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Influence of Hydrothermal Treatment on Hemicellulose Structure in *Cryptomeria japonica*

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The influence of hydrothermal treatment (HTT) on the hemicellulose, *i.e.* glucomannan/galactoglucomannan (GM/GGM) and arabinoglucurono-xylan (AGX) in Japanese cedar was examined using mild temperatures (95 °C and 120 °C) for its kiln drying. Based on infrared spectra, only a slight change due to HTT was observed in the hemicellulose chemical structure. The quantitative changes of the major chemical components including lignin, cellulose, and hemicellulose also exhibited slight differences among samples. However, the molecular chain-length composition of GM/GGM and AGX in the cell walls decreased clearly with HTT, as well as with an increase in temperature. Also, it was found that the strength of the interaction between cellulose and hemicellulose molecules such as GM/GGM and AGX in cell walls decreased depending on the increase in HTT temperature. These results showed that the polymeric structure of GM/GGM and AGX was degraded by hydrolysis at 95 °C of HTT and proceeded further at 120 °C of HTT.

INTRODUCTION

Wood is a promising biomass resource that can be reproduced by natural growth and is useful for both structural and non-structural materials. Japanese cedar (*Cryptomeria japonica* D. Don) is one of the most important softwood species in Japan, which occupies 58% volume of domestic roundwood supply in 2019 (Annual Report on Forest and Forestry in Japan, 2021). Its large dimension square lumber has been used for structures of low-rise residential buildings, and recently its bundled lumber has been used for structures of mid-to-high-rise and non-residential. To supply quality-controlled timber with guaranteed moisture content, various kiln drying methods have been proposed. One of the common methods in wood industry is "high-temperature setting drying," consisting of the three processes; steaming at 95 °C, high-temperature treatment at 120 °C, and medium or low temperature treatment below 90 °C, which suppresses surface checks through thermal softening and relaxation of drying stress (Obataya *et al*. 2006; Toba *et al*. 2021; Jiang *et al*. 2022).

Hydrothermal treatment (HTT) of wood is a promising method to improve the durability, decay resistance, and dimensional stability of wood materials within the temperature range from 150 to 230 °C by adjusting its moisture content (Hillis *et al.* 1984;

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Kamdem *et al.* 2002; Tjeerdsma and Militz 2005; Sandberg *et al.* 2013). Recently, HTT processes at different temperatures have been used for the modification of wood properties (Todaro *et al.* 2012). However, physical and chemical changes in wood materials at lower temperatures of 90 to 130 °C used for the kiln drying process are not well known. While HTT can alter the chemical and physical properties of the wood materials, it is of concern that it may reduce mechanical properties (*e.g.*, brittleness) and lead to coloration of wood due to the decomposition of a cell wall structure and chemical components of cell walls such as polysaccharides (Bekhta and Niemz 2003; Obataya *et al.* 2006; Kubojima *et al.* 2015; Endo *et al.* 2016). In particular, the changes in cell wall structure are believed to affect mainly the decomposition of the hemicellulose structure in cell walls with HTT at a higher temperature. In addition, it has been suggested that hemicellulose is susceptible to heat treatment at high temperatures due to its amorphous and hydrophilic properties compared with the other main components in the cell walls (Werner *et al.* 2014; Wang *et al.* 2020).

Hemicellulose in softwood species is composed of heteropolysaccharides, including glucomannan (GM), galacotglucomannan (GGM), and arabinoglucuronoxylan (AGX). GM makes up about 70% of hemicellulose in Japanese cedar and plays an important role as a binding agent between the molecules of cellulose microfibrils and lignin in the formation of the wood cell walls (Terashima *et al.* 2009; Kim *et al.* 2010). According to a previous study, hemicellulose has an essential role in the assembly of cell wall structure, including aggregation of cellulose microfibrils via weak bonds (*e.g.*, hydrogen bonds and electrostatic interactions) between cellulose and hemicellulose molecules (Terashima *et al.* 2009). Previously, researchers used quantification and composition analysis to investigate the effect of thermal treatment on hemicellulose chemical structure (Altgen *et al.* 2018; Wang *et al.* 2019). Based on pyrolysis-gas chromatography-mass spectrometry analysis of wood materials, the production of oligomers or monosaccharides derived from hemicellulose in cell walls were mainly detected under HTT conditions (Romagnoli *et al.* 2018; Andersone *et al.* 2019). However, the investigations in hemicellulose caused by HTT have been limited to the quantification and composition analysis of oligomers or monosaccharides (Garrote *et al.* 1999; Altgen *et al.* 2018). To our knowledge, there has been no study on the changes in hemicellulose polymeric structure and interactions between hemicellulose molecules or hemicellulose, cellulose molecules, and other cell wall components under the HTT process of Japanese cedar.

The aim of this study is to investigate the effect of HTT at mild temperatures (95 and 120 °C) on hemicellulose structure, including GM/GGM and AGX in the wood cell walls. First, the changes of color parameters and chemical components between untreated (as a control) and HTT samples were examined by spectrophotometer and Fourier transform infrared spectroscopy (FTIR) for the degreased wood powders with ethanol: toluene. Then, the amount of lignin was determined by the Klason method, and the cellulose and hemicellulose content were analyzed by high-performance liquid chromatography (HPLC). Next, to elucidate the effect of HTT on hemicellulose chemical structure, AGX and GM/GGM were extracted from the wood cell walls of each wood sample using an alkaline solution. The sugar compositions and molecular-weight distribution of GM/GGM and AGX were then analyzed by HPLC and size exclusion chromatography (SEC). The authors also evaluated the strength of the interaction between the main cell wall components by using the combination of alkaline solution and delignification method. This work, therefore, provides a significant contribution to the effect of the HTT on the hemicellulose structure in the wood cell walls of Japanese cedar.

EXPERIMENTAL

Wood Materials

A Japanese cedar (70-year-old, named Ayasugi) grown in a forest environment in Kumamoto, Japan was harvested. A log of 2.0 m in length was taken at 0.6 to 2.6 m above the ground. A board including a pith zone with a thickness of 60 mm was sawn and air-dried indoors. A Wiley mill was used to prepare sapwood from 42 to 68 annual rings for the wood powder (40–80 mesh).

Methods

HTT of wood samples

Two sets of the wood powder were placed in stainless-steel mesh bags and immersed in distilled water at room temperature for 16 h. Then, each mesh bag including wet wood powder was put in a stainless-steel potable reactor (Taiatsu Techno., Japan) and heated in a constant-temperature oven for 16 h. HTT was conducted at two temperatures. One set was conducted at 95 °C (named $HTT_{95 °C}$) and the other was at 120 °C (named $HTT_{120 °C}$). After HTT treatment, the wet wood powder was air-dried. For the analysis, three samples were used: untreated wood powder, $HTT_{95 °C}$, and $HTT_{120 °C}$. The extractives in the samples were removed with ethanol: toluene (1:2, v/v) for 6 h in a Soxhlet apparatus, and the samples were served for the subsequent analysis.

Color parameter

A spectrophotometer NF555 (Nippon Denshoku Corp., Japan; D65 standard illuminant) was used to measure the color. Approximately 2 g of degreased wood powder was filled into a transparent plastic bag and spread over an area of about 4 cm square. Then, the CIELab parameters L^*, a^*, and b^* were measured directly over the plastic bag. From each treatment sample, the average color parameters were obtained among three specimens. The values of the total color change (ΔE), L^*, a^*, and b^* were collected (Yang *et al.* 2020).

Attenuated total reflectance infrared spectroscopy

IR spectra of the degreased wood powders were obtained using an FTIR spectrometer (FT/IR6600, JASCO, Japan), equipped with an attenuated total reflectance (ATR) (ATR PRO ONE VIEW, JASCO, Japan) sampling tool attached to a diamond crystal. The spectrum was acquired by accumulating 64 scans in absorbance range of 4000 to 500 cm^{-1}. Measurements were performed three times per sample. For calibration, 1424 cm^{-1} corresponding to cellulose (-C-H stretching vibration) was used according to a previous study (Guo *et al*. 2015).

Determination of chemical components

The determination of the lignin content in wood materials was carried out according to the Klason method (Yokoyama *et al.* 2002; Yeh *et al.* 2004). The relative sugar composition in the Klason lignin hydrolysate was analyzed using HPLC on a Shimadzu LC-20AT (Shimadzu, Japan) with a TSK-gel Sugar AX1 column (Tosoh., Japan) and a Shimadzu SPD-20A UV detector (310 nm, Shimadzu, Japan) (Nakamura *et al*. 1999). The mobile phase, 50 mM boric acid buffer (pH 7.8) containing 0.5% ethanolamine hydrochloric acid, was eluted at a rate of 0.35 mL min^{-1}. Relative percentages were calculated electronically from duplicate experiments. The cellulose and hemicellulose

contents were determined according to a previous study as Cellulose (%) = Glucose − (1/3 × Mannose), and Hemicellulose (%) = Arabinose + Galactose + Glucose + Mannose + Xylose) − Cellulose (Jones *et al.* 2006). The GM/GGM and AGX contents in wood samples were calculated by assuming a fixed ratio of the relevant sugar units in each type of polysaccharide (Kibblewhite *et al.* 2010). All the measurements were done twice, and the averaged data were used.

Preparation of AGX and GM/GGM from holocellulose

Holocellulose was prepared from wood samples according to the Wise method (Japan Wood Research Society 1985). The degreased wood sample (20 g) was immersed in distilled water (560 mL) and treated with sodium chlorite (8.0 g) and acetic acid (1.6 mL) at 80 °C for 1 h. After four successive treatments, the solid residue was recovered by filtration, washed with water and acetone, and air-dried. The resulting holocellulose was extracted successively with hot water to remove GGM (Hashi *et al.* 1970). Then, the AGX was isolated from the holocellulose after GGM extraction using a 10% potassium hydroxide aqueous solution in the presence of barium hydroxide (Yamasaki *et al.* 2011). The alkaline soluble fraction obtained by filtration with a glass filter was adjusted to pH 6 with glacial acetic acid and then dialyzed against ion-exchanged water, concentrated, and freeze-dried. The solid residue was stirred in 10% acetic acid for 2 h to adjust to neutral. During AGX preparation as described above, the resulting precipitate after the addition of 10% aqueous potassium hydroxide in the presence of barium hydroxide was collected by centrifugation. The sugar composition of AGX and GM/GGM was determined by means of HPLC on a TSK-gel Sugar AX1 column after hydrolysis with 2 M trifluoroacetic acid (TFA) at 120 °C for 2 h (Nakamura *et al.* 1999).

Analysis of molecular-weight distribution

The molecular-weight analysis of hemicellulose (GM/GGM and AGX) was carried out by SEC with a Shimadzu LC-10AD (Shimadzu, Japan) and a refractive index detector ERC RefractoMax 520 (IDEX Health & Science, Japan) equipped with a Superose 6 10/300 column (GE Healthcare, USA). The mobile phase, 50 mM sodium acetate buffer (pH 5.8), was eluted at a rate of 0.5 mL min^{-1}. The calibration curve was prepared by using dextran standards (M_w = 410,000, 50,000, and 12,000) (Sigma Aldrich, USA) (Brown *et al.* 2009).

Analysis of hemicellulose assembly mode in the wood cell wall

The elution analysis of cell wall hemicellulose was performed according to instructions described in a previous study (Kurata *et al.* 2018). The soluble fraction A was obtained from wet wood powders before lignin decomposition after extraction with an 18% aqueous sodium hydroxide (NaOH) solution. The other soluble fractions (B–C) were obtained from residues by extraction with 18% aqueous NaOH solution after a stepwise delignification treatment. Hemicellulose included in fractions A and B form weak bonds with other cell wall components and are in easily released forms, while hemicellulose in fractions C and D form stronger bonds than in A and B, both between individual hemicellulose molecules or between hemicellulose and other cell wall components.

The compositional and quantitative analysis of the monosaccharides in fractions A–D were carried out using HPLC with a TSK-gel Sugar AX1 column after hydrolysis with 2 M TFA at 120 °C for 2 h (Yamasaki *et al.* 2011; Nakamura *et al.* 1999). The total sugar content in each fraction was determined by the phenol-sulfuric acid method

according to the previous method (Rao and Pattabiraman 1989). For calibration, a standard curve obtained from known amounts of glucose was used.

RESULTS AND DISCUSSION

Color Changes with Hydrothermal Treatment of Wood Materials

Table 1 shows the changes in lightness (L^*), color coordinates (a^* and b^*), and total color difference (ΔE) of Japanese cedar subjected to HTT at 95 and 120 °C. Compared to the untreated sample, the value of L^* from HTT samples decreased clearly which means that the color darkened with raising the HTT temperature. The value of ΔE also increased substantially with the raising of the HTT temperature. On the other hand, the a^* and b^* values decreased for $HTT_{95\ °C}$ and increased for $HTT_{120\ °C}$, and their changes were smaller. A previous study that investigated color differences and physical properties due to heat treatment for spruce also reported that L^* decreased monotonically with weight loss by heat treatment, but a^* and b^* values had wider variations even at the same weight loss (Endo *et al*. 2016).

The darkening of the wood with raising of HTT temperature might be due to chemical changes in cell wall components. It is suggested that cell wall polysaccharides such as hemicellulose are susceptible to hydrolysis by HTT and may be converted to furan compounds and other by-products.

Table 1. Color Parameters in Control and HTT Samples

Samples	Color Parameters			
	L^*	a^*	b^*	ΔE
Control	69.70 ± 1.0	8.24 ± 0.6	23.62 ± 0.4	-
$HTT_{95\ °C}$	61.46 ± 0.6	5.78 ± 0.1	18.83 ± 0.6	9.91 ± 0.8
$HTT_{120\ °C}$	56.47 ± 0.9	8.90 ± 0.3	20.61 ± 0.1	14.07 ± 1.8

Results represent the means ± standard deviation from three independent experiments.

Analysis of FTIR Spectrum

In order to investigate the chemical changes of cell wall components with HTT, the ATR-FTIR spectrum of control and HTT samples were measured. As shown in Fig. 1, all the three samples showed a broad absorption at around 3300 cm^{-1}, which can be attributed to the stretching vibration of hydrogen bonds related to the presence of the hydroxy group in cell wall components. The intensities at around 3300 cm^{-1} were at a similar level between untreated samples and both HTT samples. For cellulose structure in the cell wall framework, the bands at 1424 cm^{-1} corresponding to CH_2 wagging vibrations in cellulose were also at a similar level between all samples.

The presence of extractives in the cell walls may be related to changes in IR absorption for the aromatic backbone as well as for lignin. Therefore, IR spectra of each wood powder was measured after the removal of extractives with ethanol:toluene (1:2, v/v). The intensity of 1508 cm^{-1}, corresponding to the aromatic skeleton vibration of the lignin structure, was also unchanged between all samples (Fig. 1).

Next, the absorbance (C=O stretching of unconjugated groups) of FTIR spectra at 1734 cm^{-1} was examined because it reflects changes in various functional groups (*e.g.*, carbonyl groups and ester groups) of lignin and hemicellulose. Previous studies have

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suggested that high temperature treatment under wet conditions is responsible for the decomposition of carbonyl groups such as acetic acid or glucuronic acid in the hemicellulose structure (Tjeerdsma and Militz 2005; Altgen *et al*. 2018). In the present measurements, the relative intensity of the 1734 cm^{-1} band assigned to the carbonyl group of the glucuronic acid unit in AGX showed only a slight change between samples, suggesting that significant degradation of xylan did not occur.

In contrast, based on the relative absorbance ratio in FTIR spectra, the relative absorbance ratio (809 cm^{-1}/1424 cm^{-1}) of the GM-derived absorption band at 809 cm^{-1} decreased from 1.53 (control), 1.50 (HTT$_{95\ °C}$) to 1.36 (HTT$_{120\ °C}$) with raising of the temperature of HTT (Table 2). The decrease in the absorption of mannose residue, which constitutes the main chain of GM/GGM, indicates that the GM/GGM in the cell wall might have already degraded under HTT$_{95\ °C}$ in the present study. The relative absorbance ratio of 1030 cm^{-1}/1424 cm^{-1}, corresponding to cell wall polysaccharides, decreased with raising the temperature of HTT. Our results indicate that the main chain structure of the hemicellulose, including GM/GGM and AGX, slightly changed with the HTT process at 95 °C and at 120 °C.
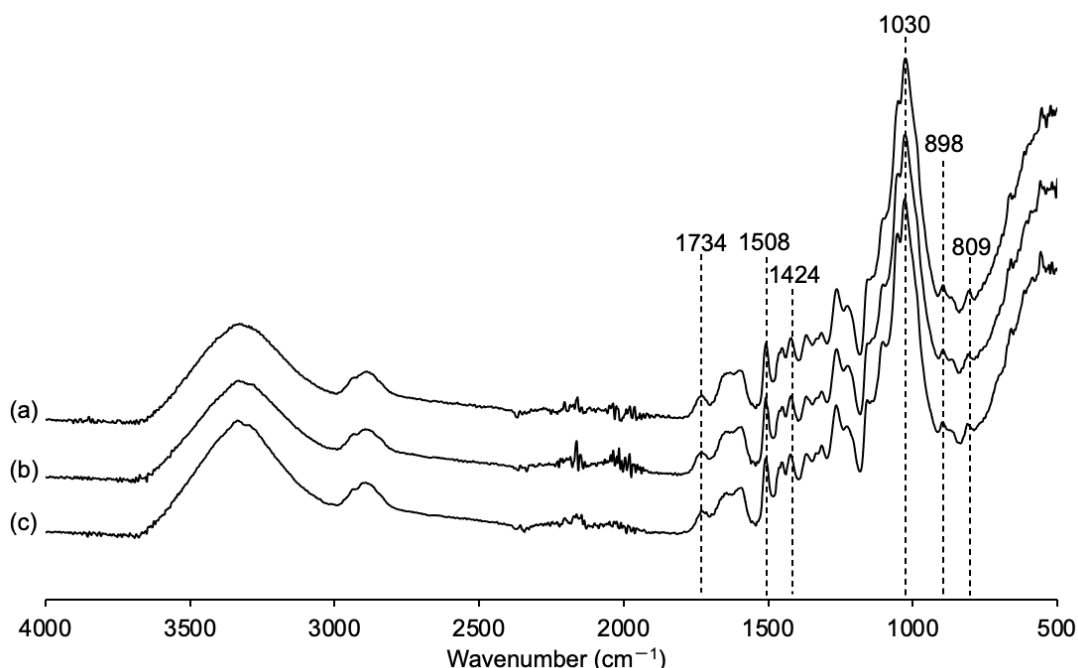
Fig. 1. ATR-FTIR spectra of (a) control, (b) HTT at 95 °C, and (c) HTT at 120 °C

Table 2. FTIR Absorbance Ratios in Control and HTT Samples

Intensity ratio	Control	HTT$_{95\ °C}$	HTT$_{120\ °C}$
$I_{1734/1424}$	0.31	0.35	0.33
$I_{1508/1424}$	0.95	1.00	0.96
$I_{1030/1424}$	4.22	4.04	3.93
$I_{809/1424}$	1.53	1.50	1.36

Changes in Chemical Components and Sugar Compositions

Chemical components in control, HTT$_{95\ °C}$, and HTT$_{120\ °C}$ samples are compiled in Table 3. No difference was observed in the content of lignin between all samples. The

proportion of hemicellulose decreased slightly in HTT$_{120\ °C}$ from control and HTT$_{95\ °C}$. Moreover, the content of GM/GGM and AGX exhibited almost no change in all samples. As shown in Table 4, the neutral sugar composition in control and HTT samples also slightly changed. These results showed that the contents of the main chemical components and the ratios of neutral sugar compositions were not influenced by the HTT conditions in the present study.

Table 3. Chemical Components in Control and HTT Samples

Polymers in cell wall (%)	Control	HTT$_{95\ °C}$	HTT$_{120\ °C}$
Klason lignin (%)	30.5 ± 0.2	30.3 ± 0.1	30.9 ± 0.8
Cellulose (%)	45.1 ± 0.1	45.7 ± 0.1	46.1 ± 0.2
Hemicellulose (%)	24.4 ± 0.2	24.0 ± 0.1	23.1 ± 0.6
GM/GGM[a] (%)	69.2 ± 1.2	69.9 ± 0.3	70.5 ± 0.4
AGX[a] (%)	30.9 ± 1.2	30.1 ± 0.3	29.5 ± 0.4

Results represent the means ± is standard deviation from three independent experiments.
[a] Percentage in hemicellulose fraction.

Table 4. Neutral Sugar Compositions in Control and HTT Samples

Sugar composition	Control	HTT$_{95\ °C}$	HTT$_{120\ °C}$
Mannose (%)	16.0	15.4	15.5
Arabinose (%)	1.7	1.7	1.2
Galactose (%)	3.3	3.5	3.3
Xylose (%)	8.6	8.5	8.6
Glucose (%)	70.4	70.9	71.3

Results represent the average value from two independent experiments.

Changes in Neutral Sugar Composition of GM/GGM and AGX

To examine the effect of temperatures of HTT on hemicellulose chemical structure, GM/GGM and AGX were isolated from each holocellulose in wood samples. Then, the neutral sugar composition of GM/GGM and AGX (Tables 5 and 6) were determined. In general, GM/GGM consists of approximately three mannose residues per glucose residue, and a galactose residue is occasionally linked to the backbone of GM/GGM (Kim *et al.* 2010). The mean ratio of mannose, glucose, and galactose in the polymer of GM/GGM was 3.2:1:0.2 in the control sample, 3.0:1:0.2 in HTT$_{95\ °C}$, and 3.1:1:0.2 in HTT$_{120\ °C}$, suggesting that the ratios of GM/GGM backbone were not affected by the HTT in this study.

AGX was isolated from control and HTT samples, and the mean rate of arabinose side-chain substitution in AGX was determined (Table 6). The mean rate of arabinose side-chain substitution, determined based on neutral sugar analysis, was 14.3 units per 100 xylose units in control. The ratio of arabinose side-chain substitution in AGX from HTT$_{95\ °C}$ and HTT$_{120\ °C}$ was 11.3 and 1.0 units per 100 xylose backbone units, respectively. According to a previous report, AGX contains generally one Ara residue per 5 to 12 xylose residues (Yamasaki *et al.* 2012). Our results suggested that the HTT at 120 °C had a relatively huge effect on arabinose side-chain substitution in the AGX backbone.

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Table 5. Relative Neutral Sugar Compositions of GM/GGM in Control and HTT Samples

Sugar composition	Control	HTT$_{95\ °C}$	HTT$_{120\ °C}$
Mannose (%)	61.7	65.1	67.1
Arabinose (%)	3.0	1.8	1.0
Galactose (%)	4.7	3.9	2.4
Xylose (%)	11.5	8.2	7.0
Glucose (%)	19.0	21.0	22.5

Results represent the average value from two independent experiments. The sugar composition in the GM fraction was described as results of the alkaline soluble fraction based on HPLC analysis.

Table 6. Relative Neutral Sugar Compositions of AGX in Control and HTT Samples

Sugar composition	Control	HTT$_{95\ °C}$	HTT$_{120\ °C}$
Mannose (%)	3.1	3.0	4.7
Arabinose (%)	10.8	8.7	0.8
Galactose (%)	5.8	7.3	6.0
Xylose (%)	75.9	77.1	82.2
Glucose (%)	4.4	3.9	6.3

Results represent the average value from two independent experiments. The sugar composition in the AGX fraction was described as results of the alkaline soluble fraction based on HPLC analysis.

Changes in Molecular-Weight Distribution of GM/GGM and AGX

Next, the molecular chain-length compositions of GM/GGM and AGX in control and HTT samples were determined by using SEC analysis. As shown in Fig. 2 (a), GM/GGM in control had a unimodal molecular-weight distribution of around 220 kDa and a shoulder peak of around 12 kDa. In the HTT$_{95\ °C}$ sample, the molecular chain-length of GM/GGM was similar to that of control, but the shoulder peak around 12 kDa disappeared. On the other hand, in the HTT$_{120\ °C}$ sample, the molecular-weight distribution of GM/GGM shifted to the lower molecular region, indicating that the GM/GGM backbone in cell walls was hydrolyzed with HTT at 120 °C. It was hypothesized that during the HTT at 95 °C, the disintegration of the GM polymeric structure, which was not visible in the quantitative changes of the primary chemical components, had already happened.

The AGX accumulated in control consisted of long-chain molecular assemblies with a peak around 220 kDa and short-chain molecules of with a peak around 30 kDa as shown in Fig. 2 (b). The molecular-weight distribution of AGX did not change in HTT$_{95\ °C}$ when compared with that of control. On the other hand, in AGX isolated from HTT$_{120\ °C}$, the long-chain-length assemblies around 220 kDa clearly disappeared and shifted to the short-chain-length of AGX around 50 kDa, suggesting the cleaving of xylose residue linked by β-(1→4) glycosidic bond and arabinose side-chain in AGX with hydrolysis under the HTT process. At a higher temperature of 120 °C, HTT had a significant impact on the molecular-weight distribution of AGX in cell walls. Several reports have previously shown that the thermal stability of xylan is lower than that of GM (Werner *et al.* 2014; Wang *et al.* 2020). Based on the molecular-weight analysis, the effect of HTT on GM/GGM and AGX indicated that the de-polymerization of the main chain is preferentially caused by hydrolysis rather than the degradation of the GM/GGM backbone.
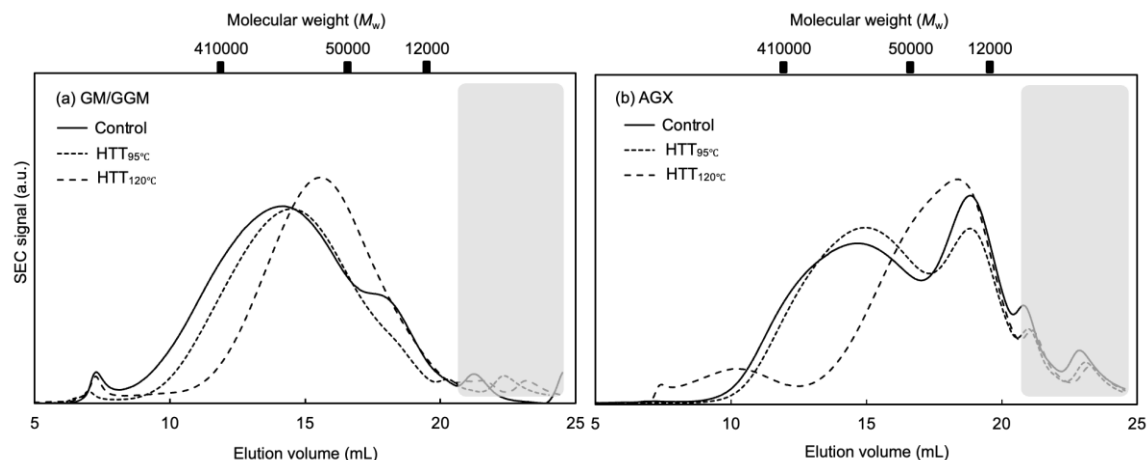
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Fig. 2. Molecular-weight distribution of (a) GM/GGM and (b) AGX. The gray zone represents salts in the exclusion volume

Changes in Assembly Mode of Hemicellulose

In the present study, to evaluate the strength of the interaction between cell wall chemical components, the changes were examined in elution sugar content extracted by an 18% aqueous NaOH solution from wood materials subjected to a stepwise delignification. The proportion of elution sugar content between the control and HTT samples is presented in Table 7. The amount of total elution sugar was 228.7 mg in the control, corresponding to ca. 20% of the total amount of hemicellulose as shown in Table 1. However, in the HTT samples, the total eluted sugar increased significantly with raising the temperature of HTT. These results show that the assembly mode of chemical components in the cell wall was influenced by the HTT of the wood samples.

As shown in Table 8, the amount of GM/GGM included in fractions A and B increased significantly along with HTT and raising the HTT temperature. The GM/GGM of HTT$_{95\,°C}$ in fraction B increased clearly to 16.6% from 9.6% in the control and 29.2% in HTT$_{120\,°C}$. This result indicates that the GM/GGM in the HTT$_{95\,°C}$ interacts weakly with other cell wall components and changes to an accumulated state that is easy to release. The amount of GM/GGM in fraction D, which contained the GM/GGM presumably assembled through strong interactions between hemicellulose molecules or between hemicellulose and other cell wall components, appeared to decrease with the temperature at 120 °C. Considering the decomposition of the main chain of GM/GGM in the cell wall with the heat treatment in the presence of moisture, the interactions of the matrix components between cellulose and hemicellulose decreased, resulting in the dissolution of more hemicellulose from the wood cell wall. Moreover, the amount of AGX in fraction A slightly increased from 2.1% in control to 4.2% in HTT$_{95\,°C}$. As shown in Table 8, the changes in AGX assembly mode in the cell wall microstructure with HTT were different from those of GM/GGM. The AGX content in HTT$_{120\,°C}$ increased significantly compared with that of an untreated sample as a control, whereas 14.5% of the AGX in HTT$_{120\,°C}$ was eluted from the cell wall without delignification, indicating that the interaction between cellulose and hemicellulose in the cell wall was altered with HTT at high temperature due to the significant decrease in molecular weight of AGX in HTT$_{120\,°C}$.

Table 7. Changes in Assembly Mode of Hemicellulose in Elution Sugar Analysis

Samples	Elution Sugar Content in Fractions (mg)				Total Elution Sugar Content (mg)
	A	B	C	D	
Control	11.3	47.7	90.5	79.2	228.7
HTT$_{95\,°C}$	25.0	90.0	88.8	73.1	276.9
HTT$_{120\,°C}$	81.8	118.4	75.6	29.4	305.2

Results represent the average value from two independent experiments.

Table 8. Percentages of GM/GGM and AGX Contents in Each Fraction

Hemicellulose Type	Samples	Elution Sugar Content in Fractions (%)			
		A	B	C	D
GM/GGM	Control	1.9	9.6	25.6	28.4
	HTT$_{95\,°C}$	3.5	16.6	24.2	22.7
	HTT$_{120\,°C}$	11.7	29.2	23.0	8.9
AGX	Control	2.1	10.3	14.6	7.4
	HTT$_{95\,°C}$	4.2	15.3	9.1	4.6
	HTT$_{120\,°C}$	14.5	9.4	2.7	0.6

Results represent the average value from two independent experiments.

CONCLUSIONS

This study investigated the effects of HTT at temperatures of 95 and 120 °C on cell wall components in Japanese cedar. In addition, the influence of HTT on the hemicellulose structure (GM/GGM and AGX) and the changes in the assembly mode of hemicellulose in the cell wall were examined.

1. Chemical components (lignin, cellulose, and hemicellulose) and sugar composition slightly changed between the control and HTT samples. The absorbances of the FTIR spectra at 1734 and 809 cm^{-1}, attributed to the hemicellulose structure such as C=O stretching of unconjugated groups and the absorption of mannose residue, were also slightly changed between the control and HTT samples.

2. Based on the sugar composition of the hemicellulose, the ratio of GM/GGM did not change with HTT (95 and 120 °C), whereas the Ara side-chain ratio of AGX in the cell wall was greatly affected. The molecular weight distributions of GM/GGM and AGX in the cell walls were also significantly altered with raising HTT temperature. In addition, analysis of the hemicellulose assembly mode suggested that the degradation of the polymeric structure of GM/GGM and AGX in the cell wall with HTT at 95 and 120 °C may have decreased the strength of interactions between the chemical components of the cell wall.

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