J Wood Sci (2010) 56:331–338
DOI 10.1007/s10086-009-1099-0

ORIGINAL ARTICLE

Natthanon Phaiboonsilpa · Kazuchika Yamauchi · Xin Lu
Shiro Saka

Two-step hydrolysis of Japanese cedar as treated by semi-flow hot-compressed water

Received: August 7, 2009 / Accepted: November 19, 2009 / Published online: March 6, 2010

Abstract Two-step hydrolysis of Japanese cedar (*Cryptomeria japonica*) was studied as treated by semi-flow hot-compressed water at 230°C/10 MPa for 15 min and 280°C/10 MPa for 30 min as the first and second stages, respectively. At the first stage, hemicelluloses and paracrystalline cellulose, whose crystalline structure is somewhat disordered, were found to be selectively hydrolyzed, as well as lignin decomposition, whereas crystalline cellulose occurred at the second stage. In all, 87.76% of Japanese cedar could be liquefied by hot-compressed water and was primarily recovered as various hydrolyzed products, dehydrated, fragmented, and isomerized compounds as well as organic acids in the water-soluble portion. The remainder, 12.24%, could not be hydrolyzed and remained as the water-insoluble residue composed entirely of lignin. Based on the distribution of various products from hemicelluloses in Japanese cedar, their decomposition pathways were proposed as independent.

Key words Japanese cedar · Hot-compressed water · Hemicellulose · Cellulose · Lignin

N. Phaiboonsilpa · K. Yamauchi · X. Lu · S. Saka (✉)
Department of Socio-Environmental Energy Science, Graduate School of Energy Science, Kyoto University, Yoshida-honmachi, Sakyo-ku, Kyoto 606-8501, Japan
Tel. +81-75-753-4738; Fax: +81-75-753-4738
e-mail: saka@energy.kyoto-u.ac.jp

X. Lu[1]
College of Food Science and Technology, Nanjing Agricultural University, Nanjing 210095, China

Present address:
[1] College of Food Science and Engineering, Northwest A&F University, Yangling, Shaanxi 712100, China

This work was presented in part at the 55th annual meeting of the Japan Wood Research Society, Matsumoto, Japan, March 2009, the World Renewable Energy Congress 2009 – Asia, Bangkok, Thailand, May 2009, and the 18th annual meeting of the Japanese Institute of Energy, Sapporo, Japan, July 2009

Introduction

In an attempt to alleviate both environmental and energy security problems, ethanol produced from various types of biomass has been proposed for several decades as a promising biofuel.[1] Lignocellulosics can be utilized to obtain fermentable saccharides for bioethanol production. However, in contrast to sugar and starch, the development of bioethanol production from lignocellulosics has been impeded by their great resistance to hydrolysis, caused by the cellulose crystallinity and presence of lignin. Therefore, various biological, chemical, and/or physical treatments such as enzymatic saccharification, acid hydrolysis, alkali process, steam explosion, and supercritical and subcritical treatments have been developed.[2–7]

However, one drawback of using acid or alkali is the necessity for neutralization and separation.[2] In addition, Aida et al.[8] elucidated that an increase in water temperature (350–400°C) and pressure (40–70 MPa) not only heightened the dehydration of glucose to 5-hydroxymethylfurfural (5-HMF) but also enhanced the formation of 1,2,4-benzenetriol (BTO), resulting in a greater loss of saccharides and an inhibition effect in the subsequent fermentability. Although the liquefaction rate became extremely high as cellulose was treated by subcritical water, it was found that the hydrolysis rate of cellulose was much lower than the decomposition rate of glucose and its oligomers.[9] Economic and environmental constraints, moreover, limit the applicability of these known methods. Thus, hot-compressed water, milder conditions with a high ionic product, seems to be a promising alternative as both an environmentally friendly solvent and an attractive reaction media for a variety of applications.[10,11] Hot-compressed water refers to water at a supercritical or subcritical state, or at sufficiently high pressure and temperature.[12]

Further, it has long been realized that the hydrolysis of cellulose and hemicelluloses could not be optimized at the same treatment severity.[2,6,13,14] Therefore, a two-step treatment, with the first stage performed at low severity to hydrolyze the hemicelluloses and the second stage in which

the solid residue from the first stage is consecutively treated, but at a higher degree of severity, has gained a great amount of attention as an alternative to increase saccharide yields.[6,13,14]

Ando et al.[10] investigated the decomposition behavior of bamboo, chinquapin, and Japanese cedar as treated by two-step hot-compressed water at 180°C for 20 min and 285°C for 7 min using the semi-flow-type reactor with a flow rate of 10 ml/min under 9.8 MPa. In this report, however, the quantitative assessments of hydrolyzed saccharides and various products derived from hemicelluloses and cellulose, together with a discussion of their decomposition pathways, were not sufficiently clarified.

In our previous work,[15] two-step hydrolysis of Japanese beech (*Fagus crenata*) as one of the hardwoods treated by semi-flow hot-compressed water was conducted. As a result, hemicelluloses and cellulose were found to be independently hydrolyzed in the first and second stages of the treatment, respectively. To gain better insights into the two-step hot-compressed water treatment of various lignocellulosics, in this work, therefore, two-step hydrolysis of Japanese cedar (*Cryptomeria japonica*) as one of the softwoods treated by semi-flow hot-compressed water was investigated. Not only qualitative assessments of various hydrolyzed products, dehydrated, fragmented, and isomerized compounds as well as organic acids recovered in the water-soluble portion, but also the quantification of these products, were reported. Based on this line of informative evaluation, the independent decomposition pathways of hemicelluloses and cellulose were eventually proposed.

Materials and methods

Material and chemicals

Extractive-free wood flour of Japanese cedar (*Cryptomeria japonica*) was prepared.[15,16] The weight percentages of products were calculated on the basis of oven-dried extractive-free wood flour. Table 1 shows the chemical composition of extractive-free Japanese cedar, determined by the Klason lignin determination method[17] for lignin and the obtained acid hydrolysate for hemicelluloses and cellulose. All chemicals used in this study were of reagent grade without purification.

Table 1. Cell-wall components of extractive-free Japanese cedar used in this study[16]

Weight percent (wt%) on extractive-free wood flour basis				
Hemicelluloses		Cellulose	Lignin	
Glucomannan	Xylan		Klason lignin	Acid-soluble lignin
12.41	6.69	48.32	32.20	0.38

Hot-compressed water treatment and fractionation of the treated samples

The semi-flow hot-compressed water biomass conversion system and operational procedures as described previously[15] were used in this study. Based on our preliminary experiments[16] to explore the relationship between treatment temperature under pressurized conditions (10 MPa) and the degree of hydrolysis of hemicelluloses and cellulose, treatment temperatures applied for the two-step treatment were set at 230 and 280°C, 10 MPa, at the flow rate of 10.0 ml/min. The hot-compressed water-soluble portion was collected every 1 min by the fraction collector. After settling in ambient temperature and pressure for 12 h, the water-soluble portion was obtained by filtration with a 0.45-μm membrane before subsequent analyses. The water-insoluble residue left in the reaction cell after treatment was collected, oven-dried, and studied for its chemical composition.

Analytical methods

Analyses of the water-soluble portion were made by high-performance anion-exchange chromatography (HPAEC), high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), and capillary electrophoresis (CE). The same analysis systems and operating conditions were applied in accordance with our previous paper.[15]

Post-hydrolysis by dilute sulfuric acid was performed to estimate all recovered oligosaccharides in the water-soluble portion in terms of acid-hydrolyzed monosaccharides.[18] The obtained acid hydrolysate was neutralized with barium hydroxide, $Ba(OH)_2$, and then filtrated by a 0.45-μm membrane filter before HPLC analysis using the HPX-87P column. Distilled water was used as an eluent, and the system operated at the flow rate and temperature of 0.6 ml/min and 85°C, respectively. The amount of the oligosaccharides was then calculated based on Eq. 1 as follows:

$$\text{Oligosaccharides (wt\%)} = \text{Total monosaccharide in acid hydrolysate (wt\%)} - \text{monosaccharide in water-soluble portion (wt\%)} \quad (1)$$

Quantification of products in water-soluble portion

All the product percentages on the basis of oven-dried extractive-free weight of the original wood flour were calculated by using the peak area[15] of chromatograms obtained from HPAEC, HPLC, CE, and GC-MS (Figs. 1–6). Not all plots of product percentages shown in the figures were based exactly on the real scale. Some of them were shifted by additional surpluses to make a clear perception of each production trend.

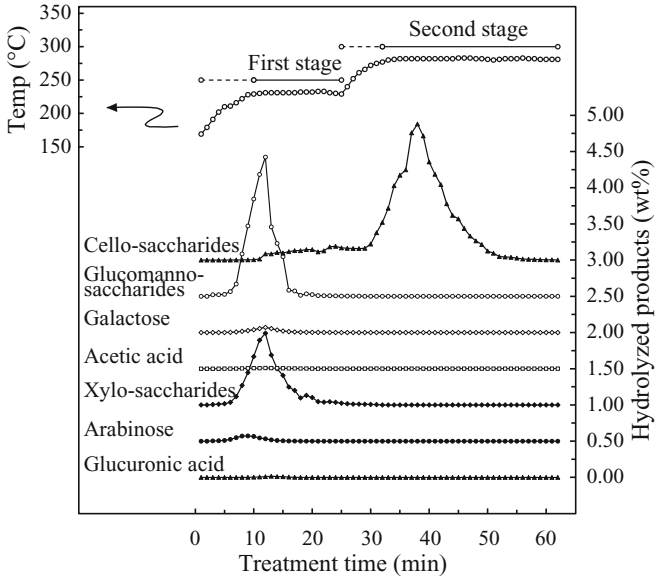

Fig. 1. Hydrolyzed products from Japanese cedar as treated by two-step semi-flow hot-compressed water at 230°C/10 MPa/15 min and 280°C/10 MPa/30 min. *Left axis* corresponds to treatment temperature (*open circles*); *right axis* corresponds to product yield

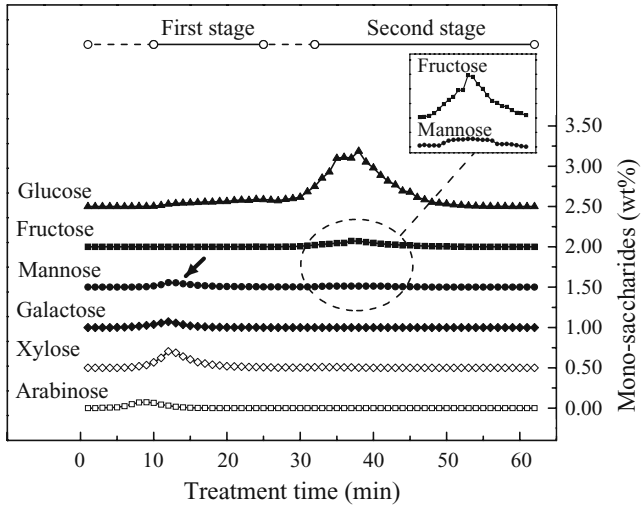

Fig. 2. Monosaccharides from Japanese cedar as treated by two-step semi-flow hot-compressed water at 230°C/10 MPa/15 min and 280°C/10 MPa/30 min. *Arrow* shows the presence of mannose in the first stage; *inserted figure* is the enlargement of the fructose and mannose peaks in the second stage

Results and discussion

Hydrolysis of major cell-wall components by hot-compressed water

As treated by the two-step hot-compressed water (230°C/10 MPa/15 min and 280°C/10 MPa/30 min), 87.76% of extractive-free Japanese cedar wood flour was liquefied and primarily recovered as various compounds in the water-soluble portion; the rest, 12.24%, was not hydrolyzed and

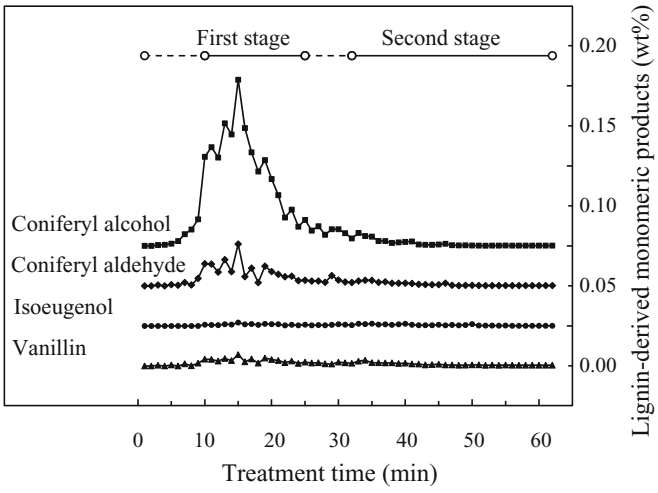

Fig. 3. Lignin-derived monomeric products from Japanese cedar as treated by two-step semi-flow hot-compressed water at 230°C/10 MPa/15 min and 280°C/10 MPa/30 min

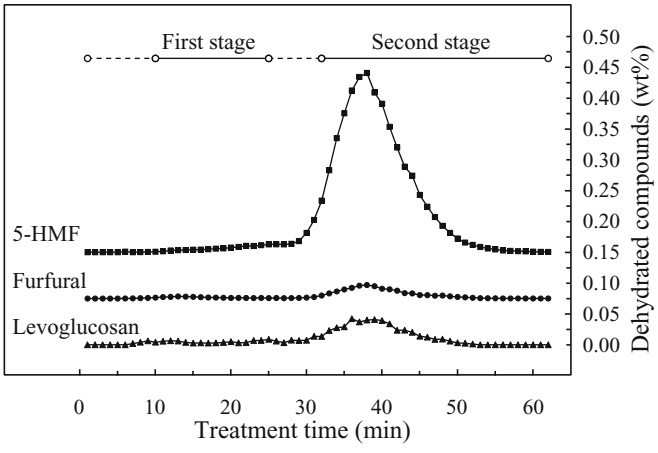

Fig. 4. Dehydrated compounds produced from Japanese cedar as treated by two-step semi-flow hot-compressed water at 230°C/10 MPa/15 min and 280°C/10 MPa/30 min. *5-HMF*, 5-hydroxymethylfurfural

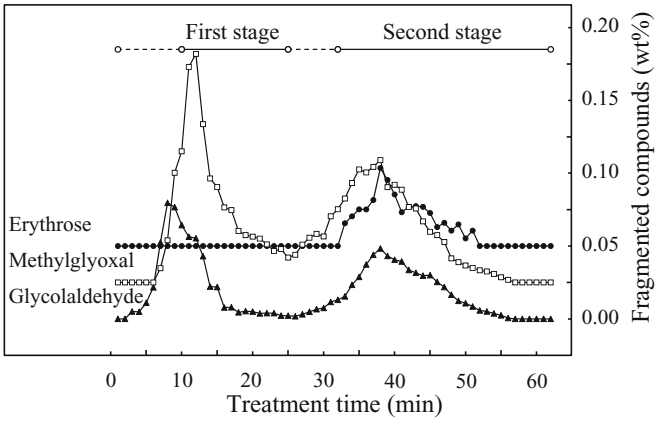

Fig. 5. Fragmented compounds produced from Japanese cedar as treated by two-step semi-flow hot-compressed water at 230°C/10 MPa/15 min and 280°C/10 MPa/30 min

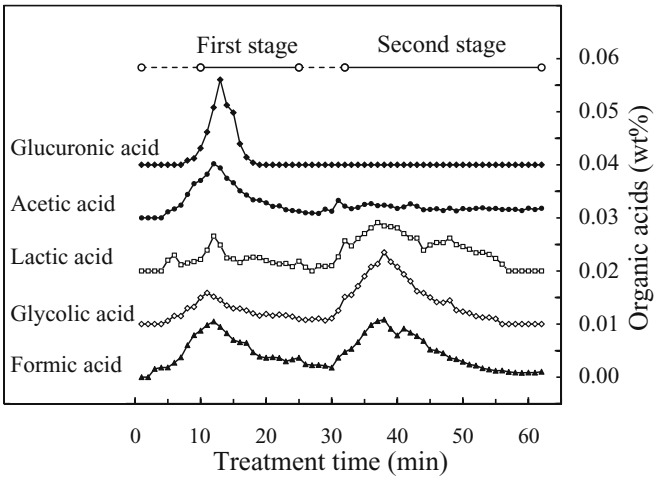

Fig. 6. Organic acids produced from Japanese cedar as treated by two-step semi-flow hot-compressed water at 230°C/10 MPa/15 min and 280°C/10 MPa/30 min

remained as the water-insoluble residue composed entirely of lignin, which was confirmed by Klason lignin determination. For the water-soluble portion obtained, the compounds produced were studied.

As seen in the temperature profile in Fig. 1, two-step treatment was performed over the wood flour of Japanese cedar. The dashed lines indicate the transient time during an increase in temperature, whereas the solid lines depict the first and second stages of treatment at 230°C for 15 min and 280°C for 30 min, respectively, as shown in the real temperature profile. The hydrolyzed products obtained from hemicelluloses and cellulose are shown.

In the first stage (230°C/10 MPa/15 min), glucomanno-saccharides, which include mannose, glucose, and oligomeric glucomannan, galactose, and acetic acid were produced. Xylo-saccharides, which include xylose and xylo-oligosaccharides, such as xylobiose, xylotriose, xylotetraose, xylopentaose, xylohexaose, and the molecules with a higher degree of polymerization (DP), arabinose and glucuronic acid, were also recovered at this stage. In production of acetic acid and glucuronic acid, the amounts are relatively small compared to the others in Fig. 1, and their more apparent production trends can be seen later in Fig. 6. Therefore, these hydrolyzed products must originate from the major and minor hemicelluloses of the softwood, *O*-acetyl-galactoglucomannan and arabino-4-*O*-methylglucuronoxylan,[14,19,20] respectively.

Based on the evidence acquired above, *O*-acetyl-galactoglucomannan can be 0.3:1:1.7 in the molar ratio of galactose:glucose:mannose with an acetyl residue per 48 monosaccharide units of the glucomannan backbone, whereas arabino-4-*O*-methylglucuronoxylan can be 1:6.4 in the molar ratio of arabinose:xylose with a glucuronic acid residue per 118 monosaccharide units of the xylan backbone. These results are in agreement with those in the literature to some extent; however, the numbers of acetyl and acid residues are relatively smaller than the ones reported previously.[19] The reason may be that they were not fully recovered in those forms.

Figure 2 depicts various monosaccharides produced from Japanese cedar. In the first stage, mannose, galactose, xylose, and arabinose were found. However, it is obvious that the amounts of mannose and xylose are much lesser than those of glucomanno-saccharides and xylo-saccharides (see Fig. 1), indicating that most of those hydrolyzed saccharides were recovered as in the oligomeric forms.

Moreover, in Fig. 2, a small amount of mannose was observed not only at the first stage (shown by an arrow) but also at the second stage (shown by the inserted figure). Because hemicelluloses are supposed to be hydrolyzed in the first stage, the observed small peak of mannose and fructose at the second stage must be from cellulose. In addition, mannose and fructose are known as isomerized products of glucose in subcritical and supercritical water treatments.[21,22] This isomerization to mannose and fructose was ensured by the treatment of D-glucose under the same hot-compressed water conditions. Such an isomerization of glucose was also observed in our previous work on Japanese beech.[15]

Cello-saccharides, which include glucose and cello-oligosaccharides, such as cellobiose, cellotriose, cellotetraose, cellopentaose, cellohexaose, and higher DP molecules (see Fig. 1), were produced from 10 to 55 min in retention time. However, the one in the first stage (10–25 min) would not be from crystalline cellulose because in the preliminary experiment the crystalline structure of cellulose remained unchanged in such a temperature around 230°C.[16] Therefore, the cello-saccharides just between the first and second stages (10–25 min) could be from para-crystalline cellulose, whose crystalline structure is somewhat disordered.[23]

In contrast to hardwoods, the dominating hemicellulose in softwoods is glucomannan, which can produce a significant amount of glucose as the hydrolyzed product. However, there was a production of glucose and its oligomers also from the hydrolysis of para-crystalline cellulose, leading to indistinguishable sources of them in the first stage. Because most of the hydrolyzed saccharides from glucomannan were recovered in the form of oligomeric glucomannan, as described previously, the glucose from glucomannan is assumed to be negligible. Moreover, on the assumption that a single glucose residue links to the mannose residue evenly in the native glucomannan backbone, therefore, the oligomeric glucomannan can be differentiated from the cello-oligosaccharides such as cellobiose, cellotriose, cellotetraose, cellopentaose, and cellohexaose. Thus, those cello-oligosaccharides (DP1–5) detected by HPAEC analysis can be assumed to be exclusively from para-crystalline cellulose. Consequently, the amount of glucose derived from oligomeric glucomannan could be estimated by the following equation:

$$\text{Glucose from oligomeric glucomannan (wt\%)} = \text{total glucose in acid hydrolysate (wt\%)} - \text{cello-oligosaccharides (DP1–5) in water-soluble portion (wt\%)} \quad (2)$$

The hemicelluloses were assumed to be completely hydrolyzed in the first stage. On the other hand, cello-saccharides produced in the second stage were only from

cellulose and thus determined ordinarily. By this line of calculation, the glucomanno-saccharides and cello-saccharides could be computed and plotted as shown in Fig. 1.

From the results as in Figs. 1 and 2, this two-step process has been proved to be efficient to separately hydrolyze hemicelluloses and crystalline cellulose. Although there was the hydrolysis of para-crystalline cellulose overlapping with the hemicelluloses in the first stage, a relative trace amount in its production compared with that from the hemicelluloses could be neglected. Therefore, these two important chemical components can be successfully chased independently by this process.

As to lignin, it was found that its monomeric products were first recovered before the temperature reached 230°C (Fig. 3). This finding accords with the literature reporting that lignin degradation was probably achieved at a temperature below 200°C.[14] Among these compounds, coniferyl alcohol, coniferyl aldehyde, isoeugenol, and vanillin were primarily found in the first stage as hydrolyzed monomeric lignin-derived products. It was certainly proved that all of them were derived from the guaiacyl unit of softwood lignin. Apart from these monomeric lignin-derived products, the amounts of its dimeric, trimeric, and higher products were estimated by subtracting the monomeric products from the whole soluble lignin, as described in our previous paper.[15] More details on lignin study will be published elsewhere.

Decomposition of hydrolyzed products from hemicelluloses and cellulose

After the hydrolysis of hemicelluloses and cellulose as already discussed, the hydrolyzed products are further decomposed if the treatment is prolonged by hot-compressed water. Generally, they are further decomposed by dehydration and fragmentation reactions.[24] Because the decomposed products would lead to an inhibitory effect on the later fermentation process for ethanol production,[25] it is important to know the decomposition pathway.

Figure 4 shows the obtained dehydrated products of 5-HMF, furfural, and levoglucosan. Generally, 5-HMF is considered as a dehydrated product from hexoses such as glucose, mannose, and galactose, whereas furfural is derived from pentoses such as xylose and arabinose.[14,21,26] Levoglucosan, particularly, is a dehydrated product from glucose.[21] As softwood hemicelluloses are composed of both hexoses and pentoses, not only furfural but also 5-HMF and levoglucosan were possibly found in the first stage. Nonetheless, fewer amounts were obtained compared with those in the second stage, because the more severe conditions in the second stage can enhance the degree of dehydration.

However, furfural was unexpectedly produced during the second stage. The same finding has been realized in our previous work.[15] Therefore, this obtained result can confirm that furfural is not only produced from pentoses but also from hexoses such as glucose under the hot-compressed water treatment. The possible formation of furfural without pentoses is possibly via the 5-carbon ketoses pathway, as proposed in the literature.[27]

In Fig. 5, methylglyoxal and glycolaldehyde can be seen in both first and second stages, although erythrose occurs in the second stage only. Therefore, at the first stage, pentoses such as xylose and arabinose from xylan hemicellulose would be decomposed to glycolaldehyde and glyceraldehyde, and then glyceraldehyde was dehydrated to methylglyoxal, as observed in the glyceraldehyde pathway of hexose fragmentation.[28] One molecule of pentose can give one glycolaldehyde and one methylglyoxal according to the carbon balance. Therefore, the ratio among their weight percentages should be approximately 1:1. However, it is obvious that much more methylglyoxal was produced, and thus there should be another source for methylglyoxal production. Undoubtedly, hexoses such as glucose, mannose, and galactose, from glucomannan hemicellulose, could be fragmented into methylglyoxal produced via the glyceraldehyde/dihydroxyacetone pathway in hexose fragmentation,[21,29] leading to the additional amount of methylglyoxal compared to the glycolaldehyde.

At the second stage, on the other hand, glycolaldehyde and erythrose formation via retro-aldol condensation[21,30] occurred in the glycolaldehyde/erythrose pathway, whereas methylglyoxal was produced via the glyceraldehyde/dihydroxyacetone pathway in hexose fragmentation, as already mentioned. However, under the conditions applied, glyceraldehyde and its isomerized dihydroxyacetone in the glyceraldehyde pathway were not detected in either stage; this would be because the dehydration reaction of glyceraldehyde is so fast consecutively to methylglyoxal and/or organic acids.[28]

Production of organic acids

Organic acids are considered as decomposition products of the dehydrated and fragmented compounds.[21] As seen in Fig. 6, the produced organic acids are glucuronic acid, acetic acid, lactic acid, glycolic acid, and formic acid. However, glucuronic acid was the hydrolyzed product from the glucuronic acid residue in the minor softwood hemicellulose, arabino-4-*O*-methylglucuronoxylan, whereas acetic acid found in the first stage mostly came from hydrolysis of the acetyl group in the major one, *O*-acetyl-galactoglucomannan. Acetic acid in the second stage, on the other hand, must be a result of the decomposition of cellulose and/or lignin.[15,31] In addition, production of lactic acid, glycolic acid, and formic acid was found in both stages of the treatment, thus indicating that the decomposition of dehydrated and fragmented compounds took place. Acrylic acid and levulinic acid were not detected under the conditions applied.

Overall compounds produced from hemicelluloses, cellulose, and lignin

Table 2 summarizes the yields of the produced compounds from hemicelluloses, cellulose, and lignin separately as

Table 2. Summary of various products produced from Japanese cedar as treated by two-step semi-flow hot-compressed water at 230°C/10 MPa/15 min and 280°C/10 MPa/30 min

Compound	Yield (wt%)						
	First stage[a]			Second stage[a]			Total
	Hemicellulose	Cellulose	Lignin	Hemicellulose	Cellulose	Lignin	
Hydrolyzed products from hemicelluloses and cellulose	**19.24**	**2.95**	**–**	**0.25**	**17.77**	**–**	**40.21**
Glucomanno–saccharides	11.54	–	–	0.17[d]	–	–	11.71
Galactose	1.33	–	–	–	–	–	1.33
Acetic acid	0.09	–	–	–	0.07[e]	–	0.16
Xylo–saccharides	5.37	–	–	0.08[d]	–	–	5.45
Arabinose	0.85	–	–	–	–	–	0.85
Glucuronic acid	0.06	–	–	–	–	–	0.06
Cello–saccharides	–	2.94[b]	–	–	17.05	–	19.99
Fructose	–	0.01[c]	–	–	0.65[e]	–	0.66
Dehydrated compounds	**0.22**	**–**	**–**	**–**	**3.84**	**–**	**4.06**
5–HMF	0.10	–	–	–	3.12	–	3.22
Furfural	0.03	–	–	–	0.22	–	0.25
Levoglucosan	0.09	–	–	–	0.50	–	0.59
Fragmented compounds	**1.63**	**–**	**–**	**–**	**2.25**	**–**	**3.88**
Erythrose	–	–	–	–	0.45	–	0.45
Methylglyoxal	1.07	–	–	–	1.19	–	2.26
Glycolaldehyde	0.56	–	–	–	0.61	–	1.17
Organic acids	**0.23**	**–**	**–**	**–**	**0.45**	**–**	**0.68**
Lactic acid	0.05	–	–	–	0.14	–	0.19
Glycolic acid	0.06	–	–	–	0.15	–	0.21
Formic acid	0.12	–	–	–	0.16	–	0.28
Lignin–derived products	**–**	**–**	**13.90**	**–**	**–**	**6.46**	**20.36**
Vanillin	–	–	0.06	–	–	0.05	0.11
Isoeuginol	–	–	0.02	–	–	0.02	0.04
Coniferaldehyde	–	–	0.15	–	–	0.05	0.20
Coniferyl alcohol	–	–	0.81	–	–	0.11	0.92
Dimeric, trimeric, and higher products	–	–	12.86	–	–	6.23	19.09
Total	21.32	2.95	13.90	0.25	24.31	6.46	69.19
Unknowns							18.57
Water-insoluble residue							12.24

[a] Including the transient time
[b] Cello-saccharides produced from hydrolysis of para-crystalline cellulose
[c] Fructose, as an isomerized product of glucose, is considered as the hydrolyzed product from cellulose
[d] Glucomanno-saccharides and xylo-saccharides from the hemicelluloses incompletely hydrolyzed in the first stage; in case of glucomanno-saccharides, mannose derived from an isomerization of glucose is included as well
[e] Acetic acid produced from decomposition of cellulose

treated by two-step hot-compressed water at 230°C/10 MPa/15 min and 280°C/10 MPa/30 min. The results clearly show that the water-soluble portion contained 40.14% (= 40.21%–0.07%) hydrolyzed products as in forms of various saccharides, uronic acid, and acetic acid [19.49% (= 19.24% + 0.25%) from hemicelluloses and 20.65% (= 2.95% + 17.77%–0.07%) from cellulose], and their decomposed compounds (dehydrated and fragmented compounds plus organic acids) are 8.69% (= 4.06% + 3.88% + 0.68% + 0.07%), while lignin-derived products were 20.36% (= 13.90% + 6.46%). Apart from these, 18.57% unidentified products in the water-soluble portion were obtained and considered as unknowns. On the other hand, the water-insoluble residue, which was only 12.24%, was composed entirely of lignin.

The hydrolyzed saccharides from *O*-acetyl-galactoglucomannan hemicellulose included glucomanno-saccharides, such as mannose, glucose, and oligomeric glucomannan, galactose, and acetic acid, whereas those from arabino-4-*O*-methylglucuronoxylan hemicellulose were xylose, xylo-oligosaccharides, arabinose, and glucuronic acid. On the other hand, saccharides from cellulose included glucose and cello-oligosaccharides as well as fructose, an isomerized product from glucose.

Recently, Matsunaga et al.[32] treated Japanese cedar with subcritical water by using the semi-flow-type reactor. It was reported that the highest total recovered saccharide yield of about 37.26 wt% on extractive-free wood flour basis could be achieved at 310–320°C, 25 MPa, and 65 g/min flow-rate without any pretreatment to improve the wettability of the wood meal. In comparison with the total hydrolyzed compounds obtained in this study (40.14 wt%), the two-step hot-compressed water treatment has been proved to give higher yields of saccharides.

Because the chemical composition of Japanese cedar on an extractive-free basis is 19.10%, 48.32%, and 32.58% for hemicelluloses, cellulose, and lignin, respectively, the total products from hemicelluloses accounted for 112.93 [= (21.32 + 0.25)/19.10 × 100] wt%, lignin for 100.06 [= (13.90 + 6.46 + 12.24)/32.58 × 100] wt%, whereas cellulose was 56.42 [= (2.95 + 24.31)/48.32 × 100] wt% on each constituent basis.

In hemicelluloses, if the figure is calculated without a water molecule jointly, it will be around 100%. For lignin, there existed lignin-derived products (13.90 + 6.46) and water-insoluble residue (12.24) so that most of the lignin-derived products were identified. On the other hand, the residue of Japanese cedar after the two-step treatment did not show any cellulose, as inspected by the Klason lignin determination. Thus, the total products from cellulose were relatively low in comparison with hemicelluloses and lignin. However, this missing part of cellulose is not yet known.

Decomposition pathway of hemicelluloses

Cellulose hydrolysis as treated by hot-compressed water has been well and adequately studied.[33,34] In our previous work,[15] moreover, its decomposition pathway has been reported. However, there is no hydrolysis pathway of softwood hemicelluloses reported in detail. In this article, the pathways of both major and minor hemicelluloses in softwood as treated with hot-compressed water were proposed independently.

Figure 7 shows the decomposition pathway of the major hemicellulose in softwood, *O*-acetyl-galactoglucomannan. The dehydration reaction could be confirmed because dehydrated products (5-HMF and levoglucosan) from hexoses (glucose, mannose, and galactose) were detected in the first stage, while the formation of the fragmentation product (methylglyoxal) could be attributed to the fragmentation reaction of theses hexoses as well. The other possible fragmented product, erythrose from hexoses, on the other hand, was not detected in the first stage. It could be, therefore, inferred that the 6-carbon carbohydrates might be stable or the decomposition into erythrose might not occur at the lower temperature of the first stage. In addition, although a large amount of glucomanno-saccharides was produced in the first stage, much less glucose was formed, as described earlier. As a result, fructose was scarcely produced at this stage, as seen in Table 2. The other reason that might explain this low fructose yield is that the isomerization reaction preferentially proceeds in a higher temperature under the studied conditions. For this reason, thus the isomerization of glucose to fructose was not included in this proposed pathway of the hemicellulose.

The decomposition pathway of the minor hemicellulose in softwood, arabino-4-*O*-methylglucuronoxylan, is shown in Fig. 8. In the same manner, its dehydration and fragmentation reactions were confirmed by the production of furfural, glycolaldehyde, and methylglyoxal from the constituent pentoses in this hemicellulose such as xylose and arabinose.

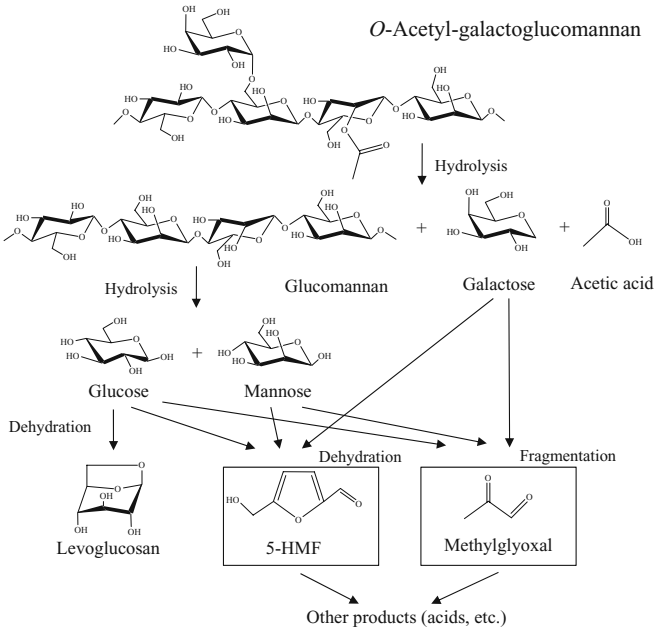

Fig. 7. Proposed decomposition pathway of *O*-acetyl-galactoglucomannan in Japanese cedar as treated by two-step semi-flow hot-compressed water at 230°C/10 MPa/15 min and 280°C/10 MPa/30 min

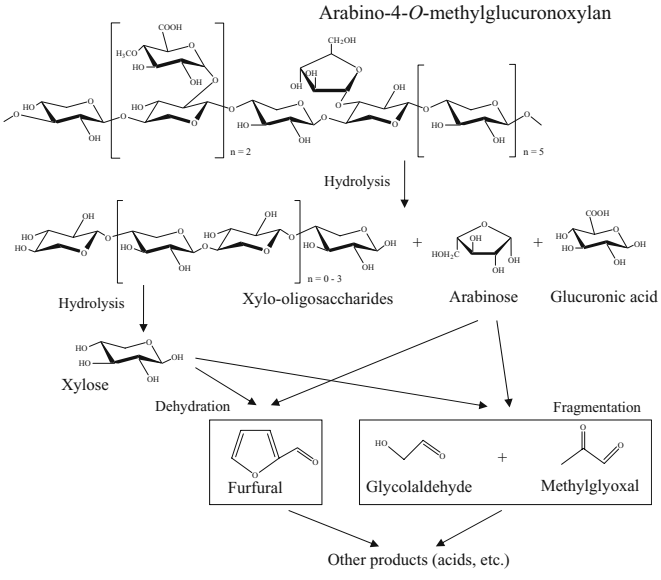

Fig. 8. Proposed decomposition pathway of arabino-4-*O*-methylglucuronoxylan in Japanese cedar as treated by two-step semi-flow hot-compressed water at 230°C/10 MPa/15 min and 280°C/10 MPa/30 min

Concluding remarks

The two-step semi-flow hot-compressed water treatment was applied to the hydrolysis of Japanese cedar. The two-step treatment was found to be effective to obtain hydrolyzed products from hemicelluloses and cellulose, separately. As a result, the predominant softwood hemicellulose, *O*-acetyl-galactoglucomannan (glucomannan), was hydrolyzed to be glucomanno-saccharides, such as mannose,

glucose, and oligomeric glucomannan, galactose, and acetic acid, whereas the minor component of softwood hemicelluloses, arabino-4-*O*-methylglucuronoxylan (xylan), was xylo-saccharides, such as xylose and xylo-oligosaccharides, glucuronic acid, and arabinose. The para-crystalline cellulose was found to be readily hydrolyzed at 230°C/10 MPa into cello-saccharides, such as glucose and cello-oligosaccharides, in the first stage, starting from the retention time of 10 min. This result was evidently attributed to the same finding in our previous study on Japanese beech[15] that there existed also the hydrolysis of para-crystalline cellulose at the same conditions in the first stage, while crystalline cellulose was hydrolyzed at 280°C/10 MPa. In addition, glucose, a hydrolyzed product of cellulose, was found to be isomerized to fructose and mannose during the second stage. Lignin was decomposed at an even lower temperature to produce coniferyl alcohol and its aldehyde and fragmented compounds. These lines of information are very important and useful to utilize efficiently various kinds of lignocellulosic materials for biochemicals and biofuels.

Acknowledgments This work has been done as part of the NEDO project (FY 2007–2010) "Eco-ethanol production from lignocellulosics with hot-compressed water treatment followed by acetic acid fermentation and hydrogenolysis," and under the partial financial support of the Global Center of Excellence (GCOE) Program, Kyoto University. The authors gratefully acknowledge all their support.

References

1. Solomon BD, Barnes JR, Halvorsen KE (2007) Grain and cellulosic ethanol: history, economics, and energy policy. Biomass Bioenerg 31:416–425
2. Galbe M, Zacchi G (2002) A review of the production of ethanol from softwood. Appl Microbiol Biotechnol 59:618–628
3. Walch E, Zemann A, Schinner F, Bonn G, Bobleter O (1992) Enzymatic saccharification of hemicellulose obtained from hydrothermally pretreated sugar cane bagasse and beech bark. Bioresour Technol 39:173–177
4. Torget R, Werdene P, Himmel M, Grohmann K (1990) Dilute acid pretreatment of short rotation woody and herbaceous crops. Appl Biochem Biotechnol 24–25:115–126
5. Targo ski Z (1985) Alkali process for enhancing susceptibility of autohydrolysed beech sawdust to enzymatic hydrolysis. Enzyme Microb Technol 7:126–128
6. Heitz M, Capek-Ménard E, Koeberle PG, Gagné J, Chornet E, Overend RP, Taylor JD, Yu E (1991) Fractionation of *Populus tremuloides* at pilot plant scale: optimization of steam pretreatment conditions using the STAKE II technology. Bioresour Technol 35:23–32
7. Saka S, Konishi R (2001) Chemical conversion of biomass resources to useful chemicals and fuels by supercritical water treatment. In: Bridgwater AV (ed) Progress in thermochemical biomass conversion. Blackwell, Oxford, pp 1338–1348
8. Aida TM, Sato Y, Watanabe M, Tajima K, Nonaka T, Hattori H, Arai K (2007) Dehydration of D-glucose in high temperature water at pressures up to 80 MPa. J Supercrit Fluids 40:381–388
9. Sasaki M, Kabyemela B, Malaluan R, Hirose S, Takeda N, Adschiri T, Arai K (1998) Cellulose hydrolysis in subcritical and supercritical water. J Supercrit Fluids 13:261–268
10. Ando H, Sakaki T, Kokusho T, Shibata M, Uemura Y, Hatate Y (2000) Decomposition behavior of plant biomass in hot-compressed water. Ind Eng Chem Res 39:3688–3693
11. Rogalinski T, Ingram T, Brunner G (2008) Hydrolysis of lignocellulosics biomass in water under elevated temperatures and pressures. J Supercrit Fluids 47:54–63
12. Ehara K, Takada D, Saka S (2005) GC-MS and IR spectroscopic analyses of the lignin-derived products from softwood and hardwood treated in supercritical water. J Wood Sci 51:256–261
13. Söderström J, Pilcher L, Galbe M, Zacchi G (2002) Two-step steam pretreatment of softwood with SO_2 impregnation for ethanol production. Appl Biochem Biotechnol 98–100:5–21
14. Bobleter O (1994) Hydrothermal degradation of polymers derived from plants. Prog Polym Sci 19:797–841
15. Lu X, Yamauchi K, Phaiboonsilpa N, Saka S (2009) Two-step hydrolysis of Japanese beech as treated by semi-flow hot-compressed water. J Wood Sci 55:367–375
16. Phaiboonsilpa N, Lu X, Yamauchi K, Saka S (2009) Chemical conversion of lignocellulosics as treated by two-step semi-flow hot-compressed water. In: Proceedings of the World Renewable Energy Congress 2009—Asia, 19–21 May 2009, Bangkok, Thailand, pp 235–240
17. Dence CW (1992) The determination of lignin. In: Lin SY, Dence CW (eds) Methods in lignin chemistry. Springer, Berlin, pp 33–63
18. Yang B, Wyman CE (2008) Characterization of the degree of polymerization of xylooligomers produced by flowthrough hydrolysis of pure xylan and corn stover with water. Bioresour Technol 99:5756–5762
19. Timell TE (1967) Recent progress in chemistry of wood hemicelluloses. Wood Sci Technol 1:45–70
20. Lundqvist J, Teleman A, Junel L, Zacchi G, Dahlman O, Tjerneld F, Stålbran H (2002) Isolation and characterization of galactoglucomannan from spruce (*Picea abies*). Carbohydr Polym 48:29–39
21. Kabyemela BM, Adschiri T, Malaluan R, Arai K (1999) Glucose and fructose decomposition in subcritical and supercritical water: detailed reaction pathway, mechanisms, and kinetics. Ind Eng Chem Res 38:2888–2895
22. Srokol Z, Bouche AG, van Estrik A, Strik RCJ, Maschmeyer T, Peters JA (2004) Hydrothernal upgrading of biomass to biofuel: studies on some monosaccharides model compounds. Carbohydr Res 339:1717–1726
23. Larsson PT, Wickolm K, Iversen T (1997) A CP/MAS ^{13}C NMR investigation of molecular ordering in celluloses. Carbohydr Res 302:19–25
24. Kruse A, Dinjus E (2007) Hot compressed water as reaction medium and reactant 2. Degradation reactions. J Supercrit Fluids 41:361–379
25. Palmqvist E, Hahn-Hägerdal B (2000) Fermentation of lignocellulosic hydrolysates. II: Inhibitors and mechanisms of inhibition. Bioresour Technol 74:25–33
26. Antal MJ, Leesomboon T, Mok WS, Richards GN (1991) Mechanism of formation of 2-furaldehyde from D-xylose. Carbohydr Res 217:71–85
27. Kallury RKMR, Ambidge C, Tidwell TT, Boocock DGB, Agblevor FA, Stewart DJ (1986) Rapid hydrothermolysis of cellulose and related carbohydrates. Carbohydr Res 158:253–261
28. Kabyemela BM, Adschiri T, Malaluan R, Arai K (1997) Degradation kinetics of dihydroxyacetone and glyceraldehyde in subcritical and supercritical water. Ind Eng Chem Res 36:2025–2030
29. Watanabe M, Aizawa Y, Iida T, Levy C, Aida TM, Inomata H (2005) Glucose reactions within the heating period and the effect of heating rate on the reactions in hot compressed water. Carbohydr Res 340:1931–1939
30. Kabyemela BM, Adschiri T, Malaluan RM, Arai K, Ohzeki H (1997) Rapid and selective conversion of glucose to erythrose in supercritical water. Ind Eng Chem Res 36:5063–5067
31. Yoshida K, Kusaki J, Ehara K, Saka S (2005) Characterization of low molecular weight organic acids from beech wood treated in supercritical water. Appl Biochem Biotechnol 121–124:795–806
32. Matsunaga M, Matsui H, Otsuka Y, Yamamoto S (2008) Chemical conversion of wood by treatment in semi-batch reactor with subcritical water. J Supercrit Fluids 44:364–369
33. Ehara K, Saka S (2002) A comparative study on chemical conversion of cellulose between the batch-type and flow-type systems in supercritical water. Cellulose 9:301–311
34. Sasaki M, Furukawa M, Minami K, Adschiri T, Arai K (2002) Kinetics and mechanism of cellobiose hydrolysis and retro-aldol condensation in subcritical and supercritical water. Ind Eng Chem Res 41:6642–6649