 178.20 and 196.20 were obtained from the 1st stage water-soluble portion, suggesting β - β or β -5, and β -O-4 linkages, respectively.

2.3.4 Lignin-derived products in precipitates

The lignin-derived products in the precipitates in both stages are composed of the higher molecular weight products than water-soluble portion (Fig. 2-4), and their structure was rich in ether linkage according to the yield of vanillin by alkaline nitrobenzene oxidation analysis (Fig. 2-5). These results suggest that some micropores were produced in the cell wall due to the decomposition of hemicelluloses and the cleavage of ether linkages in lignin in the 1st stage and that the micropores would have facilitated the elution of some lignin-derived large molecular products, which maintain ether linkages (Yamauchi et al., 2013). Hydrophobic lignin could be eluted in hot-compressed water due to the low dielectric constant of hot-compressed water, whereas some large molecular compounds precipitated after hot-compressed water returned to ordinary water under ambient condition. In the 2nd stage, the cellulose- and lignin-derived oligomeric compounds were also eluted in hot-compressed water.

2.3.5 Lignin-derived products in water-insoluble residue

Approximately one-third of original lignin still remained as the water-insoluble residue after two-step treatment, and the water-insoluble residue is 0.875 g/g in lignin concentration as shown in Table 2-2. The ratio of condensed type linkages was observed to increase as the hot-compressed water treatment was prolonged. Consequently, lignin in the water-insoluble residue after two-step treatment was rich in condensed type due to the cleavage of ether type linkages and possible recondensation reaction.

2.4 Summary

Decomposition behavior of lignin in Japanese cedar (*Cryptomeria japonica*) as treated by two-step semi-flow hot-compressed water was discussed based on the analyses of the lignin-derived products fractionated during the treatment.

For the 1st stage (230 °C/10 MPa/15 min), in which hemicelluloses and a part of 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 ether linkages in lignin, some micropores would occur in the cell wall, which would have facilitated the elution of the some lignin-derived large molecular products together with the hemicelluloses-derived products. The hydrophobic lignin could be, thus, eluted in hot-compressed water due to the low dielectric constant of hot-compressed water. After its elution, some large molecules were precipitated under ambient condition. Since the precipitated lignin contained ether linkages, it would be considered to maintain the structure of original lignin. On the other hand, lignin-derived products in the water-soluble portion were of low molecular weight up to heptamers. It was found that identified monomeric compounds maintained methoxyl and phenolic hydroxyl groups such as coniferyl alcohol, coniferyl aldehyde, and vanillin.

For the 2nd stage (270 °C/10 MPa/15 min), in which the rest of cellulose was mostly decomposed, a part of lignin was decomposed and eluted. Similar to the 1st stage, some large molecules of lignin-derived products would be eluted and then precipitated under ambient condition. The rest of the remaining lignin to be one-third of the original one had resistance to hot-compressed water and remained as the water-insoluble residue, which was mostly composed of lignin. It is interesting that the remaining lignin rich in condensed type as compared with untreated wood can be expected to be utilized as materials resistant to any external attacks.

Chapter 3

Comparative Study on Topochemistry of Delignification from Japanese Cedar and Japanese Beech by Hydrothermal Treatment

3.1 Introduction

Woody biomass has been gaining attention as an alternative resource to fossil fuel because of its abundance and noncompetition with foodstuff. In order to utilize the woody biomass as biofuels and chemicals, various chemical and physical degradation methods have been examined. Among them, the hydrothermal treatments such as the steam explosion, subcritical and supercritical water, and hot-compressed water treatments are expected as a promising technology due to its catalyst-free and environmentally benign characteristics. As a feature of hydrothermal treatment, an increase in temperature of water at a high pressure results in a decrease in its dielectric constant and an increase in its ionic product (Ando et al., 2000; Rogalinski et al., 2008).

Phaiboonsilpa et al. and Lu et al. treated Japanese cedar (*Cryptomeria japonica*) and Japanese beech (*Fagus crenata*) with semi-flow hot-compressed water (1st stage: 230 °C/10 MPa/15 min, 2nd stage: 270 °C/10 MPa/ 15 min), and the hemicelluloses and cellulose were decomposed in the 1st and 2nd stages, respectively (Lu et al., 2009; Phaiboonsilpa et al., 2010). Lignin has been decomposed and eluted in hot-compressed water at both stages as discussed in *Chapter 2*.

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

Therefore, the delignification behaviors under the treatment of pulping such as kraft (Aguayo et al., 2014; Saka et al., 1982a), sulphite (Koch et al., 2003; Mendonca et al., 2004; Rehbein et al., 2010; Whiting and Goring, 1981), soda methods (Saka et al., 1982a) have been studied. In addition, some researchers studied the topochemistry of wood with hydrothermal treatment as one of the pretreatments (Biziks et al., 2013; Milagres et al., 2011; Mou et al., 2014). However, the delignification behavior of woody biomass by hydrothermal treatment has not been discussed yet. Thus, the aim of this study is to elucidate the topochemical differences in the delignification between softwoods and hardwoods as treated by hot-compressed water.

3.2 Materials and methods

3.2.1 Hot-compressed water treatment

Extractive-free wood flour of Japanese cedar (*Cryptomeria japonica*) and Japanese beech (*Fagus crenata*) were prepared as described in 2.2.1. All chemicals used in this study were of reagent grade without purification, purchased from Nacalai Tesque. Ltd., Kyoto, Japan.

Hot-compressed water treatment was conducted with two-step (1st stage: 230 °C/10 MPa/15 min and 2nd stage: 270 °C/10 MPa/15 min) as shown in Fig. 3-1. Each step of the treatment includes time up for 10 and 5 min for the 1st and 2nd stages, respectively, followed by time at for 15 min. The pressure was increased up to 10 MPa by the pressure pump, and controlled with a back-pressure regulator. A half gram of oven-dried extractive-free wood flour was placed in the reaction vessel, for which the two-step semi-flow hot-compressed water treatment was carried out as described in 2.2.1.

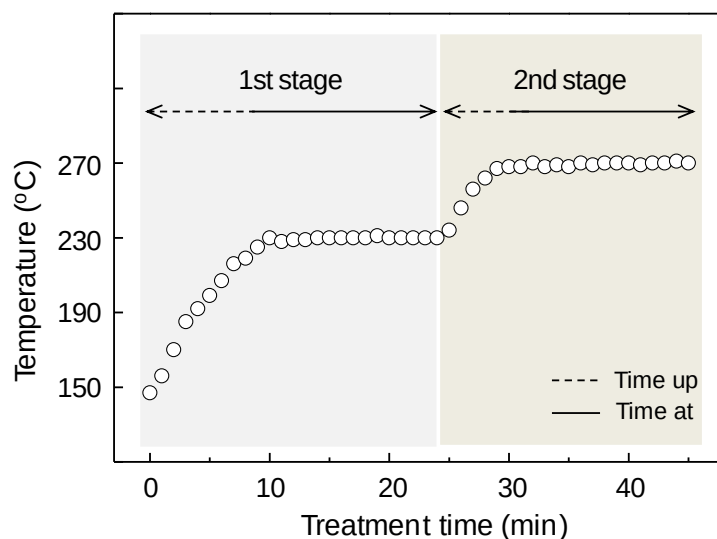


Fig. 3-1 The treatment temperature of hot-compressed water for 1st stage (230 °C/10 MPa) and 2nd stage (270 °C/10 MPa)

3.2.2 Analytical methods

The lignin content of insoluble residue was examined as the combined yields of Klason lignin and acid-soluble lignin (Dence, 1992). For acid-soluble portion, the amounts of various monosaccharides were determined by high-performance anion-exchange chromatography (HPAEC: ICS-3000, Thermo Fisher Scientific, Massachusetts, United States). The analysis condition is shown in 2.2.3. The cellulose and hemicelluloses contents in the insoluble residues were then estimated based on the amounts of monosaccharides (Phaiboonsilpa et al., 2010).

In order to evaluate the chemical characteristics of residual lignin, alkaline nitrobenzene oxidation was conducted and the total yields of vanillin and syringaldehyde were analyzed with GC (GC-2014: Shimadzu Co., Kyoto, Japan) (Chen, 1992).

The obtained insoluble residues of the 1st and 2nd stage treatments were gradually dehydrated with ethanol-water mixtures with increasing ethanol concentration up to 99.5 %. After the ethanol was replaced by propylene oxide, the residues were 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, Illinois, United States). For UV microscopy, the obtained sections were placed on quartz slides, mounted with glycerin, and then covered with quartz cover slip. They were, then, examined under MSP-800 (Carl Zeiss, Oberkochen, Germany) at a wavelength of 280 nm with a wavelength width of ± 10 nm. For TEM observations, ultrathin sections were mounted on copper grids, stained with 1 % aqueous solution of KMnO_4 , and observed under a JEM-1400 (JEOL Ltd., Tokyo, Japan) at an accelerating voltage at 100 kV.

Methoxyl content of residual lignin was analyzed according to general method (Baker, 1996). After insoluble residues were treated with 57 wt% of hydroiodic acid, the yield of obtained methyl iodide was determined with gas chromatography to study the average methoxyl content of the residual lignin.

3.3 Results and discussion

3.3.1 Yield of insoluble residue and its delignification rate

The hot-compressed water treatment was conducted under constant pressure (10 MPa) with two-step treatment consisting of the 1st stage (230 $^{\circ}\text{C}$ /10 MPa) and 2nd stage (270 $^{\circ}\text{C}$ /10 MPa) as shown in Fig. 3-1. The 1st stage consists of 10 min time up towards the maximum temperature 230 $^{\circ}\text{C}$ for 15 min, while the 2nd stage consists of 5 min time up towards the maximum temperature 270 $^{\circ}\text{C}$ for 15 min.

Table 3-1 shows the yield of the obtained insoluble residues from Japanese cedar and Japanese beech treated by hot-compressed water as mentioned above. The insoluble residues at the end of the time up and time at for both stages were collected and examined. For both species, more than half of the initial wood was decomposed and removed with

hot-compressed water at the end of the 1st stage, and at the end of the 2nd stage, 13 wt% of Japanese cedar and 7 wt% of Japanese beech remained as insoluble residue.

The chemical composition of the insoluble residues is also shown in Table 3-1. It seems apparent that the insoluble residues consist of lignin together with cellulose and hemicelluloses without any chars. For both species, hemicelluloses were decomposed completely, while cellulose was partly remained as insoluble residues after two-step 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 3-1. 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.

Table 3-1 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 %)
			Cellulose	Hemicelluloses	Lignin ^{a)}	
Japanese cedar	Untreated	—	0.49	0.19	0.32 (= 0.31 + 0.009)	0
	1st stage	10 min	0.57	0.11	0.32 (= 0.32 + 0.002)	30
	Time at	15 min	0.59	0.03	0.37 (= 0.37 + 0.002)	44
	2nd stage	5 min	0.53	0	0.47 (= 0.47 + 0.002)	52
	Time at	15 min	0.12	0	0.87 (= 0.87 + 0.004)	65
	Untreated	—	0.46	0.28	0.26 (= 0.22 + 0.034)	0
Japanese beech	1st stage	10 min	0.56	0.15	0.29 (= 0.26 + 0.029)	29
	Time at	15 min	0.70	0.01	0.29 (= 0.28 + 0.005)	49
	2nd stage	5 min	0.71	0	0.29 (= 0.28 + 0.005)	60
	Time at	15 min	0.23	0	0.77 (= 0.77 + 0.006)	77
	Untreated	—	0.46	0.28	0.26 (= 0.22 + 0.034)	0
	1st stage	10 min	0.56	0.15	0.29 (= 0.26 + 0.029)	29

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

3.3.2 Alkaline nitrobenzene oxidation

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. (Ehara et al., 2002) treated dimeric model compounds of β -O-4 and 5-5' types with the subcritical water (330 °C/ 50 MPa/10 s), and ether linkages were readily cleaved, while condensed type linkages had resistance to subcritical water.

Based on this information, alkaline nitrobenzene oxidation analysis was conducted for the insoluble residues in order to compare the relative proportion of ether linkages of the residual lignin. The obtained yields of the products from insoluble residue of Japanese cedar and Japanese beech are shown in Fig. 3-2. Vanillin derived from guaiacyl (G) lignin and syringaldehyde from syringyl (S) lignin were mainly obtained as decomposed products. Just comparison, the yields of obtained products from the untreated wood are also shown.

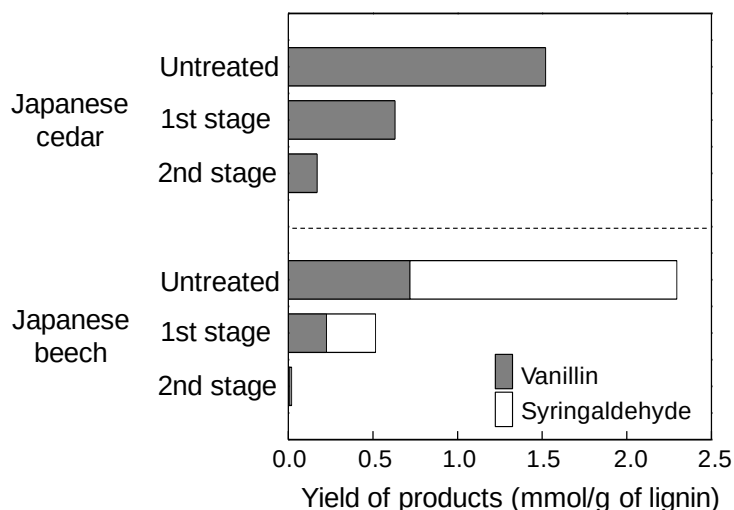


Fig. 3-2 The yield of alkaline nitrobenzene oxidation products for insoluble residue of Japanese cedar and Japanese beech at the end of the 1st and 2nd stages of the two-step hot-compressed water treatment

For both species, the yields of products decreased as hot-compressed water treatment was prolonged. The obtained products would mainly be derived from ether type linkages of lignin. Considering the previous experiment of lignin model compounds, this result would indicate that the relative proportion of ether linkages in the residual lignin was decreased by hot-compressed water treatment.

Consequently, lignin in the final residues would be rich in condensed type lignin and/or unknown structures produced in the hot-compressed water treatment. This result would be owing to the fact that hot-compressed water cleaves only ether linkages of lignin but not condensed type linkages, as observed in a study on the lignin model compounds (Ehara et al., 2002).

3.3.3 Microscopic observation of insoluble residue

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

Such an observation can be made more clearly in the high magnified TEM images as shown in Figs. 3-4 and 3-5, in which the middle lamella at cell corner (ML_{CC}) region remained as insoluble residue with some residual lignin fraction from the cell walls, all

of which are stained with KMnO_4 specific for lignin. However, in case of Japanese cedar (Fig. 3-4), lignin in tracheid secondary cell walls partly remained. From these lines of evidence, lignin in tracheid secondary cell wall of Japanese cedar had higher resistance to hot-compressed water than that in fiber cell wall of Japanese beech.

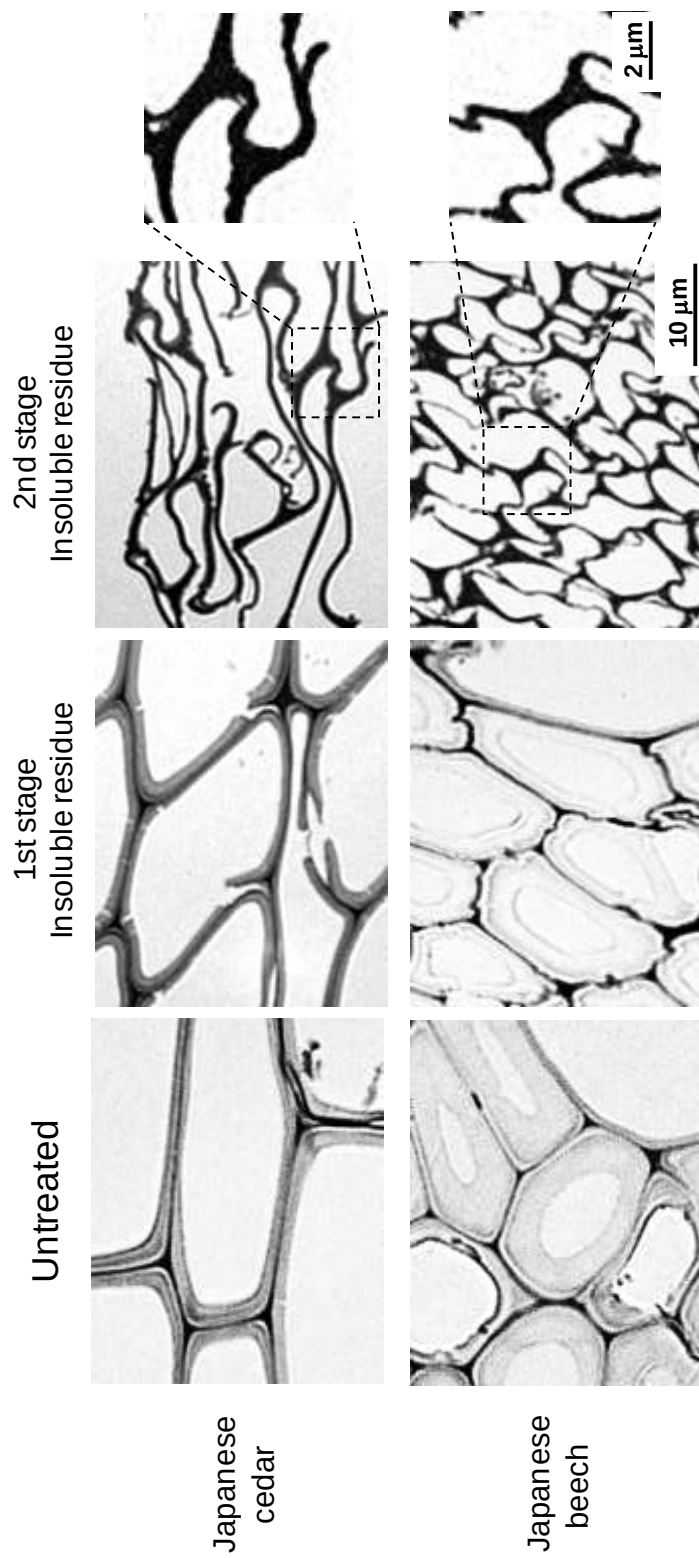


Fig. 3-3 UV micrographs taken at 280 nm of insoluble residues at the end of 1st and 2nd stages from Japanese cedar (upper) and Japanese beech (bottom)

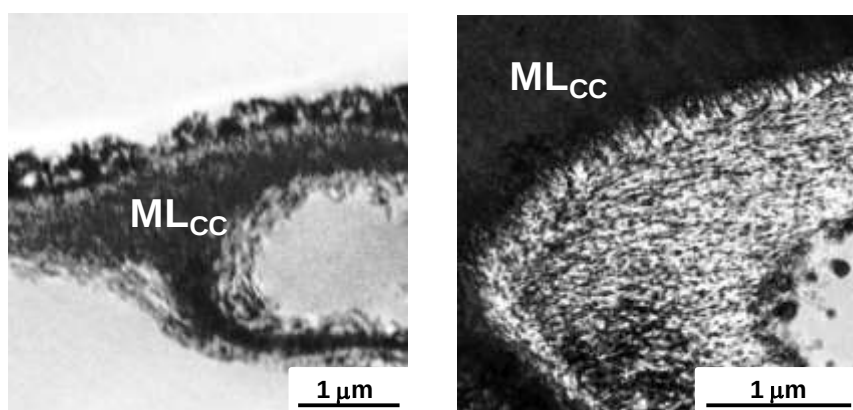


Fig. 3-4 KMnO_4 -stained TEM micrographs of insoluble residue for Japanese cedar.

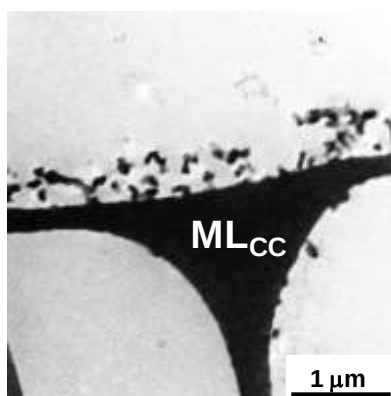


Fig. 3-5 KMnO_4 -stained TEM micrograph of insoluble residue for Japanese beech.

3.3.4 Methoxyl content of insoluble residue

Lignin is composed of up to three different phenylpropane (C₉) units, which are guaiacyl (G), syringyl (S) and p-hydroxyphenyl (H) lignins with, respectively, one, two and no methoxyl groups per aromatic ring. The distribution of these phenylpropane units is different from one morphological region to another. For example, in case of softwood tracheid, Whiting and Goring elucidated that lignin in the secondary wall is mainly composed of G lignin, whereas that in middle lamella, especially at cell corner, is composed of H and G lignins according to the methoxyl content analysis (Whiting and Goring, 1982). On the other hand, hardwood lignin is composed of G and S lignins, distributed differently from one morphological region to another. Saka and Goring examined the distribution of lignin in white birch (*Betula papyrifera*) with ultraviolet microscopy coupled with energy-dispersive X-ray analysis (UV-EDXA) and elucidated that G : S ratio of lignin in fiber S₂ layer, which occupied most of the cell wall, is 12 : 88, while G : S ratio of the cell corner middle lamella is 88 : 12 (Saka and Goring, 1988). Thus, lignin in middle lamella has lower methoxyl content per C₉ unit than that in secondary wall for both softwood tracheid and hardwood fiber.

The methoxyl content of residual lignin was, therefore, analyzed and shown in Fig. 3-6 as lignin-based wt%. In order to make the discussion understandable clearly, the methoxyl content per phenylpropane unit is also shown in Fig. 3-6. Sarkanen and Hergert (Sarkanen and Hergert, 1971) indicated that normal hardwood lignins are composed of G and S lignins in varying ratios with the methoxyl content per phenylpropane units in a range of 1.2~1.5. Japanese beech used in this study was 0.21 % ($\div 1.35/\text{C}_9$ unit) in its content as in Fig. 3-6. On the other hand, normal softwood lignins are mainly composed of G lignin with a trace amount of H lignin, as observed to be 0.16 % ($\div 0.96/\text{C}_9$ unit) for Japanese cedar. If both lignins in cell wall and middle lamella were uniformly removed at the same rate, the average methoxyl content per C₉ unit should be the same during hot-compressed water treatment. However, the methoxyl content per C₉ unit was decreasing for both species as the delignification was progressed. According to the analysis of the

lignin-derived products in water-soluble portion, the methoxyl residue was not removed from aromatic ring by hot-compressed water treatment (Table 2-3). Thus, this result in Fig. 3-6 indicates that the relative proportion of middle lamella lignin has increased, compared with secondary cell wall lignin, as observed in Fig. 3-3 through Fig. 3-5 for insoluble residues.

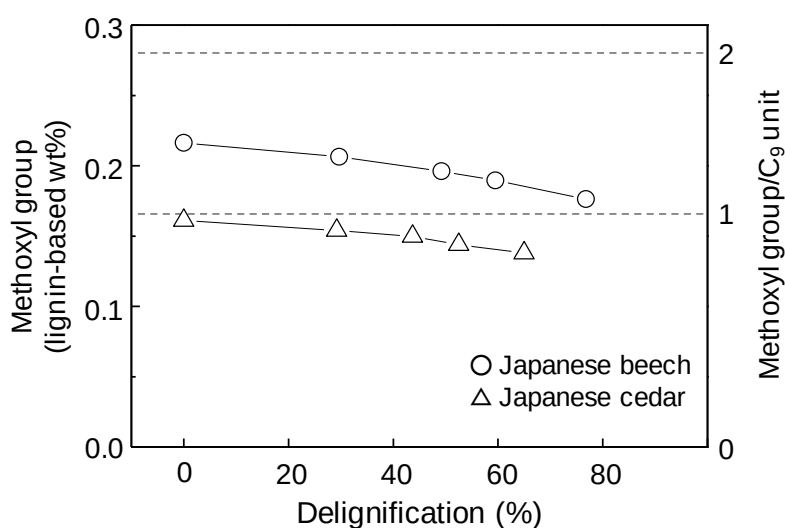


Fig. 3-6 Methoxyl group per C₉ unit of residual lignin for Japanese cedar and Japanese beech

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

A part of lignin in the insoluble residue might be intermolecularly condensed during

hot-compressed water treatment. If the condensed type linkages in insoluble residue are the result of condensation during the treatment, condensation reaction would be occurred not only in the middle lamella but also in the secondary wall. However, as described already, the delignification behavior was different from each other. Thus, the distribution of the remaining lignin must be reflected from that in the original condensed type lignin itself. Moreover, the semi-flow type of the hot-compressed water treatment may minimize its possibility due to prompt removal of the delignified products.

3.4 Concluding remarks

The difference in delignification from Japanese cedar and Japanese beech as treated by hot-compressed water was investigated from a topochemical viewpoint. Due to the preferential cleavage of ether linkages of lignin, lignin in secondary walls of the tracheid and fiber was preferentially decomposed and removed in hot-compressed water. Consequently, lignin in middle lamella remained as insoluble residue after treatment. Although some alternation of the lignin through condensation might result during hot-compressed water treatment and obscure the data, overall the results obtained would suggest that middle lamella lignin is rich in condensed type. For secondary wall lignin, on the other hand, Japanese cedar tracheids would have relatively higher condensed type lignin than Japanese beech fibers. These results, thus, indicate that the decomposition behaviors of softwood and hardwood differ due to their intrinsic characteristics of lignin. Such information must elucidate the importance of topochemistry in the decomposition behaviors of lignin.

Chapter 4

Characterization of the Precipitated Lignin from Japanese Beech by Hydrothermal Treatment

4.1 Introduction

Lignin, a major component of woody biomass, is the second most abundant natural macromolecule on the earth, and thus has been expected as an important resource for producing energy and value-added aromatic chemicals for many decades. However, lignin is not yet sufficiently utilized because it is entangled with cellulose and hemicelluloses in an artful manner to reinforce the morphological structure of woody biomass, resulting in difficulties in its decomposition and isolation. In the given situation, hydrothermal treatments such as steam explosion, supercritical and subcritical water, and hot-compressed water treatments have emerged as attractive approaches for the decomposition of woody biomass due to their catalyst-free and environmentally benign nature. Hydrothermal treatment provides high ionic product and has a low dielectric constant, endowing it with features of high hydrolysis ability and high solubility, which renders the hydrophobic compounds into water (Bröll et al., 1999; Savage, 1999).

Although the hot-compressed water treatment is conducted under milder conditions compared to other hydrothermal processes, it still has a high ability to decompose woody biomass, and can be carried out with three general types of systems; batch, flow and semi-flow (percolation) types. In the batch and flow systems, however, undesirable degradations of the products from woody biomass often occur at high temperature. In contrast, the semi-flow system can minimize such degradations, because once the products from woody biomass are liquefied in hot-compressed water, they can immediately flow out of the reaction vessel and be cooled down to quench the reaction (Sakaki et al., 2002; Liu and Wyman, 2003).

With these advantages, our research laboratory has developed the two-step semi-flow hot-compressed water treatment for the decomposition of woody biomass (Lu et al., 2009; Phaiboonsilpa et al., 2010). In this process, woody biomass is treated at 230 °C/10 MPa for 15 min as the 1st stage mainly to decompose hemicelluloses and a part of lignin. The treatment is further continued at 270 °C/10 MPa for 15~30 min as the 2nd stage to decompose crystalline cellulose. After this two-step treatment, water-soluble portion and insoluble residue can be obtained, and their amounts vary greatly depending on the biomass species. The water-soluble portion contains largely monosaccharides and oligosaccharides from hemicelluloses and cellulose together with lignin-derived products, while insoluble residue is rich in lignin, which has tolerance against hot-compressed water as discussed in *Chapter 3*.

With focusing on the 1st stage treatment, as mentioned above, hemicelluloses and lignin are mainly decomposed and liquefied in hot-compressed water, and these products keep dissolving in water even after being cooled. However, some precipitates are formed in the water-soluble portion after 12 h standing under ambient conditions. The obtained precipitates are rich in lignin-derived products as discussed in the previous work (Yamauchi et al., 2013). However, details of their structures still remain unclear.

In this study, therefore, the precipitated lignin, which is the lignin-derived products in the precipitates, was characterized by various chemical and spectroscopic analyses, and compared with milled wood lignin (MWL), which is widely considered to be a good representative of the original lignin.

4.2 Materials and methods

4.2.1 Samples and chemicals

The sapwood of Japanese beech (*Fagus crenata*) was milled in a Wiley mill (Thomas Scientific, New Jersey, United States) and the obtained wood flour was sieved to collect

the fraction between 0.15 mm to 1.0 mm mesh screens. The sieved flour was extracted with acetone using Soxhlet apparatus and dried at 105 °C for 24 h before experiment. This oven-dried extractive-free wood flour is referred to hereinafter as “original wood”. The milled wood lignin (MWL) was prepared from Japanese beech by the method of Björkman (Björkman, 1956). The wood flour was milled for 48 h using vibratory ball mill VS-1 (Irie Shokai Co., Ltd., Tokyo, Japan). All chemicals used in this study were of reagent grade without purification, purchased from Nacalai Tesque, Inc., Kyoto, Japan.

4.2.2 Semi-flow hot-compressed water treatment

The semi-flow hot-compressed water treatment was carried out as described in the previous paper (Phaiboonsilpa et al., 2010). Approximately 0.5 g of the original wood was placed in a reaction vessel. The treatment temperature was started from 150 °C, linearly increased to 230 °C in 10 min, and kept at 230 °C for 15 min by using electric heaters. Ion-exchanged water was continuously supplied to the reaction vessel at a flow-rate of 10 mL/min by a high pressure pump and maintained at 10 MPa by a back pressure regulator. The decomposed products liquefied in hot-compressed water were flowed out of the reaction vessel through a sintered metallic filter, then cooled down by a cooler jacket, and finally collected as the water-soluble portion. After the treatment, solid products remaining in the reaction vessel were also collected as the insoluble residue. After the obtained water-soluble portion was kept standing for 12 h under ambient conditions, the precipitates formed in the water-soluble portion were separated by a membrane filter (pore size: 0.45 µm, Merck Millipore Ltd., Massachusetts, United States).

4.2.3 Analytical methods

The lignin contents in the precipitates, MWL and original wood were determined as the

combined yield of Klason lignin and acid-soluble lignin (Dence, 1992). The amounts of monosaccharides in the clear filtrate obtained after the lignin determination were also determined by high-performance anion-exchange chromatography (HPAEC: ICS-3000, Thermo Fisher Scientific, Massachusetts, United States) under the following conditions: column, CarboPac PA-1 (4 mm x 250 mm, Thermo Fisher Scientific, Massachusetts, United States); mobile phase, a gradient-programmed mixture of distilled water, 0.2 M NaOH and 1.0 M sodium acetate; flow-rate, 1.0 mL/min; column temperature, 35 °C; detector, electrochemical cell (Thermo Fisher Scientific, Massachusetts, United States).

For the chemical structural analysis, gel permeation chromatography (GPC), Fourier transform infrared spectroscopy (FT-IR) and alkaline nitrobenzene oxidation analysis were performed. Before GPC analysis, the precipitates and MWL were acetylated to facilitate the solubility (Gellerstedt, 1992). GPC was conducted with LC-10A (Shimadzu Co., Kyoto, Japan) under the following conditions: column, Shodex KF-801, KF-802, KF-802.5 and KF-803 (Showa Denko, Tokyo, Japan) connected in series; eluent, tetrahydrofuran; flow-rate, 0.6 mL/min; column temperature, 50 °C; detector, ultraviolet (UV) light at 205 nm. For the mass calibration, polystyrene standards (molecular weight (MW): 162, 580, 2960 and 5000) were used. FT-IR spectra were recorded using IRAffinity-1S (Shimadzu Co., Kyoto, Japan) by the KBr pellet method. Alkaline nitrobenzene oxidation was performed using the general method (Chen, 1992) and the obtained products were quantified by gas chromatography analysis using GC-14B (Shimadzu Co., Kyoto, Japan) after trimethylsilyl derivatization.

In addition, the contents of methoxyl and phenolic hydroxyl groups were determined by the hydroiodic acid method (Baker, 1996) and the periodate oxidation method (Lai, 1992), respectively, for the precipitates, MWL and original wood.

Two dimension-nuclear magnetic resonance (2D-NMR) were performed for both precipitates and MWL. The samples (100 mg) were dissolved in DMSO-*d*₆ (700 µm) and subjected to 2D-NMR analysis on a Bruker AC-400 (400 MHz) (Bruker Corp., Massachusetts, United States). Heteronuclear single quantum coherence (HSQC) experiments were acquired.

4.3 Results and discussion

4.3.1 Fractionation of Japanese beech as treated by semi-flow hot-compressed water

Table 4-1 shows the yield of the products obtained from Japanese beech as treated by semi-flow hot-compressed water (150~230 °C/10 MPa/25 min). After the treatment, the amount of the insoluble residue was 45.6 wt% on the original wood basis. Therefore, the rest of the part 54.4 (= 100 – 45.6) wt% is thought to be decomposed and liquefied in hot-compressed water as the water-soluble portion, some of which was precipitated to be 9.0 wt% on original wood basis after 12 h standing. The others as the rest of the water-soluble portion was 45.4 (= 54.4 – 9.0) wt%.

The lignin contents of these portions are also shown in Table 4-1. The original wood of Japanese beech contains 25.5 wt% lignin, about half of which (13.0 wt%) remained in the insoluble residue, while the water-soluble portion [12.5 (= 25.5 – 13.0) wt%] is composed of the precipitates (8.1 wt%) and others (water-soluble portion after 12 h standing) (4.4 wt%).

After all, the yield of the precipitates was 9.0 wt%, in which 8.1 wt% was lignin-derived products, thus, the lignin concentration of the precipitates is high to be 0.90 (= $8.1 / 9.0 \times 100$) g/g. In addition, the lignin-derived products in the precipitates correspond to 31.8 (= $8.1 / 25.5 \times 100$) wt% of the whole lignin in the original wood. The lignin-derived products in the precipitates are referred to hereinafter as “precipitated lignin”. Since the MWL prepared from Japanese beech was 8.8 wt% in yield on the whole lignin basis, the yield of the precipitated lignin (31.8 wt%) is about 3.6 times higher than that of the MWL.

Table 4-2 shows the chemical composition of the precipitates from Japanese beech. This was compared with the MWL from Japanese beech. The precipitates contain 90.0 wt% lignin-derived products, which is a sum of 81.0 wt% Klason lignin and 9.0 wt% acid-soluble lignin. The rest of 10.0 wt% is composed mainly of cellulose- and hemicelluloses-derived polysaccharides. In case of the MWL, however, the acid-soluble

Table 4-1 Yield and lignin content of the insoluble residue and water-soluble portion from Japanese beech as treated by semi-flow hot-compressed water.

Sample	Yield (wt% on wood basis)	Lignin content (wt% on wood basis)
Original wood	100.0	25.5
Insoluble residue	45.6	13.0
Water-soluble portion	54.4	12.5
Precipitates	9.0	8.1
Others	45.4	4.4

Table 4-2 Chemical composition of the precipitates and MWL from Japanese beech

Sample	Chemical composition (wt% on dried sample basis)			
	Lignin-derived products	Cellulose-derived		Hemicelluloses-derived
	Klason lignin	Acid-soluble lignin	saccharides ^{a)}	saccharides ^{a)}
Precipitates	81.0	9.0	3.9	4.4
MWL	84.6	3.4	1.0	4.7
				Others
				1.7
				6.3

a) Cellulose- and hemicelluloses-derived saccharides were estimated based on the amounts of monosaccharides in hydrolysate by Klason lignin determination.

fraction is very small compared with the precipitates, even though the lignin content is almost the same to be 88.0 (= 84.6 + 3.4) wt% as the precipitates to be 90.0 (= 81.0 + 9.0) wt%.

4.3.2 Characterization of the precipitated lignin

Fig. 4-1 shows the molecular weight distributions of the acetylated precipitates and MWL by GPC analysis measured at a wavelength of 205 nm. Since UV light was used as a detector, the GPC spectra should indicate the molecular weight distributions of the lignin-derived products. As a result, the precipitated lignin consists of relatively high molecular weight compounds ranged from a few hundred to thousands in polystyrene equivalent molecular weight. This might mean that the high molecular weight fraction in the water-soluble portion are aggregated and thus precipitated as kept at ambient conditions for 12 h. In comparison with the MWL, the molecular weight peak of the precipitated lignin is much lower than that of MWL.

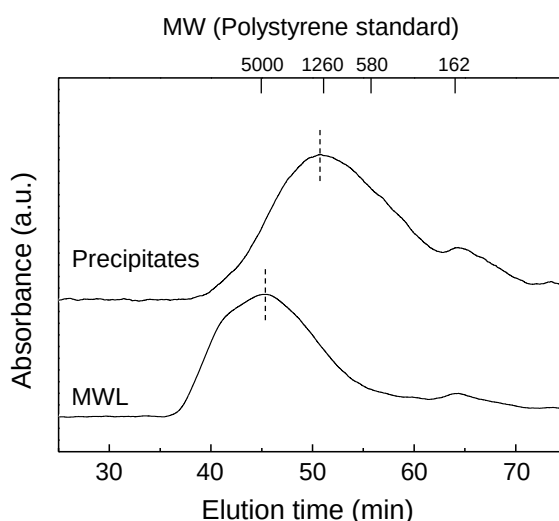


Fig. 4-1 GPC chromatograms of the UV light at 205 nm for the acetylated precipitates and MWL from Japanese beech.

For the chemical and spectroscopical analyses, FT-IR spectra were obtained as shown in Fig. 4-2, in which the peak assignment was done in accordance with the literatures (Faix, 1991; Faix and Beinoff, 1988). The precipitates and MWL have similar spectra, which are typical patterns of lignin. For example, peaks (*symbol a*) from aromatic ring at 1430, 1510 and 1600 cm^{-1} and ones (*symbol s*) from syringyl (S) lignin at 1120 and 1325 cm^{-1} are found in both spectra. In addition, a peak at 1730 cm^{-1} (*symbol c*) from carbonyl group is also observed in both spectra. Larsson and Miksche (1972) reported that the carbonyl content of MWL was increased by solvolysis during the preparation. Therefore, the carbonyl content in the precipitates is also considered to be increased through the hot-compressed water treatment. On the other hand, a significant difference can only be found at 1270 cm^{-1} (*symbol g*), which is derived from guaiacyl (G) lignin, indicating that the precipitates contain much less G lignin than the MWL. Since MWL is considered to be from all the morphological regions (Whiting and Goring, 1981), the presence of G lignin in MWL seems appropriate, whereas the precipitates containing higher S lignin suggest that the precipitated lignin is mainly originated from the fiber secondary wall.

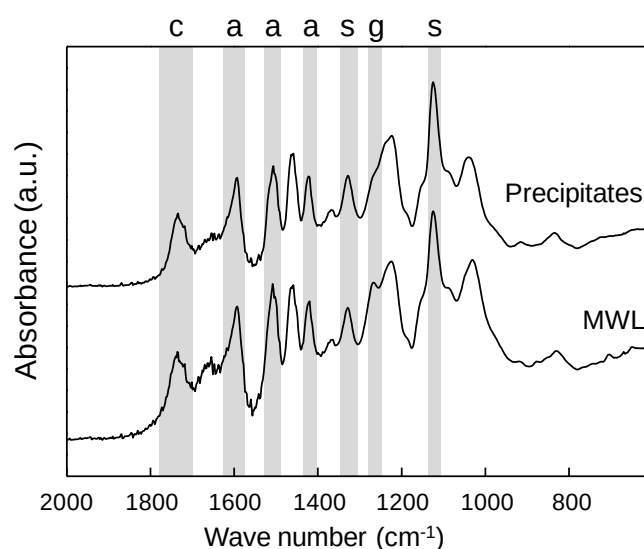


Fig. 4-2 FT-IR spectra of the precipitates and MWL from Japanese beech. For peak symbols, c; carbonyl group, a; aromatic ring, s; syringyl ring, g; guaiacyl ring.

Fig. 4-3 shows the yields of alkaline nitrobenzene oxidation products from the precipitates, MWL and original wood. In all cases, syringaldehyde and vanillin were main products from S and G lignins, respectively, and the ratios of syringaldehyde /vanillin (S/V) are also shown in parentheses. Compared with the original wood, the yield of the oxidation products from the MWL is slightly higher, while its S/V ratio is lower compared with the original wood. However, it can be said that these values are close to the original wood, even though they depend on the time of milling and extraction method in the MWL preparation (Chang et al., 1975; Lee et al., 1981). On the other hand, the yield of oxidation products from the precipitates (4.3 mmol/g of lignin) is quite higher than those from the MWL and original wood, and even higher than other S lignin-rich hardwoods such as Eucalyptus (dos Santos et al., 2014). This high yields could indicating that the precipitated lignin contains higher ether type linkages. Besides, its S/V ratio is higher than the MWL and original wood. Thus, it would contain more S lignin, the fact of which is a good agreement with a finding in Fig. 4-2.

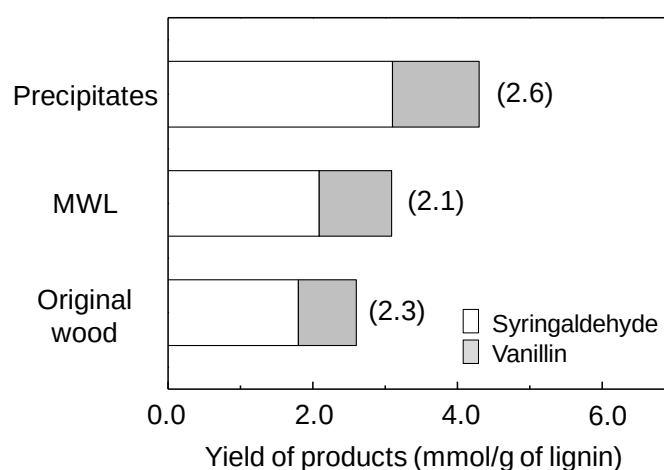


Fig. 4-3 The yield of alkaline nitrobenzene oxidation products from the precipitates, MWL and original wood from Japanese beech. The number in the parenthesis shows the S/V ratio.

In order to verify this result, the number of methoxyl groups per C₉ unit was obtained as in Table 4-3; the precipitated lignin contains higher methoxyl content than others. This also indicates clearly that it contains more S lignin.

Table 4-3 The number of methoxyl groups per C₉ unit of lignin in the precipitates, MWL and original wood from Japanese beech.

Sample	The number of methoxyl groups /C ₉ unit
Precipitates	1.62
MWL	1.38
Original wood	1.35

Based on these lines of evidence, it can be concluded that the precipitated lignin is richer in S lignin, while the MWL is almost the same as the original wood. From topochemical perspectives, S and G lignins are not uniformly distributed in the secondary wall and the compound middle lamella of hardwoods. In hardwood fibers, for example, lignin in the secondary wall is mainly composed of S lignin, whereas that in the compound middle lamella is of G lignin (Fergus and Goring, 1970a; Kirk et al., 1975; Saka and Goring, 1988). Furthermore, the authors elucidated in the previous study that lignin in the secondary wall was preferentially decomposed by the hot-compressed water treatment, while that in the middle lamella remained as the insoluble residue as described in *Chapter 3*. Therefore, the precipitated lignin would be derived mainly from the fiber secondary wall, while the MWL is from both the secondary wall and the compound middle lamella (Whiting and Goring 1981; Maurer and Fengel, 1992a, b; Terashima et al., 1992).

On the other hand, Fig. 4-4 shows the number of the phenolic hydroxyl groups per 100 C₉ units of lignin in the precipitates, MWL and original wood. Its number in the MWL is slightly higher than the original wood. In a similar manner to the carbonyl group,

the phenolic hydroxyl group was reported to be increased by solvolysis during the MWL preparation (Pew, 1957). Furthermore, the number of the phenolic hydroxyl groups is more increased in the precipitates as compared with the MWL. This increase would be caused by the preferential cleavage of ether type linkages in hot-compressed water (Ehara et al., 2002). From topochemical viewpoints, as Yang and Goring reported that the number of phenolic hydroxyl groups of the secondary wall and the compound middle lamella are 12.0 and 6.0 per 100 C₉ units, respectively (Yang and Goring, 1980). From this reason, the precipitated lignin is expected to contain much phenolic hydroxyl groups that would be mainly derived from the fiber secondary wall.

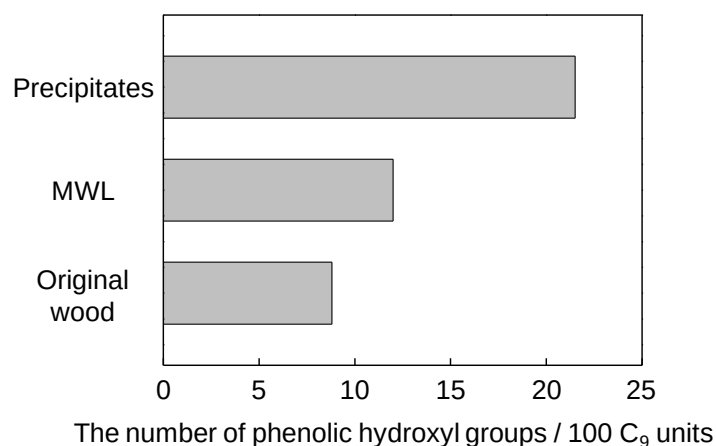


Fig. 4-4 The number of phenolic hydroxyl groups per 100 C₉ units of lignin in the precipitates, MWL and original wood from Japanese beech.

For further investigation of the chemical structures, the precipitates and MWL were subjected to 2D hetero-nuclear single quantum coherence (HSQC) NMR analysis. Their aromatic and aliphatic regions were shown in Fig. 4-5 and Fig. 4-6, respectively. The assignment of each signal is based on the literature (Miyagawa 2015).

In the aromatic regions as shown in Fig. 4-5, both the precipitates and MWL contained G and S ring-derived signals. As compared to MWL, in the precipitates, S ring-

derived signals seems to be larger than G ring-derived signals. This tendency is in good agreement with the results of FT-IR and alkaline nitrobenzene oxidation.

In the aliphatic regions as shown in Fig. 4-6, both spectra include typical lignin linkages such as β -O-4, β - β and β -5 types. This indicate that the chemical structure of the precipitated lignin are relatively similar to that of MWL, which is also in good agreement with FT-IR spectra.

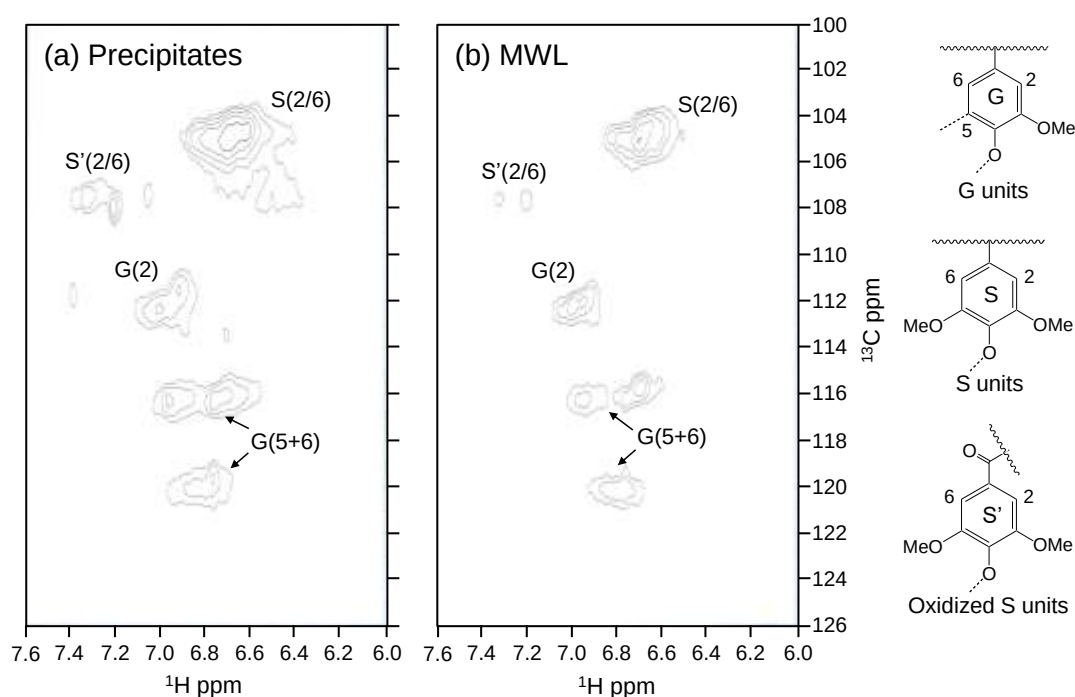


Fig. 4-5 Aromatic regions of HSQC spectra of (a) precipitates and (b) MWL in DMSO- d_6

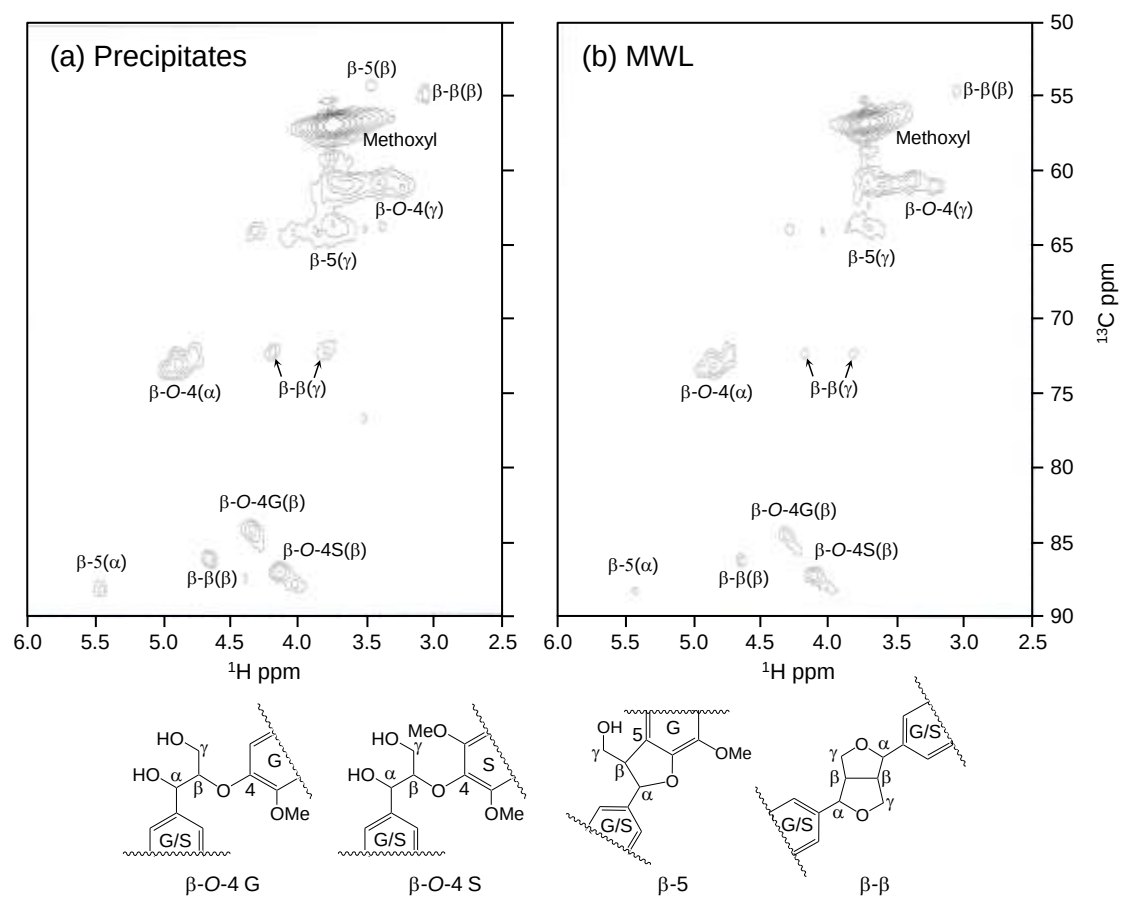


Fig. 4-6 Aliphatic regions of HSQC spectra of (a) precipitates and (b) MWL in DMSO- d_6

4.4 Summary

The precipitates were prepared by the semi-flow hot-compressed water treatment of Japanese beech (*Fagus crenata*) at 150~230 °C/10 MPa totally for 25 min. The yield of the precipitates was 9.0 wt% on original dried wood basis, and the lignin concentration was 0.90 g/g. It corresponds to about 1/3 of the whole lignin in Japanese beech. Such precipitated lignin was characterized and compared with MWL.

GPC analysis indicated that the precipitated lignin consisted of relatively high molecular weight compounds ranged from a few hundred to thousands. From FT-IR analysis, the precipitates were similar to the MWL but significant difference observed was richer in S lignin than the MWL and original wood. In addition, the precipitated lignin contained more ether linkages of lignin with more syringyl lignin moiety, and more phenolic hydroxyl groups. From such evidence, it would indicate that the precipitated lignin is derived mainly from the fiber secondary wall, while the MWL is from both the secondary wall and compound middle lamella (Whiting and Goring, 1981; Maurer and Fengel, 1992a, b; Terashima et al., 1992).

The high amount of ether type linkages and phenolic hydroxyl groups present in the precipitated lignin would be due to the relatively low temperature and short residence time of the semi-flow hot-compressed water treatment, which can suppress excessive degradations and condensation reactions on lignin-derived products. Thus, the semi-flow hot-compressed water treatment can offer an efficient method to isolate lignin in the fiber secondary wall.

Chapter 5

Decomposition Behaviors of Corn Cob by Hydrothermal Treatment

5.1 Introduction

Corn (*Zea mays*) is the most produced crops in the world with the yield of 968.9 million tonnes in 2015-2016 (World Agricultural Production, 2016). As a result of the utilization of corn grain, a large quantity of corn cob was by-produced. Based on the production quantity of corn grain, that of corn cob was estimated under the following three assumptions: 1) the moisture content in the corn grain is 15.5 grain-based wt% (Zych, 2008); 2) the ratio by weight between the corn grain and corn stover is 1.0 (Graham et al., 2007); 3) the weight proportion of corn cob to corn stover is 20 wt% (Sokhansanj et al., 2002). Based on these assumptions, the global annual production of corn cob in 2015-2016 was estimated to be 163.7 million tonnes, showing the potential of corn cob as a resource for the production of energy and chemicals.

In fact, as to its utilization, a variety of researches have been conducted to produce chemical products such as xylitol (Latif and Rajoka, 2001; Ling et al., 2011), ethanol (Beall et al., 1992; Lawford and Rousseau, 1992; Chen et al., 2007; Liu et al., 2010; Chen et al., 2010; Eylon et al., 2011) and cellulose nanofibers (Kumar et al., 2010; Shogren et al., 2011; Li et al., 2014). Moreover, it was reported that corn cob has already been practically used as a feedstock for bioethanol production in China since 2013 (Clever et al., 2014).

In the previous chapters, the decomposition behaviors of softwoods as gymnosperms and hardwoods as dicotyledonous angiosperms by hydrothermal treatment were discussed. Since the corn is classified into monocotyledonous angiosperms, its decomposition behaviors were speculated to be different from those of woody biomass.

Thus, in this chapter, the hot-compressed water treatment was applied for the corn cob in order to gain better insight into the whole major plant species.

5.2 Materials and methods

5.2.1 Samples and chemicals

Corn cob used in this study was harvested in Langfang city, Habei province, China. After drying the corn cob, it was pulverized with a Wiley mill (Thomas Scientific, New Jersey, United States) and afterwards sieved to obtain particles with diameter between a range of 0.15 and 1.0 mm. All of the chemicals used in this study were of reagent grade without purification.

5.2.2 Chemical composition analysis

Quantitative determination for chemical composition of corn cob was conducted. Fig. 5-1 shows the quantitative approaches applied in this study, which were based on the methodology proposed by Rabemanolontsoa et al. (2011).

The corn cob sample powder was extracted with acetone in a Soxhlet apparatus. The amount of extractives was measured by comparing the dried sample weights before and after the extraction. Inorganics were quantitated by incinerating corn cob in a muffle furnace at 600 °C for 4 h. Holocellulose was quantitatively determined with a modified Wise method (Wise et al., 1946). Cellulose was then determined by measuring the insoluble portion left after the holocellulose had been treated with 17.5 % aqueous sodium hydroxide (Kuroda, 2000). Lignin was measured with a modified Klason method (Dence 1992). Perchloric acid method was used to measure the amount of starch (Humphreys and Kelly, 1961). As to the determination of protein, Kjeldahl nitrogen method was used and

the nitrogen factor was set at 6.25 with Super kjel 1400 (ACTAC Project Services Corp., Tokyo, Japan) (Thiex et al., 2002).

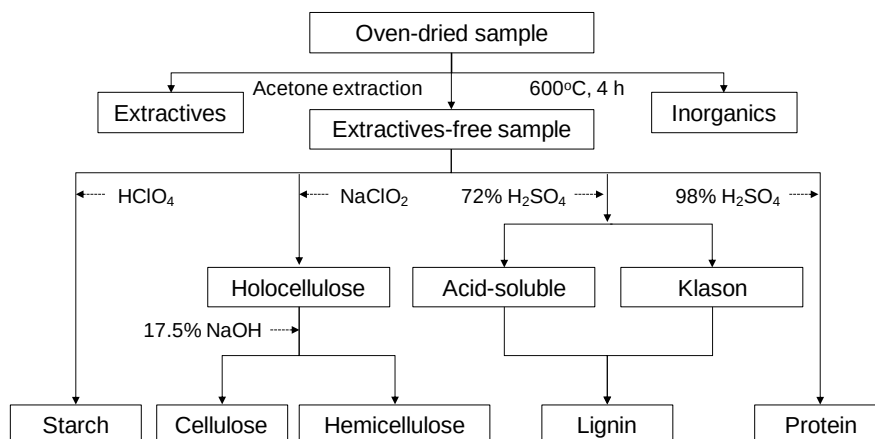


Fig. 5-1 Methods employed for chemical composition determination for corn cob
(Rabemanolontsoa et al., 2011)

5.2.3 Hot-compressed water treatment

Half a gram of corn cob powder was placed in a reaction vessel for which the two-step hot-compressed water treatment was performed as described in 2.2.2. Two-step hot-compressed water treatment condition was 230 °C/10 MPa/15 min for the 1st stage and 270 °C/10 MPa/30 min for the 2nd stage. Prior to heating up to start the hot-compressed water treatment, cold-water extraction was carried out at the condition of 20 °C/10 MPa for 30 min. After the hot-compressed water treatment, the insoluble residue left over in the reaction vessel was separated from hot-compressed water-soluble portion and dried at 105 °C for 12 h to measure its oven-dried weight.

5.2.4 Analytical methods of hot-compressed water-soluble portion

Prior to the analyses, the water-soluble portion was filtered with a membrane filter (pore size: 0.45 μm). Then, high-performance liquid chromatography (HPLC), high performance anion-exchange chromatography (HPAEC) and gas chromatography-mass spectrometry (GC-MS) were used to conduct a series of analyses.

The HPLC system (LC-10A, Shimadzu Co., Kyoto, Japan) was equipped with a refractive index detector/ultraviolet-visible (UV-Vis) detector. The columns used were KS-801 (Showa Denko, Tokyo, Japan), Ultron PS-80P (Shimadzu Co., Kyoto, Japan) and HPX-87H (Bio-Rad Laboratories, Inc., California, United States). Column KS-801 was used to detect 5-hydroxymethylfurfural (5-HMF) and furfural. The eluent was distilled water, the flow-rate was set at 1.0 mL/min and the oven temperature at 80 °C. Column Ultron PS-80P was for the detection of levoglucosan and fragmented compounds. The eluent was also distilled water and the other parameters were set at the same condition as KS-801. Column HPX-87H was used to detect organic acids, uronic acids and methanol. The 5 mM sulfuric acid was used as the eluent at a flow-rate of 0.6 mL/min and the oven temperature was set at 45 °C. To detect the lignin-derived products in water-soluble portion in every minute, the UV absorbance of the water-soluble portion was analyzed with the same HPLC system without using a column. The UV wavelength was set at 210 nm to detect only lignin-derived products.

Dionex ICS-3000 system (Thermo Fisher Scientific, Massachusetts, United States) was used for HPAEC to detect saccharides-derived products. Before the HPAEC analysis, the water-soluble portion was post-hydrolyzed with 4 wt% sulfuric acid to convert oligosaccharides into monosaccharides. The obtained monosaccharides were then analyzed with HPAEC. The detailed analysis condition is described in 2.2.3.

GC-MS (GCMS-QP 2010 Ultra, Shimadzu Co., Kyoto, Japan) analysis was performed to identify and quantitatively determine the lignin-derived monomeric products in the water-soluble portion. The detailed analysis condition is described in 2.2.3.

5.3 Results and discussion

5.3.1 Chemical composition

Table 5-1 shows the chemical composition of corn cob. It can be seen that totally 98.9 wt% of the corn cob was successfully quantified. Among the cell wall components, the content of hemicelluloses is the highest to be 41.0 wt%, while cellulose is the second highest component which occupies 32.1 wt% and lignin content 18.0 wt% being the sum of the Klason lignin (14.3 wt%) and acid-soluble lignin (3.7 wt%). As in hardwoods, the acid-soluble lignin in corn cob is relatively higher. Regarding the minor components, inorganics, protein, starch and extractives account for 3.3 wt%, 2.2 wt%, 1.5 wt% and 0.8 wt%, respectively.

Table 5-1 Chemical composition of corn cob

Component	Chemical composition (wt% on oven-dried corn cob basis)
Cellulose	32.1
Hemicelluloses	41.0
Lignin	18.0
Klason lignin	14.3
Acid-soluble lignin	3.7
Inorganics	3.3
Protein	2.2
Starch	1.5
Extractives	0.8
Total	98.9

5.3.2 Analysis of the hot-compressed water-soluble portion

Table 5-2 shows the summative yields of products from corn cob as extracted by cold-water followed by semi-flow hot-compressed water treatment. The 98.8 wt% of corn cob was hydrolyzed in the hot-compressed water treatment, and only 1.2 wt% remained as the insoluble residue. The amount of insoluble residue is quite low compared with other lignocellulosics such as Japanese cedar (13 wt%) and Japanese beech (7 wt%) as shown in 3.3.1.

As to the decomposed products, derivatives from hemicelluloses, cellulose and lignin were identified and quantitatively determined. Totally, 82.9 wt% of the obtained products were successfully identified, which were respectively collected from cold-water extraction (3.1 wt%), 1st stage treatment (55.0 wt%) and 2nd stage treatment (24.8 wt%). The detailed analyses of these decomposed products are described in Table 5-2.

5.3.2.1 Free sugars and inorganics in cold-water extractives

Fig. 5-2 shows the temperature profile and the saccharides yields throughout the whole treatment. Since oligosaccharides cannot be analyzed directly, the hot-compressed water-soluble portion was post-hydrolyzed with 4 wt% of sulfuric acid to convert oligosaccharides into monosaccharides. The cold-water extraction (20 °C/10 MPa/30 min) is from -30 min to 0 min in the linear graph. It can be seen that a considerable amount of glucose and fructose were recovered at the very beginning after the cold-water extraction was conducted. Furthermore, according to the HPAEC analysis of water-soluble portion without post-hydrolysis, little sucrose were detected in water-soluble portion.

Table 5-2 Summative yields of products from corn cob as extracted by cold-water (20 °C/10 MPa/30 min) followed by two-step semi-flow hot-compressed water treatment at 230 °C/10 MPa/15 min and 270 °C/10 MPa/30 min

Products	Yield (wt% on oven-dried corn cob basis)			
	Cold-water extraction	1st stage	2nd stage	Total
Free sugars	1.1	-	-	1.1
From hemicelluloses				
Σ	-	33.5	0.3	33.8
Xylo-saccharides	-	27.2	0.3	27.5
Arabinose	-	4.4	-	4.4
Acetic acid	-	0.2	-	0.2
Glucuronic acid	-	0.2	-	0.2
Methanol	-	0.1	-	0.1
Galactose	-	1.3	-	1.3
Rhamnose	-	0.1	-	0.1
From cellulose				
Cello-saccharides	-	3.7	16.3	20.0
From lignin				
Σ	-	15.2	2.0	17.2
Coniferyl alcohol	-	0.05	0.003	0.05
Sinapyl alcohol	-	0.14	0.006	0.14
<i>p</i> -Coumaryl alcohol	-	0.003	-	0.003
Ferulic acid	-	0.01	0.01	0.02
<i>p</i> -Coumaric acid	-	0.46	0.06	0.52
Dimeric, trimeric and oligomeric products	-	14.5	1.9	16.4
Dehydrated compounds				
Σ	-	0.2	2.4	2.6
Levogluconan	-	0.01	0.13	0.14
5-HMF	-	0.05	1.74	1.79
Furfural	-	0.13	0.54	0.67
Fragmented compounds				
Σ	-	2.2	2.8	5.0
Methylglyoxal	-	0.7	0.2	0.9
Glycolaldehyde	-	1.5	0.6	2.1
Erythrose	-	-	2.0	2.0
Organic acids				
Σ	-	0.03	0.9	0.9
Lactic acid	-	0.01	-	0.01
Glycolic acid	-	0.02	0.9	0.9
Inorganics	2.0	0.9	0.1	3.0
Total	3.1	55.0	24.8	83.6
Unknown				15.2
Insoluble residue				1.2

As to the inorganics, 2.0 wt% of inorganics eluted in the cold-water extraction, which accounts for 60.6 ($= 2.0 / 3.3 \times 100$) % of the total recovered inorganics. As to the rest of the inorganic contents, 27.3 ($= 0.9 / 3.3 \times 100$) % was eluted in 1st stage and 3.0 ($= 0.1 / 3.3 \times 100$) % was in 2nd stage of the hot-compressed water treatment. Consequently, the rest 9.1 ($= 0.3 / 3.3 \times 100$) % is considered to be left in the residue after the treatment.

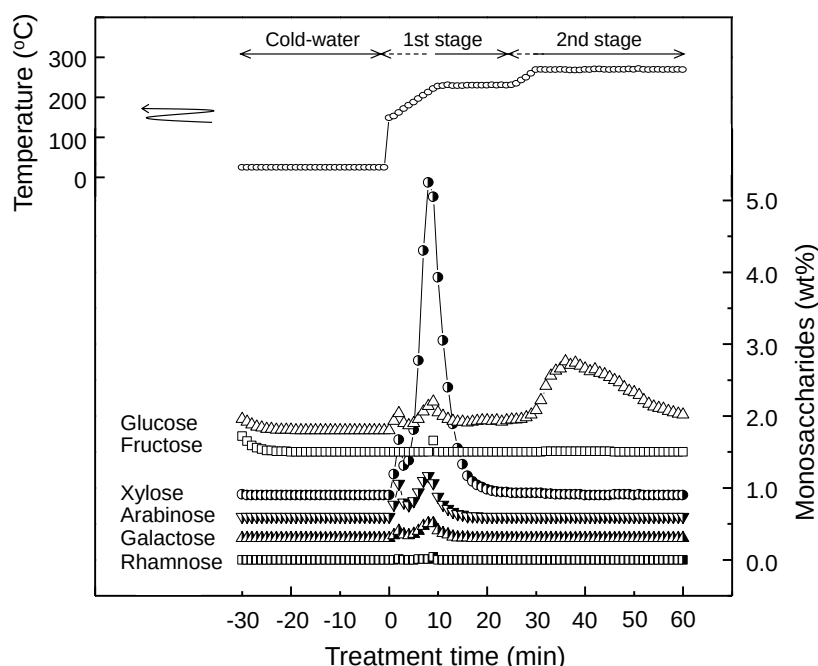


Fig. 5-2 Monosaccharides obtained by post-hydrolysis on water-soluble portion from corn cob as treated by cold-water at 20 °C/10 MPa/30 min followed by two-step hot-compressed water at 230 °C/10 MPa/15 min and 270 °C/10 MPa/30 min

5.3.2.2 Hydrolysis of major cell wall components

As treated by two-step semi-flow hot-compressed water (1st stage: 230 °C/10 MPa/15 min, 2nd stage: 270 °C/10 MPa/30 min), 98.8 wt% of the initial corn cob was decomposed and dissolved into water, while 1.2 wt% remained as insoluble residue, which is mainly

composed of lignin (0.8 wt%) and inorganics (0.3 wt%).

Fig. 5-2 demonstrates the yield changes of monosaccharides obtained by post-hydrolysis in the water-soluble portion from corn cob as treated by two-step hot-compressed water. During the 1st stage treatment (230 °C/10 MPa/15 min), xylose, galactose, arabinose, rhamnose, glucose and fructose were all obtained. The detailed amounts of the monosaccharides are shown in Table 5-2; xylose, arabinose, galactose and rhamnose were quantitatively determined to be 27.2, 4.4, 1.3 and 0.1 wt%, respectively. In addition, acetic acid, glucuronic acid and methanol were also retrieved during the 1st stage. The detailed structure of hemicelluloses in corn cob was not identified yet, while considering that the main hemicelluloses of corn stover is *O*-acetyl-4-*O*-methyl-glucuronoarabinoxylan (Naran et al., 2009), these obtained products would be derived from *O*-acetyl-4-*O*-methyl-glucuronoarabinoxylan.

Glucose is found to be produced in both 1st and 2nd stages of hot-compressed water treatment. Since there is no glucomannan in hemicelluloses of corn cob, the glucose should be derived from cellulose. Since the treating condition of the 1st stage is not expected to decompose crystalline cellulose, the glucose appeared in the 1st stage is probably derived from para-crystalline cellulose.

Fig. 5-3 shows the changes of UV absorbance of water-soluble portion from corn cob during the semi-flow hot-compressed water treatment. Compared to saccharides, the lignin-derived products can absorb UV light due to its aromatic structure. Thus, this graph should show the changes of the relative concentration of lignin-derived products in every minute. As a result, the UV absorbance rapidly increased until 10 min, where the treatment temperature achieved at 230 °C. Then, the UV absorbance gradually decreased as the treatment was prolonged. When it came to the point of 25 min, when the 1st stage treatment ended, the UV absorbance decreased to a fairly low level and basically kept decreasing slowly and steadily thereafter. Accordingly, corn cob lignin was mainly decomposed in the 1st stage, especially during the temperature rising up to 230 °C.

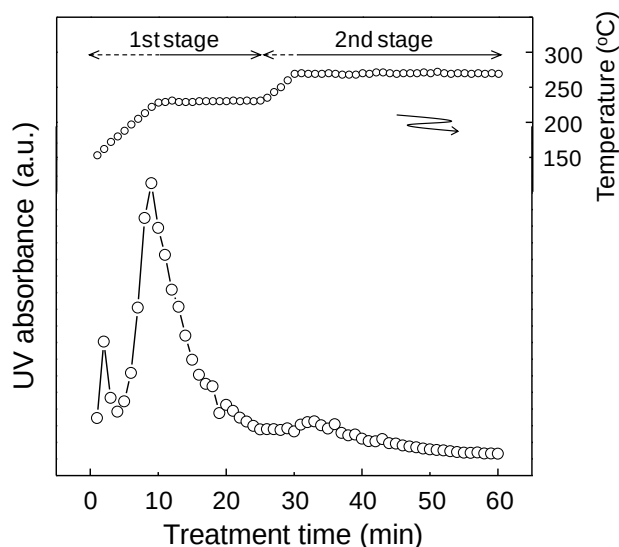


Fig. 5-3 Relative UV absorbance of water-soluble portion from corn cob as treated by two-step semi-flow hot-compressed water at 230 °C/10 MPa/15 min and 270 °C/10 MPa/30 min ($\lambda = 210$ nm)

In terms of the lignin-derived products, sinapyl alcohol from syringyl propane (S), coniferyl alcohol from guaiacyl propane (G), and *p*-coumaryl alcohol from *p*-hydroxyphenyl propane (H) units were all retrieved mainly during the 1st stage treatment as shown in Table 5-2. In addition, *p*-coumaric acid was also obtained. It was reported that *p*-coumaric acid is associated with lignin in monocotyledons, comprising nearly 20 % of lignin in corn stover (Ralph et al., 1994). As a characteristic component connecting hemicelluloses with lignin in the form of ester and ether linkages in monocotyledonous plants (Higuchi et al., 1967), ferulic acid was obtained as well. Some other lignin-derived monomers were detected, whereas their yields were quite small. Besides the lignin-derived monomeric products discussed above, the amounts of dimeric, trimeric and oligomeric products were estimated by subtracting the amount of monomeric products out of the total water-soluble lignin. Thus, it was found that most of the lignin-derived products were dimers, trimers or oligomers. Consequently, 95.6 ($= 17.2 / 18.0 \times 100$) % of lignin was decomposed and dissolved into hot-compressed water and only 4.4 (0.8 /

18.0 × 100) % remained as residual lignin. The delignification yield (95.6 %) was much higher than those of Japanese cedar (65 %) and Japanese beech (77 %) (Table 3-1).

5.3.2.3 Degradation products from hemicelluloses and cellulose

As the hot-compressed water treatment progressed, the hydrolyzed products from saccharides were further dehydrated and fragmented (Kruse et al., 2007).

Fig. 5-4 shows the yields of dehydrated compounds which are furfural, 5-hydroxymethylfurfural (5-HMF) and levoglucosan. Basically, it is regarded that furfural is derived from the dehydration of pentoses by elimination of 3 mol of water, while 5-HMF and levoglucosan are produced when hexoses such as glucose, galactose and rhamnose get further dehydrated (Antal et al., 1991; Bobleter, 1994; Kabyemela et al., 1999).

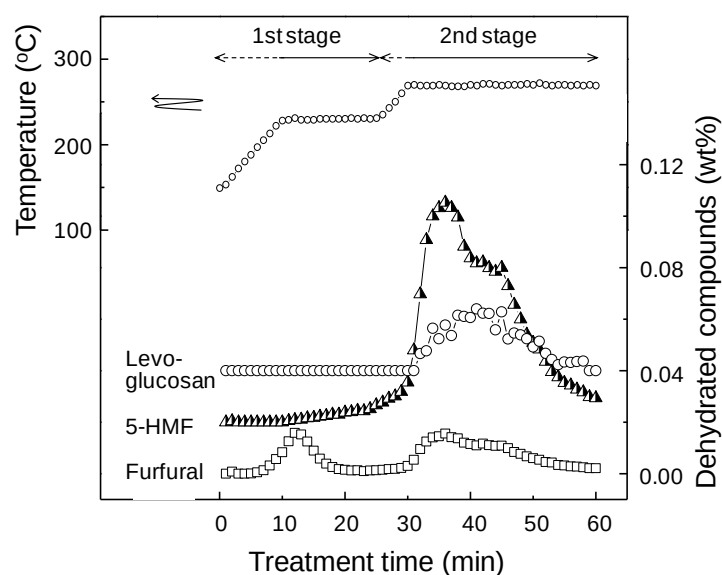


Fig. 5-4 Yields of dehydrated compounds from corn cob as treated by two-step semi-flow hot-compressed water at 230 °C/10 MPa/15 min and 270 °C/10 MPa/30 min

Based on Table 5-2, a considerable amount of furfural was detected in the 1st stage treatment. The furfural would be produced from xylose, which is the main monosaccharides from hemicelluloses in corn cob. However, a part of furfural was also recovered in the 2nd stage treatment. Similar phenomenon occurred in the cases of Japanese cedar and Japanese beech (Lu et al., 2009; Phaiboonsilpa et al., 2010). These lines of findings indicate that furfural can also be produced from hexoses such as glucose under the condition of hot-compressed water treatment. The way in which furfural is produced without pentose is considered to be the five-carbon ketose pathway (Kullury et al., 1986).

In regards to the production of 5-HMF and levoglucosan, most of them were collected in the 2nd stage treatment where crystalline cellulose was decomposed under a more severe condition. Nonetheless, a small amount of them was also detected in the 1st stage. They are considered to be derived from the para-crystalline cellulose which could be decomposed at a milder condition.

Fig. 5-5 shows the yields of fragmented compounds. Methylglyoxal and glycolaldehyde were detected in both the 1st and 2nd stages, whereas erythrose only appeared in the 2nd stage. The reactions occurring in the 1st stage were probably as follows: pentoses such as xylose and arabinose from hemicelluloses were first decomposed into glycolaldehyde and glyceraldehyde, and then glyceraldehyde was further dehydrated into methylglyoxal. These reactions were observed in the glyceraldehyde pathway of hexose fragmentation (Kebyemela et al., 1997a).

In the 2nd stage, a considerable amount of erythrose was also recovered. The reactions occurred in the 2nd stage were considered as follows: glycolaldehyde and erythrose were formed via retro-aldol condensation in the glycolaldehyde/erythrose pathway (Kabyemela et al., 1997b, 1999). Methylglyoxal was produced from hexoses such as glucose and galactose, through the glyceraldehydes/dihydroxyacetone pathway in the hexane fragmentation (Kebyemela et al., 1999; Watanabe et al., 2005).

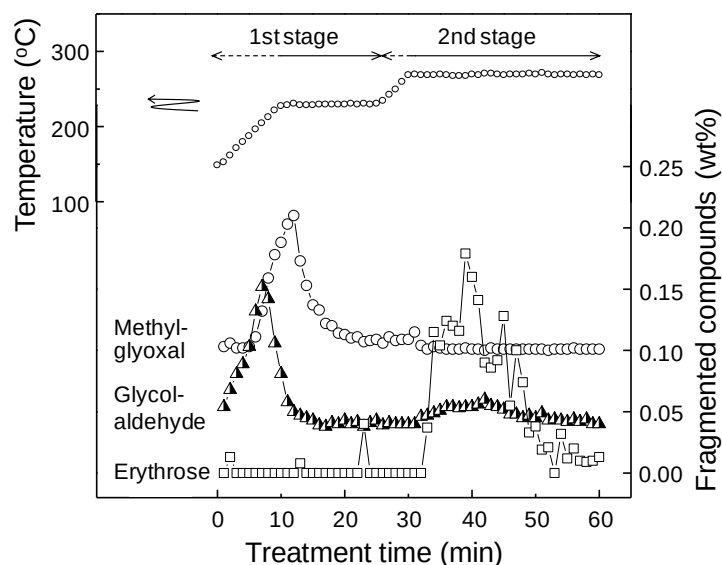


Fig. 5-5 Yields of fragmented compounds from corn cob as treated by two-step semi-flow hot-compressed water at 230 °C/10 MPa/15 min and 270 °C/10 MPa/30 min

5.3.2.4 Production of organic acids

Fig. 5-6 shows the yields of organic acids which are acetic acid, lactic acid and glycolic acid. Organic acids are regarded as the further decomposition products from dehydrated and fragmented compounds (Kabyemela et al., 1997a). Among them, the acetic acid would be derived from acetyl residue associated with hemicelluloses, and the other acids would be derived from saccharides or lignin-derived products.

According to the previous work (Yoshida et al., 2009), when dehydrated compounds such as furfural and 5-HMF, and fragmented compounds such as methylglyoxal and glycolaldehyde were treated with supercritical water (380 °C/25 MPa/240 s), the amount of organic acids produced from the fragmented compounds was far more than that produced from the dehydrated compounds. Accordingly, the organic acids would be mainly derived from fragmented compounds.

It was also reported that under the supercritical water treatment (380 °C/25 MPa/240

s), organic acids can be produced from lignin through the decomposition of the propyl side-chains on phenylpropane units, rather than the aromatic moiety (Yoshida et al., 2005). However, based on the observation of the lignin-derived products in the hot-compressed water-soluble portion with GC-MS analysis, most of the identified lignin monomers maintained propyl side-chains, due probably to the relatively milder treating conditions. Thus, it is likely that the organic acids would be mainly derived from fragmented compounds instead of the propyl side-chains.

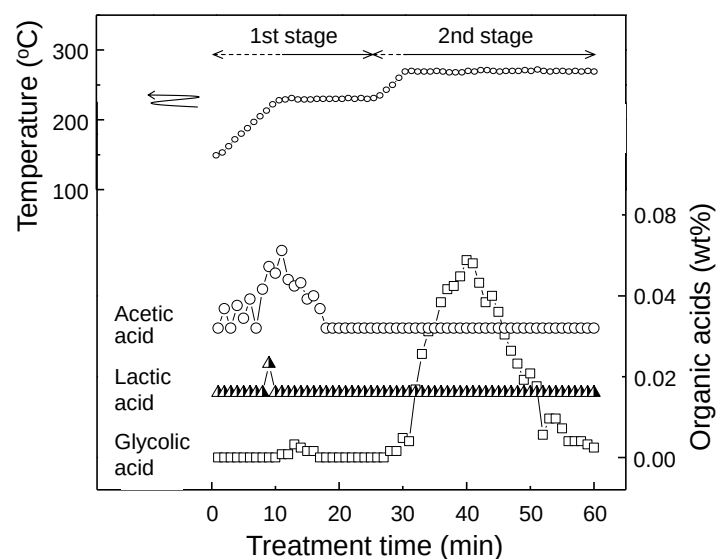


Fig. 5-6 Yields of organic acids from corn cob as treated by two-step semi-flow hot-compressed water at 230 °C/10 MPa/15 min and 270 °C/10 MPa/30 min

5.4 Summary

A two-step hydrolysis was applied on the corn cob and the characterization of the decomposed products was performed.

On the whole, 98.8 wt% of the corn cob was decomposed into water-soluble portion and only 1.2 wt% remained as the insoluble residue.

During the cold-water extraction (20 °C/10 MPa/30 min) prior to the hot-compressed water treatment, a considerable amount of free sugars (1.1 wt%) and inorganics (2.0 wt%) were recovered.

In the 1st stage (230 °C/10 MPa/15 min), most of the hemicelluloses were decomposed into water-soluble products such as xylose, arabinose, galactose, rhamnose, acetic acid, glucuronic acid and methanol. These products were considered to be derived from *O*-acetyl-4-*O*-methyl-glucuronoarabinoxylan. Moreover, lignin was mainly decomposed and dissolved in water during the 1st stage. As the lignin-derived products, sinapyl alcohol, coniferyl alcohol and *p*-coumaryl alcohol were all obtained. In addition, *p*-coumaric acid and ferulic acid which were not obtained in either Japanese cedar or Japanese beech, were also recovered.

In the 2nd stage (270 °C/10 MPa/30 min), the crystalline cellulose and a part of lignin were decomposed. Furthermore, due to the more severe condition, dehydrated compounds, fragmented compounds and organic acids were also recovered.

The yields of water-soluble portion from corn cob turned out to be much higher compared with Japanese cedar and Japanese beech. Notably, the delignification in corn cob was more progressed compared to Japanese cedar and Japanese beech.

Based on these lines of evidence, it was found that the hot-compressed water is a powerful medium to decompose corn cob. Also, the obtained information is very useful and important for the efficient utilization of various kinds of lignocellulosics for the production of biochemicals and biofuels.

Chapter 6

Comparative Study on Topochemistry of Delignification from Japanese Beech as Treated by Subcritical Water and Subcritical Phenol

6.1 Introduction

Lignin as the second most abundant macromolecule in terrestrial plants (on the same order of magnitude as hemicelluloses) is an important potential source of energy and value-added aromatic chemicals. There are many degradation methods for isolation and utilization of lignins. Among them, supercritical and subcritical fluid technologies are attractive approaches for the conversion of biomass into chemicals with lower molecular masses. With this approach, the dielectric constant and ionic products, which are important parameters in a reaction, can be controlled by the parameters of temperature and pressure. In supercritical water, the dielectric constants of the products are decreasing and their ionic products are increasing at higher severity of the treatment (Bröll et al., 1999; Savage, 1999). Lower dielectric constants lead to a better solubility of some hydrophobic compounds in supercritical and subcritical water, which can decompose lignin (Erdocia et al., 2014; Liu and Wyman, 2003; Tirtowidjojo et al., 1988; Zhang et al., 2008). For example, treatment of Japanese beech (*Fagus crenata*) in a semi-flow reactor system with subcritical water at 230~270 °C led to the decomposition of around 80 wt% lignin (Table 3-1) In addition, other woods could also be delignified with the same approach (Ogura et al., 2013; Phaiboonsilpa et al., 2013, 2011).

The supercritical and subcritical treatment processes of woody biomass were investigated in a variety of solvents. Yamazaki et al. treated Japanese beech with straight-chain alcohols under subcritical conditions at 270 °C/10 MPa and it was demonstrated that the lignin became liquefied in all alcohols tested (Yamazaki et al., 2006).

Supercritical and subcritical phenols were also investigated in this context (Mishra and Saka, 2013, 2011a, 2011b). Phenol is a good solvent for the organosolv pulping in the presence of a catalyst (e.g. HCl) (April et al., 1979; Brauns and Hibbert, 1933; Kang et al., 2012; Kratzl et al., 1962; Saisu et al., 2003; Sano and Sasaya, 1985; Schweers and Rechy, 1973; Schweers, 1974; Vega and Bao, 1992; Zhang et al., 2006). Cresol is an also effective solvent in the presence of a catalyst (Funaoka and Abe, 1989; Sakakibara et al., 1984; Sano et al., 1989a, 1989b; Takagi et al., 1989). However, it was also found that subcritical phenol does not need a catalyst for delignification (Lee and Ohkita, 2003) and phenol-liquefied woods are well suited for mixing with phenol-formaldehyde resins for various applications (Lee et al., 2012, 2014).

Mishra and Saka treated Japanese beech with phenol, anisole, *m*-cresol, catechol and 2,4-dimethylphenol under subcritical condition at 270 °C and found that the ability of these reactants for wood liquefaction is dependent on the number of phenolic hydroxyl groups, but phenol was the best compound for selective lignin decomposition (Mishra and Saka, 2012). However, some aspects of the delignification behavior of wood under supercritical conditions remain unclear, for example, the influence of the topochemistry of lignin distribution in plants on the delignification processes is not well-investigated.

The aim of this chapter is, therefore, the observation of the delignification behavior of Japanese beech (*Fagus crenata*) in subcritical water and subcritical phenol under consideration of topochemical details. The results were observed by ultraviolet (UV) microscopy and UV microspectrophotometry (UMSP) and alkaline nitrobenzene oxidation.

6.2 Materials and methods

6.2.1 Wood samples and chemicals

The sapwood of Japanese beech (*Fagus crenata*) was milled in a Wiley mill (Thomas

Scientific, New Jersey, United States) and extracted with acetone for 8 h. Approximately 150 mg of extractive-free wood flour and 4.9 mL of water or phenol were placed in a 5.0 mL reaction vessel made of Inconel-625. The vessel was then sealed and immersed in a molten salt bath, which was preheated to 230 °C. After an adequate reaction time, the reaction vessel was quenched in a water bath. The resulting reaction mixture was then filtered through a 0.45 µm membrane filter (Merck Millipore Ltd., Massachusetts, United States) to give an insoluble residue and a soluble portion. The insoluble residue was then washed with water and methanol, and dried at 105 °C for 12 h to measure its oven-dried weight. All chemicals used in this study were of reagent grade without any purification, purchased from Nacalai Tesque, Inc., Kyoto, Japan.

6.2.2 Analytical methods for water- and phenol-insoluble residues

The lignin-derived products in each fraction were examined in terms of their combined yield of Klason lignin and acid-soluble lignin (Dence, 1992). The acid-soluble portions were analyzed by high performance anion exchange chromatography (HPAEC; Dionex ICS-3000 system: Nippon Dionex, Osaka, Japan) for monosaccharides' analysis. The contents of cellulose and hemicelluloses were estimated based on the amounts of glucose and the other monosaccharides, respectively. The HPAEC was equipped with the CarboPac PA-1 column (4 mm × 250 mm) and EC detector for pulsed amperometric detection; 35 °C and He flow rate of 1.0 mL/min under atmospheric pressure. The mobile phase was a gradient-programmed mixture of distilled water, 0.2 M NaOH, and 1.0 M sodium acetate.

Alkaline nitrobenzene oxidation analysis was performed according to Chen, 1992 and the total yields of vanillin and syringaldehyde were obtained by gas chromatography (GC; GC-2014, Shimadzu Co., Kyoto, Japan).

The distribution of residual lignin was observed by UV microscopy according to the procedure as described in 3.2.2. The residues were embedded in epoxy resin, and the

samples were cut into 0.5 μm thick sections with a diamond knife mounted on a Leica Reichert Supernova Microtome (Illinois, United States). The sections were placed on quartz slides, mounted with glycerin and covered with quartz coverslip before examination by MSP-800 system (Carl Zeiss, Oberkochen, Germany) at 280 ± 5 nm. The morphological regions of the objects were analyzed on a UMSP based on photometric point-by-point measurements (spot size: $1 \times 1 \mu\text{m}^2$). The change of UV absorbance at 240 nm was observed. The measurements were repeated at least three times on each morphological region.

6.2.3 Analytical methods for water- and phenol-soluble products

The water-soluble products obtained in subcritical water (230 $^{\circ}\text{C}$ /2.9 MPa/10 min) were obtained by evaporation under vacuum. In contrast, the phenol-soluble products obtained under the subcritical phenol treatment (230 $^{\circ}\text{C}$ /1.2 MPa/30 min) were separated by precipitation from the unreacted free phenol with a large excess of diethyl ether, which is an appropriate anti-solvent (Mishra and Saka, 2012). The water- and phenol-soluble products obtained were dried in an oven at 105 $^{\circ}\text{C}$ overnight, and their lignin contents were determined. The methoxyl contents of the lignin were analyzed by a standard method (Baker, 1996).

6.3 Results and discussion

6.3.1 Delignification in subcritical water and subcritical phenol

The chemical compositions of the insoluble residues are presented in Fig. 6-1. The supercritical treatment processes in water and phenol were both performed at 230 $^{\circ}\text{C}$ with reaction pressures of 2.9 MPa and 1.2 MPa, respectively, because of the differences in

their densities in the batch-type reaction vessel. Given that the critical points of phenol and water are 374 °C/22.1 MPa and 421 °C/6.1 MPa, respectively, the solvents existed in their subcritical states under the conditions listed above (Ambrose, 1963).

During the subcritical water treatment, around half of lignin in the Japanese beech is remaining after 10 min. It is noteworthy that no more delignification occurred beyond this point, with half of the lignin remaining as an insoluble residue after 30 min. However, in the subcritical phenol treatment, the lignin gradually decomposed and was subsequently removed in the phenol solvent, with very little lignin remaining as an insoluble residue after 30 min. In terms of the hemicelluloses, very little decomposition or removal was observed in subcritical phenol, whereas most of the hemicelluloses were removed following only 2 min of subcritical water treatment. Notably, neither of these two processes had any effect on the level of cellulose. Thus, the crystalline structure of cellulose is highly resistant to the subcritical water and phenol treatments at 230 °C.

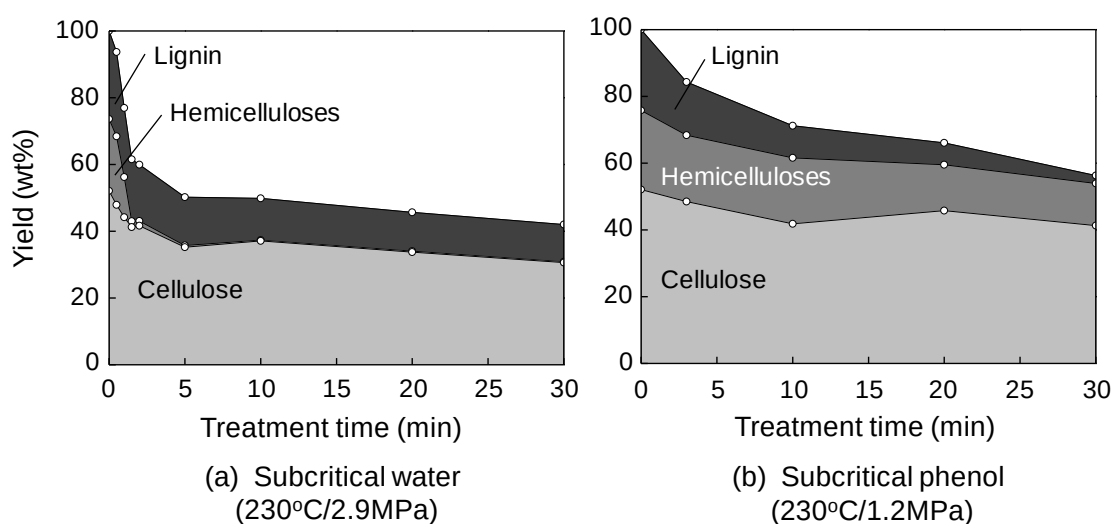


Fig. 6-1 Yields of the insoluble residues obtained from Japanese beech under (a) subcritical water (230 °C/2.9 MPa) and (b) subcritical phenol (230 °C/1.2 MPa) conditions

Table 6-1 Residue yields, chemical compositions and delignification rates from Japanese beech as treated by subcritical water and subcritical phenol

Treatment	Treatment time (min)	Residue yield (wt% on wood basis)	Chemical composition (wt% on wood basis)			Delignification rate (% on lignin basis)
			Cellulose	Hemicelluloses	Lignin	
Subcritical water	0	100.0	51.9	23.7	24.4	0.0
	0.5	93.7	49.3	21.0	23.4	4.1
	1	76.9	45.7	12.0	19.2	21.3
	1.5	61.5	42.7	1.7	17.1	29.9
	2	59.9	42.9	1.4	15.6	36.1
	5	50.2	37.5	0.5	12.2	50.0
	10	49.8	37.9	0.4	11.5	52.9
Subcritical phenol	20	45.7	33.7	0.3	11.6	52.3
	30	42.0	30.6	0.2	11.2	54.1
	0	100.0	51.9	23.7	24.4	0.0
	2	91.7	51.0	21.7	19.0	22.1
	3	84.3	48.5	19.8	16.0	34.4
	5	75.9	46.2	18.4	11.3	53.7
	10	71.3	42.8	18.8	9.7	60.2
	20	66.1	45.8	13.6	6.7	72.5
	30	56.3	41.3	12.7	2.3	90.6

The yields of the residue, the chemical components, and delignification rates of the insoluble residues are presented in Table 6-1. The delignification rate was calculated as: $\text{Delignification (\%)} = (L_{\text{ini}} (\%) - L_{\text{res}} (\%)) / L_{\text{ini}} \times 100 (\%)$, where L_{ini} and L_{res} are the lignin contents in the initial stage and in the residue, respectively.

Concerning the subcritical water treatment, the delignification rates were 36.1 and 52.9 % after 2 min and 10 min treatment times, respectively. Notably, no further delignification was observed after 10 min. For the subcritical phenol treatment, the lignin content decreased with increasing treatment time. Furthermore, the calculated delignification rates showed that 34.4, 60.2, and 90.6 % of the lignin was removed from the wood after 3, 10, and 30 min treatment times, respectively. These results demonstrate the higher delignification efficiency of subcritical phenol.

6.3.2 UV microscopy of the insoluble residues

UV micrographs are presented in Fig. 6-2, where the darker regions in the images indicate higher lignin concentrations. It is clearly seen how large parts of the secondary wall are destroyed. In the course of subcritical water treatment, the secondary wall of fibers and vessels were partly compressed after 2 min with a delignification rate of 36.1 %. However, in the subcritical phenol treatment, the structure of the secondary wall layer remained unchanged in the first 3 min with a delignification rate of 34.4 %. Prolonged phenol treatment led to compression and to lower levels of UV absorbance after 10 min with a delignification rate of 60.2 %. In contrast, the secondary wall remained after 10 min under the subcritical water conditions with a delignification rate of 52.9 %, although the cells were partly detached. Under these conditions, the compound middle lamella seems to contain more lignin than the secondary wall and the delignification process slowed down and stopped. However, under subcritical phenol conditions the delignification reached 90.6 % after 30 min, while the secondary wall still remained. Interestingly, the compound middle lamella was nearly completely delignified with only traces of lignin left in the

corner region of the cell wall.

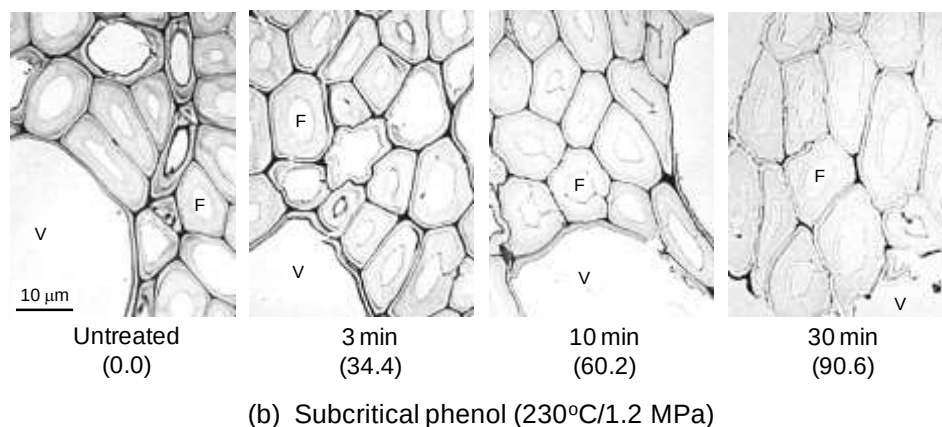
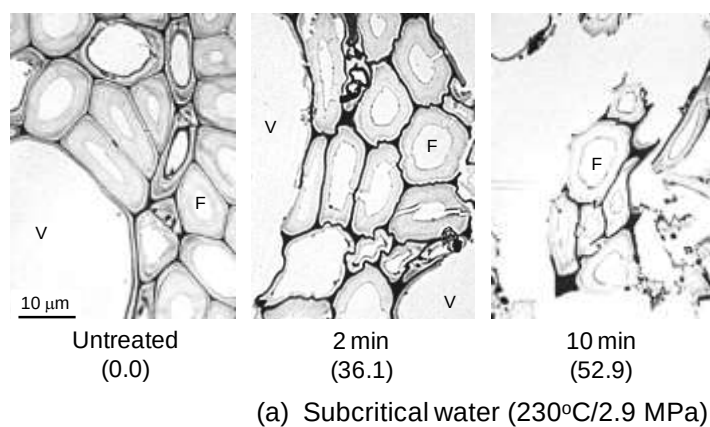


Fig. 6-2 UV micrographs taken at a wavelength of 280 nm of the insoluble residues obtained from Japanese beech by (a) subcritical water (230 °C/2.9 MPa) and (b) subcritical phenol (230 °C/1.2 MPa). The values in parenthesis indicate the rate of delignification of each sample. (F; Fiber, V; Vessel)

As known, hardwood lignin is composed of guaiacyl (G) and syringyl (S) propane units, and the ratio G/S may vary from one morphological region to another (Saka and Goring, 1988). The UV absorption coefficients of G and S lignins are different at 280 nm, while these coefficients of G and S lignins are quite similar at 240 nm (Aulin-erdtman and Sanden, 1968; Fergus and Goring, 1970a). Therefore, the UV absorbances were measured in the different morphological regions at 240 nm (Fig. 6-3). Assuming the same thicknesses of the thin section cuts, the UV absorbances are proportional to the lignin concentration according to the Lambert-Beer law. In Japanese beech, the distribution of G and S lignins is not uniform within an annual ring (Takabe et al., 1992). To consider this effect, the latewood terminal zone was not included into the UMSP analysis.

For the subcritical water treatment, the relative lignin absorbance in the secondary wall decreased rapidly compared to that of compound middle lamella. Accordingly, delignification occurs preferentially in the secondary wall, especially in that of the fibers, but the lignin in the lignin concentration of the compound middle lamella remained almost constant. This explains why the delignification rate did not exceed 55 % (Fig. 6-3). For the subcritical phenol treatment, the delignification occurs preferentially in the secondary wall, especially in that of the fibers. This tendency is also known from several other delignification processes (Saka et al., 1982b; Whiting et al., 1981). The UV absorbance decrement of the compound middle lamella finally stopped after 30 min (Table 6-1).

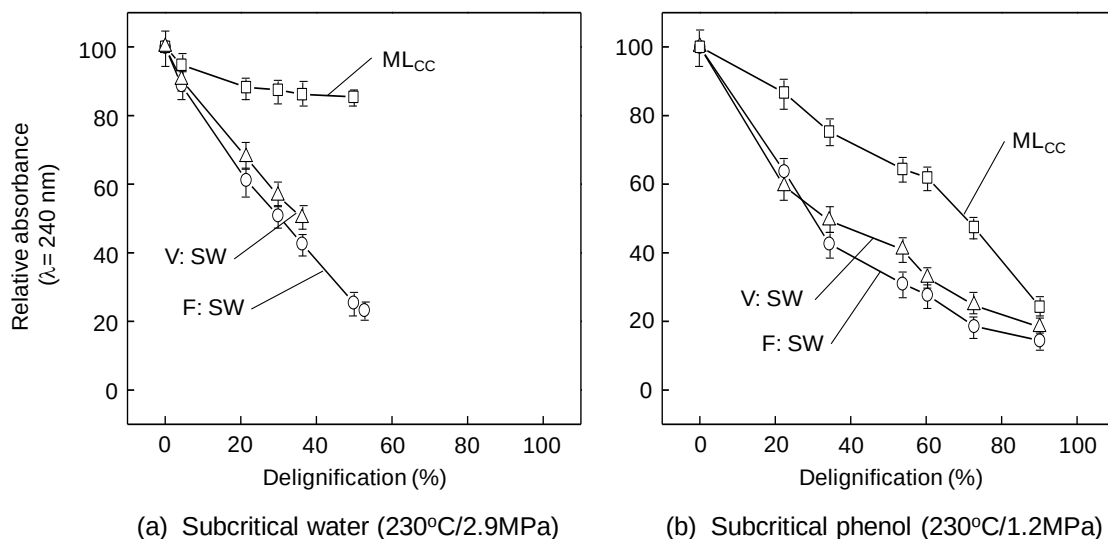


Fig. 6-3 Relative UV absorbance properties of the three morphological regions of Japanese beech treated by (a) subcritical water (230 °C/2.9 MPa) and (b) subcritical phenol (230 °C/1.2 MPa) at a wavelength of 240 nm.

F; Fiber, V; Vessel; SW; Secondary wall, ML_{CC}; cell corner middle lamella

6.3.3 Alkaline nitrobenzene oxidation analysis of insoluble residues

The ether type linkages (e.g. β -O-4 linkage) in lignin are dominant over the condensed type linkages (e.g. 5-5' linkage). The former are more easily susceptible to degradation processes as demonstrated by Ehara et al. (2002) based on dimeric model compounds with β -O-4 and 5-5' type linkages treated with subcritical water (330 °C/50 MPa/10 s). In contrast, the condensed type linkages show a considerable resistance under these conditions.

The alkaline nitrobenzene oxidation was performed to estimate the condensed and non-condensed moieties of the residual lignins left after both treatments (Fig. 6-4) as it is known that the nitrobenzene oxidation products are from the ether linkages. Expectedly, vanillin and syringaldehyde are the main products for the G and S lignin units,

respectively. The product yields decreased with increasing treatment times. Accordingly, ether linkages are preferably split under both treatment conditions. This is especially true for the subcritical water treatment.

The syringaldehyde/vanillin (S/V) ratios in the range of 2.1~2.3 are constant during the subcritical phenol treatment. However, the ratios are decreasing from 2.2 to 1.6 in the course of subcritical water treatment, which is interpreted that S ether type lignin units are preferentially decomposed. As known, the secondary wall of fibers is mainly composed of S units, while the compound middle lamella consists mainly of G units (Fergus and Goring, 1970b; Saka and Goring, 1988). Thus the S/V ratios in subcritical phenol treatment reflect the delignification of both secondary wall and compound middle lamella, but the subcritical water seems to delignify mainly the secondary wall only. This corresponds to the UV microscopic observations. One of the conclusions is that subcritical phenol is more able to react with condensed type lignin units.

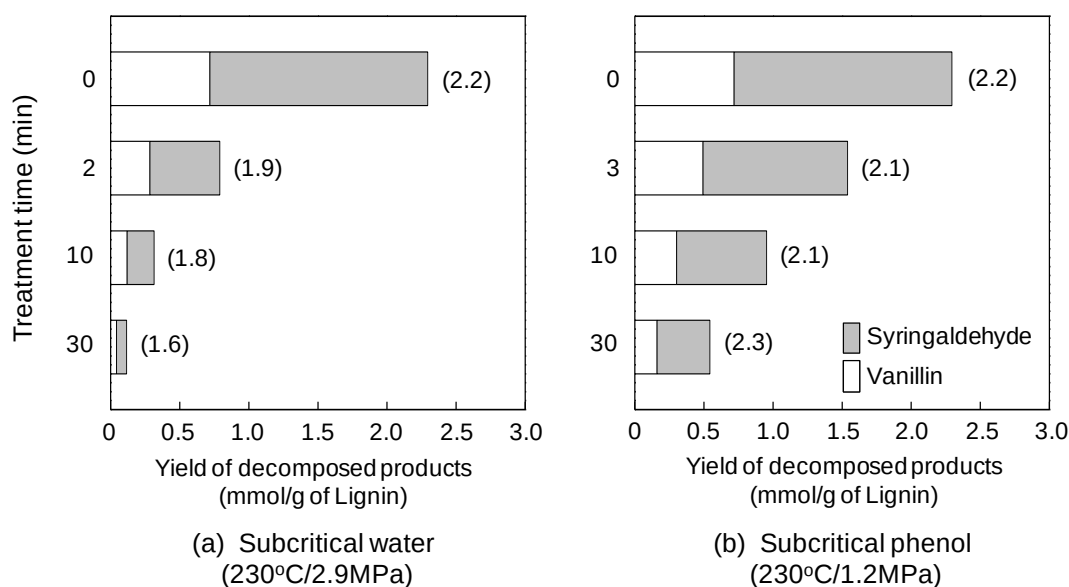


Fig. 6-4 Yields of the alkaline nitrobenzene oxidation reactions for the insoluble residues of the (a) subcritical water (230 °C/2.9 MPa) and (b) subcritical phenol (230 °C/1.2 MPa) treatments. The syringaldehyde/vanillin (S/V) ratios of obtained products are also shown.

6.3.4 Analysis of water-soluble and phenol-soluble products

The water-soluble products were obtained by simply evaporation of the solvents water and methanol. To allow for the separation of the phenol-soluble products from the free phenol as solvent, the solution was precipitated in a large excess of diethyl ether. The yields of the obtained products are listed in Table 6-2. In the subcritical water treatment, 81.2 ($= (40.8/50.2) \times 100$) wt% of the water-soluble portion was collected after evaporation, with 76.7 ($= (9.9/12.9) \times 100$) wt% yield based on lignin. In the case of the subcritical phenol, 71.6 ($= (31.3/43.7) \times 100$) wt% of the phenol-soluble portion was collected as precipitates, with 65.6 ($= (14.5/22.1) \times 100$) wt% yield based on lignin.

Table 6-2 Yield, lignin content and methoxyl content of the residues obtained by precipitation and solvent evaporation.

Material	Yield (wt%)	Lignin content (wt%)	Methoxyl group/C ₉
Subcritical water			
Water-soluble	50.2 ^{a)}	12.9	-
Evaporation residue	40.8	9.9	1.42
Subcritical phenol			
Phenol-soluble	43.7 ^{a)}	22.1	-
Precipitated	31.3	14.5	0.84
Untreated wood	100	24.4	1.35

^{a)} Calculated by subtracting the yield from residue of initial wood.

The methoxyl contents of the collected lignins (Table 6-2) are presented based on phenylpropane (C₉) units. The methoxyl content in case of precipitated phenol-soluble lignin was 0.84/C₉, which is essentially lower than that of the untreated wood lignin (1.35/C₉) and that of water-soluble lignin (1.42/C₉). For a hardwood lignin, the methoxyl

content 0.84/C₉ is very low, which can only be explained by phenolation in the course of the phenol treatment. In fact, some phenol-added dimeric lignin-derived products were detected by GC-MS. Lin et al. also demonstrated the phenolation of lignin model compounds under subcritical phenol treatment conditions (Lin et al., 1997a, 1997b).

The precipitated phenol-soluble lignin was dissolved in seven different solvents, including ethyl acetate (dielectric constant $\epsilon = 6.0$), tetrahydrofuran (7.5), phenol (9.8), pyridine (12.3), acetone (21), methanol (33) and water (80), as illustrated in Fig. 6-5. High molecular weight compounds were only found among the precipitated phenol-soluble products indicating their high affinity to phenol. In summary, treatment with subcritical phenol leads to degradation and phenolation and the phenolated products are well soluble in phenol. In contrast, lignin in the compound middle lamella is highly resistant to subcritical water.

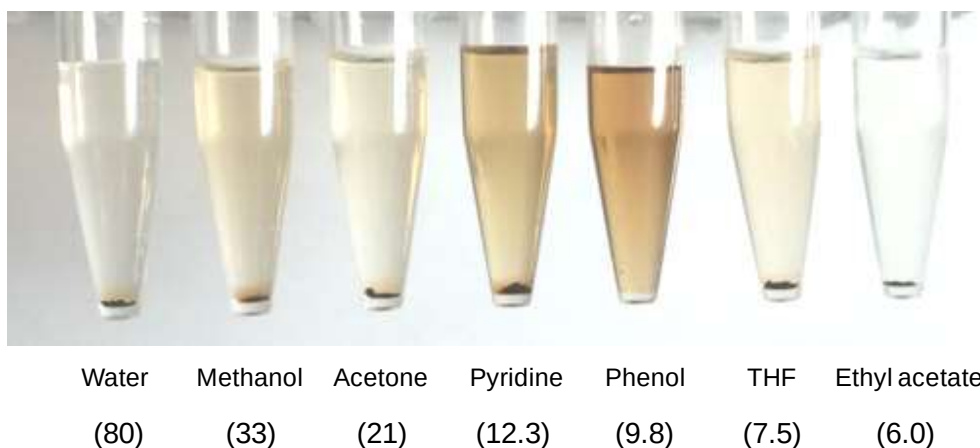


Fig. 6-5 Solubility of the phenol-soluble products in various solvents. The values in parenthesis indicate the dielectric constants of the associated solvents under ambient conditions.

6.4 Concluding remarks

Based on the preferential cleavage of the ether type lignin linkages, the lignin in the secondary wall of fibers and vessels are easily delignified in both subcritical water and subcritical phenol. Lignin in the compound middle lamella is more resistant to subcritical water than subcritical phenol. As indicated in the literature, lignin is simultaneously phenolated and decomposed in subcritical phenol, resulting in the solvation of the products. The lignin in compound middle lamella is rich in condensed type linkages, and thus, was more easily liquefied in subcritical phenol than in subcritical water. Although the condensation reactions cannot be ignored during subcritical treatments with water and phenol, the differences in the course of both the treatments are due more likely to the intrinsic characteristics of lignin in various morphological regions and the properties of solvents.

Chapter 7

Concluding Remarks

7.1 Conclusions

The decomposition behaviors of lignocellulosics by the hydrothermal treatment were studied. To elucidate the decomposition behaviors of lignin, the author approached them in two ways: 1) characterization of the obtained lignin-derived products, 2) discussion of delignification from a topochemical viewpoint.

First of all, in *Chapter 2*, the decomposed products from Japanese cedar as treated by two-step hot-compressed water were fractionated into three fractions, which are water-soluble portion, precipitates and insoluble residue, and the lignin-derived products in three fractions were analyzed. For the water-soluble portion, some lignin-derived monomers and dimers, which contained methoxyl and phenolic hydroxyl groups, were detected. For the precipitates, the lignin-derived products contained high ratio of ether type linkages, indicating the possibility that the precipitated lignin maintained the structure of the original lignin. On the other hand, for the insoluble residue, the residual lignin was rich in condensed type linkages. This would be due to the high resistance of the condensed type linkages against the hydrothermal treatment.

In *Chapter 3*, the topochemistry of the delignification was discussed for Japanese cedar as one of the softwoods and Japanese beech as one of the hardwoods, whose cell wall components and lignin distributions are different. As a result, for both species, the delignification was preferentially occurred from the secondary wall compared to the compound middle lamella. This result was supported by the methoxyl contents analysis of residual lignin. Furthermore, the residual lignin was rich in condensed type lignin, indicating that the lignin in the compound middle lamella would be rich in condensed type linkages. Such information must reveal the importance of topochemistry in the

clarification of decomposition behaviors of lignin.

In order to explore the utilization possibility of the obtained lignin-derived products, the author focused on the precipitated lignin among the obtained lignin-derived products by hydrothermal treatment. In *Chapter 4*, the precipitated lignin from Japanese beech was characterized and compared with the milled wood lignin (MWL). According to their structural analyses, their structures are relatively similar. However, the precipitated lignin contained higher syringyl (S) lignin as compared to the MWL. This would be because the precipitated lignin is derived mainly from the secondary wall of fiber due to the richness of S lignin in the fiber secondary wall. Furthermore, the precipitated lignin contained higher contents of phenolic hydroxyl groups and ether type linkages compared to MWL, which should increase the potential of the precipitated lignin to be utilized as the value-added aromatic materials.

In the previous chapters, the decomposition behaviors of woods by hydrothermal treatment were discussed. In *Chapter 5*, the hydrothermal treatment was applied on the corn cob, which is one of the angiosperms monocotyledons, in order to gain better insight into the whole species. As a result, 98.8 wt% of corn cob was decomposed and dissolved into hot-compressed water. Among the decomposed products, more than 80 wt% were quantitatively determined. As to the lignin, 95.6 % of the initial lignin was decomposed and dissolved into hot-compressed water. In the water-soluble portion, both monolignols derived from all of the G, S and H lignins and cinnamic acids were obtained.

Finally, in *Chapter 6*, for the further understanding of the delignification behaviors, phenol was applied for the subcritical treatment of the woody biomass for its high ability to delignify lignocellulosics. Then, the delignification behaviors of Japanese beech as treated by subcritical water and subcritical phenol were compared from a topochemical viewpoint. As a result, based on the preferential cleavage of ether type linkages in lignin, the lignin in the secondary wall of the fiber and vessel were easily delignified in both subcritical water and subcritical phenol. Lignin in the compound middle lamella showed resistance to subcritical water, while it was delignified in subcritical phenol. Through the structural analyses of water- and phenol-soluble portions, it was found that the differences

would be due to the influence of solvolysis.

In summary, these studies show that hydrothermal treatment of lignocellulosics can be expected as a new technology for the efficient utilization of lignocellulosics. Furthermore, the topochemical discussion elucidates the topochemistry of lignin when considering the chemical conversions of lignocellulosics into energy and chemicals.

7.2 Prospects for the future research

As described in *Chapter 1*, lignocellulosics can be classified into gymnosperms, angiosperms monocotyledons and angiosperms dicotyledons, and their morphological structures are different depending on the taxonomy. In this study, the topochemistry of delignification from Japanese cedar (one of gymnosperms) and Japanese beech (one of angiosperms dicotyledons) were discussed. In the future, the topochemical discussion of delignification behaviors from various angiosperm monocotyledonous plants are expected to be discussed. In addition, since the distribution of lignin in monocotyledonous plants still remains unclear, the information obtained from the hydrothermal treatment provides a clue as to elucidating of the structures and distributions of lignin in monocotyledonous plants.

Topochemical discussions elucidated the importance of topochemistry in the hydrothermal treatment. Therefore, topochemical study is expected to be a good researching approach and should be applied for different decomposition treatments such as other supercritical and subcritical solvent treatments. Furthermore, not only lignin but also hemicelluloses are not uniformly distributed within the cell wall. Thus, the decomposition behaviors of hemicelluloses are also expected to be discussed from a topochemical viewpoint.

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List of Publications

Original papers

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