Molecular weight distributions of polysaccharides and lignin extracted from plant biomass with a polar ionic liquid analysed without a derivatisation process

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Kosuke Kuroda,[a,b] Yukinobu Fukaya,[b,‡] Tatsuhiko Yamada,[c] and Hiroyuki Ohno*[,a,b]

Polysaccharides and lignin, extracted from wheat bran with 1-ethyl-3-methylimidazolium methylphosphonate, were directly analysed with high performance liquid chromatography with the ionic liquid as an eluent (HPILC). Polysaccharides and lignin were clearly detected independently with the use of both refractive index detector and UV detector. Polysaccharides with lower molecular weight were obtained at 25 °C for 2h with extraction yield of only 4 %. Higher molecular weight polysaccharides were extracted with yield of 26 % at 120 °C. Similarly, high molecular weight polysaccharides were successfully obtained even at 80 °C from bran that was pre-treated with ionic liquid at 50 °C to extract low molecular weight fraction. Furthermore, similar extraction was carried out for wood biomass. Characteristics of pine and oak were observed in molecular weight distribution of the extracted polysaccharides and lignin. We also analysed the extracts from different parts of *Prunus × yedoensis 'Somei-yoshino'*. Polysaccharides from leaves were relatively low molecular weight than those from twigs. The present HPILC method has potential to analyse molecular weight distribution of components of plants easily and fast.

Introduction

Ionic liquids (ILs)[1] are widely studied as solvents for polymers that are insoluble in conventional molecular solvents.[2-4] Especially, since precisely-designed polar ILs dissolve both cellulose and hemicellulose under mild conditions,[5,6] ILs have been studied as media to extract cellulose from plant biomass.[5,7-13] To obtain efficient use of biomass, extraction of high molecular weight (Mw) polysaccharides is necessary in spite of their little solubility. While considerably high temperature leads to a complete dissolution of biomass,[14,15] it is not sure whether ILs at various conditions may indeed extract high Mw polysaccharides and lignin. Furthermore, higher temperature may induce decomposition of components of extracts[14] but it is not also clear. From these queries, there is a strong request to accurately analyse the relation between molecular weight distribution (MwD) of extracts and extraction condition.

To date, a method to analyse MwD of extracts from biomass through derivatisation has been reported.[16,17] However, this method is not suitable for analysis of extracts because MwD of the derivatised materials may change during the derivatisation and following processes.[16] Obviously, direct analysis of extracts without derivatisation is an important goal to achieve.

Polar ILs, in spite of less favourable physico-chemical properties such as relatively high viscosity, have been used as solvents for the analysis of cellulose.[18,19] We have already proposed a method to analyse the component of polysaccharides (cellulose and hemicellulose) extracted with ILs using ^{1}H NMR.[20] The use of a no-deuterium NMR combined with a solvent suppression technique, allowed us to carry out the measurements without need of ILs deuterisation[20], this enabling the analysis of polysaccharides in various ILs. This enabled the analysis of polysaccharides in various ILs. Furthermore, we have also demonstrated the use of polar ILs as eluents to high performance liquid chromatography (called HPILC) to reveal the MwD of cellulose dissolved in ILs.[21,22] In addition, this HPILC technique allows the analysis in a very wide MwD range by a single scan; HPILC technique is expected to be an effective method to analyse polysaccharides and lignin extracted with ILs without derivatisation. It has again to be ramarked that, due to the fact that derivatisation and washing processes lose low Mw compound in many cases, MwD detected with the

conventional methods did not show the exact profile of the extracts.

In this study, we have investigated the relation between the extraction conditions (temperature, time, kind of biomass, part of a biomass) and the MwD of extracted polysaccharides and lignin.

Experimental

Materials and Instruments

1-Ethylimidazole was purchased from Kanto Chemical Co. and used after drying over KOH and distillation. Dimethyl phosphite was purchased from Tokyo Chemical Ind. Co. and was used after distillation. Cellulose (Cellulose powder C, from Advantech Co., Ltd) and lignin (Lignin dealkaline, from Tokyo chemical industry Co., Ltd) was purchased and used without pretreatment. The amounts of water of IL samples were confirmed by Karl Fischer coulometric titration (Kyoto Electronics; MKC-510N). ^{1}H- and ^{13}C-NMR spectra for analysis of polysaccharides and confirmation of structures of ILs were performed with JEOL ECX 400 (JEOL Ltd.).

Synthesis of $[C_2mim][(MeO)(H)PO_2]$

1-Ethylimidazole (100g, 1.04 mol) and dimethyl phosphite (126g, 1.14 mol) were slowly mixed under an argon gas atmosphere at room temperature without solvent. The reaction mixture was stirred at 80 °C for 24h. The resulting liquid was washed repeatedly with excess dehydrated diethyl ether. The residual liquid was dissolved in dichloromethane, and the resulting solution was passed through a column filled with neutral activated alumina. After removal of dichloromethane, the residual liquid was dried *in vacuo* at 80 °C for 24h to give $[C_2mim][(MeO)(H)PO_2]$ as a colourless liquid.

Water content of $[C_2mim][(MeO)(H)PO_2]$ was measured with Karl Fischer Coulometric Titrator (Kyoto Electronics; MKC-510N). The IL with water content of less than 2000 ppm was used as both eluent and solvent. Structure of $[C_2mim][(MeO)(H)PO_2]$ was confirmed by ^{1}H- and ^{13}C-NMR spectra (JEOL ECX-400). ^{1}H-NMR δ_H (400 MHz; $CDCl_3$; Me_4Si); 1.58 (3H, t, J = 7.3 Hz, NCH_2CH_3), 3.55 (3H, d, J = 11.9 Hz, $POCH_3$), 4.06 (3H, s, NCH_3), 4.36 (2H, q, J = 7.3 Hz, NCH_2CH_3), 6.92 (1H, d, J = 588.5 Hz, PH), 7.58 (2H, d, J = 11.3 Hz, NCHCHN), 10.66 (1H, s, NCHN). ^{13}C-NMR δ_c (100 MHz; $CDCl_3$; Me_4Si); 15.22 (NCH_2CH_3), 35.87 (NCH_3), 44.98 (NCH_2CH_3), 50.05 ($POCH_3$), 121.35 (NCHCHN), 123.17 (NCHCHN), 138.40 (NCHN).

Methods

HPLC setup

Components in the HPLC system used were high pressure durable pump (LC-20AD; Shimadzu), an injector (7725; Rheodyne) with a 5 μL loop, a UV-vis detector (SPD-20AV; Shimadzu), and a refractive index detector (Shodex RI-71; Showa Denko). Columns filled with silica gel (Shodex KW-402.5-4F, 4.6 mm (inner diameter) × 300 mm, 3 μm, and KW-405-4B, 4.6 mm (inner diameter) × 50 mm, 5 μm; Showa Denko) were used in tandem. The columns were heated at 55 °C using a column oven (CTO-10Avp; Shimadzu). The RI detector cells were maintained at 40 °C. The flow rate was set at 0.01 mL·min^{-1}. Sodium polystylenesulfonate standards from Sowa Science Corporation with molecular weight ranging from 3,000 to 2,350,000 Da were used for calibration of the SEC system because pullulan shows no UV-absorption. For data acquisition and processing we used the software package SIC-480 II XP (SIC). $[C_2mim][(MeO)(H)PO_2]$ with water less than 2000 ppm was used as an eluent under an argon atmosphere.

HPILC measurement of cellulose and lignin

Suspensions of cellulose or lignin (1.0 mg each) in 200 mg of dried $[C_2mim][(MeO)(H)PO_2]$ were prepared under dry nitrogen gas atmosphere. The mixtures were gently stirred at room temperature until the solutions became homogeneous and clear. The solutions were directly injected into HPILC and measured.

Analysis of extracts from biomass with ILs by HPILC

The milled biomass from different sources were used; wheat bran (herbaceous plant, 42-50 mesh), pine (softwood, *Picea jezoensis*, 36-200 mesh), and oak (hardwood, *Quercus crispula*, 36-200 mesh) without defatting. Detailed procedure according to *Prunus* × *yedoensis* was described below. Biomass was dried under reduced pressure before use. The dried biomass powder (70 mg) was added into 1.0 g of dried $[C_2mim][(MeO)(H)PO_2]$ and stirred at 200 rpm in an oil bath. The resulting solutions were centrifuged at 14,800 rpm (16200 G) from 10 to 60 min for removing residue. The supernatants were mixed with 70 wt% of DMSO and the resulting solutions were stirred at 80 °C for 3min. After filtration with glass filter under reduced pressure, the samples were injected to HPILC.

When we extracted polysaccharides from IL-treated bran, we subjected three IL/bran solutions to 1st extraction (temperature: 50 °C, time: 2h, stirring: 200 rpm, feed bran: 70 mg, IL: 1.0 g). They were centrifuged and the precipitation was collected. The precipitation was dispersed into 40 ml of DMSO and mixed with vortex mixer for 2min, to strip any dissolved substances adsorbed or trapped within the solid texture. The solution was centrifuged (10000 G, 10min) and the supernatant was removed. For further washing, 40 ml of methanol was added and the solution was mixed with vortex mixer for 1min. The solution was centrifuged (10000 G, 10min) and the supernatant was removed. Washing process with methanol was repeated 2 times. After drying under reduced pressure at room temperature, we collected the IL-treated bran (over 80 mg) from three samples. The IL-treated bran (70 mg) was added into 1.0 g of fresh $[C_1mim][(MeO)(H)PO_2]$ and stirred (80 °C, 2h, 200 rpm).

Pretreatment of *Prunus* × *yedoensis*

Petal, leaf, and twig of *P.* × *yedoensis* were obtained on the campus of Tokyo University of Agriculture and Technology in April, 2014. They were freeze-dried and bark of the twig was peeled. They were fragmented by hands to be almost 1 mm in diameter.

Calculation of yield

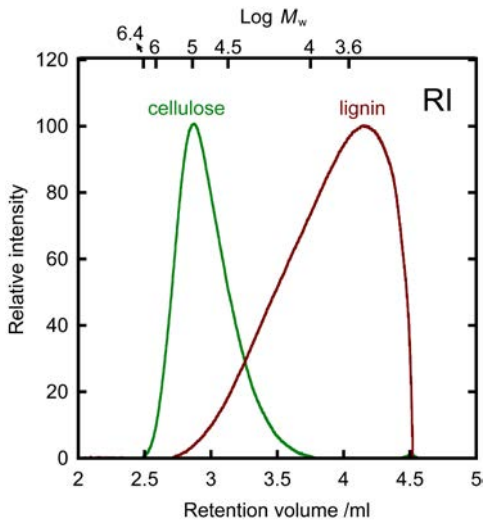
Yield was calculated based on peak area of RI-chromatograms with a hypothesis that signals appeared in RI-chromatogram are cellulose. We already reported that HPILC enabled quantitative analysis.[21] For yield calculation, following equation was used:

$$yield\ (\%) = \frac{weight\ of\ extract\ from\ chromatogram\ (mg)}{70\ (mg)}$$

Results and discussion

HPILC measurement with model polysaccharides and lignin

To analyse polysaccharides and lignin independently, combination of refractive index (RI) detector and UV detector should be effective. Since lignin has UV absorption from 500 nm, lignin is detectable with both RI and UV detectors. On the other hand, both cellulose and hemicellulose have no UV absorption, and hence, detection of polysaccharides and lignin separately should be possible by using both detectors. However, the frequently-used imidazolium-type polar ILs also have UV absorption based on the imidazolium ring. We have preliminarily performed UV spectrometry of lignin dissolved in 1-ethyl-3-methyl-imidazolium methylphosphonate ($[C_2mim][(MeO)(H)PO_2]$), as shown in Fig. S1 (see ESI). Absorption of UV light of $[C_2mim][(MeO)(H)PO_2]$ was found from 350 nm with saturation at 260 nm. Lignin dissolved in $[C_2mim][(MeO)(H)PO_2]$ shows intense UV absorption spectrum. We chose the detection wavelength of 300 nm for detection of lignin. At this wavelength, appreciable detection of lignin was possible with relatively low absorption of the imidazolium ring as the background.



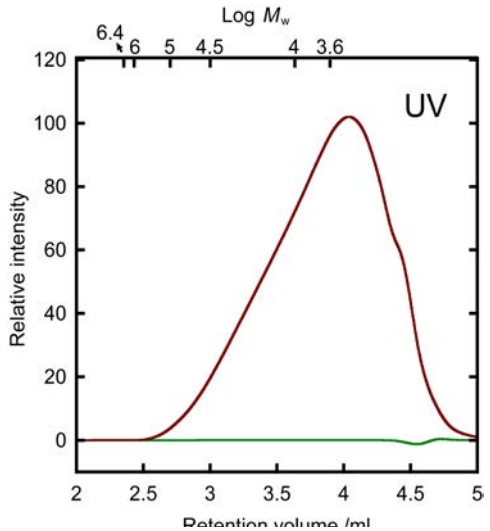


Fig. 1 RI-chromatogram (top) and UV-chromatogram (bottom) of the solutions of cellulose or lignin dissolved in $[C_2mim][(MeO)(H)PO_2]$.

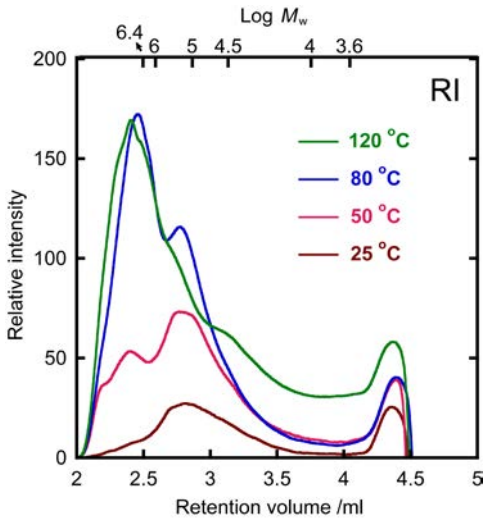
To confirm that cellulose and lignin were distinguished by use of the two detectors, solutions of cellulose or lignin in $[C_2mim][(MeO)(H)PO_2]$ (0.5 wt%) were measured using HPILC (Fig. 1). As expected, the signals of cellulose and lignin were detected with RI detector (Fig. 1, top), while with UV detector, only lignin was detected (Fig. 1, bottom). There was difference in peak of intensity of the peaks between the two detectors depending on their sensitivity (7 mV with RI detector and 265 mV with UV detector). For an easy comparison of the chromatograms, these signals were normalised based on the intensity of the peaks for lignin: maximum intensity of the peaks of lignin was calculated to be 100.

HPILC analysis of extracts from wheat bran

Fig. 2 shows MwD of extracts from wheat bran with $[C_2mim][(MeO)(H)PO_2]$ at various temperature. Wheat bran (70 mg) was added into 1.0 g of $[C_2mim][(MeO)(H)PO_2]$ and stirred for 2h. To decrease viscosity, dimethyl sulfoxide was added. The resulting solution was measured after filtration. Comparing the RI- and UV-chromatograms, we see that the former showed much higher intensity than the latter (e.g. 170 vs. 4 at 80 ^{o}C). This clearly indicates that RI-chromatograms are attributed mainly to cellulose and hemicellulose. Three peaks were observed In the RI-chromatograms, namely at 2.4,

2.8, and 4.4 ml of the retention volume. Among them, the peak at 4.4 ml was assigned to monomeric or oligomeric sugar and other low Mw compounds. Two fractions, observed in between 2.0 and 3.5 ml (Mw of over 10^4) were suggested to be attributed to mainly hemicellulose and cellulose respectively according to the literature[17]. In the UV-detected chromatograms in Fig. 2, mainly two peaks were observed at higher and lower retention volume than 3.5 ml, respectively. The peaks at higher retention volume should be assigned to both lignin and low Mw aromatic species. The peak at low retention volume are presumably considered to be lignin-carbohydrate complexes (LCCs).[17]

At lower temperature, only low Mw polysaccharides were detected with an extracted yield calculated from the chromatogram to be approximately 4 %. By increasing the extraction temperature, high Mw polysaccharides were obtained with an increase of the extracted yield. At 120 °C, the extracted amount of high Mw components was almost the same as that detected at 80 °C but with an increased yield (26 %), attributed to the extraction of low Mw polysaccharides (at around 3.0 to 4.2 ml). Additionally, some decomposition of polysaccharides was detected at 120 °C, as suggested by the decreased intensity of the signal at 2.8 ml. According to lignin (UV-chromatogram), there is no change in MwD between 25 to 80 °C, but extracted amount



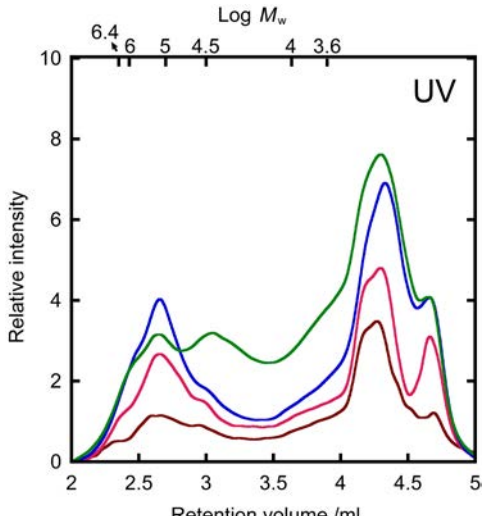


Fig. 2 Temperature-dependence of chromatogram of extracts from wheat bran with [C_2mim][(MeO)(H)PO_2] upper: with RI detector, lower: with UV detector.

increased at higher temperature. At 120 °C, decrease of the peak at 2.6 ml and appearance of new peak at 3.1 ml were observed. They are attributed to the partial decomposition of lignin. It is known that partial decomposition of lignin generally occurs at temperature over 100 °C.[23,24]

The relation between extraction temperature and extracted amount of cellulose and xylan (main hemicellulose of wheat bran) with ^{1}H NMR using a quite similar IL, 1,3-dimethylimidazolium methyl methylphosphonate, was previously investigated in our laboratory.[24] In our report, it is reported that extracted amount of only xylan increases at temperature in between 80 and 120 °C. Therefore, the increased signal between 3.0 and 4.2 ml in the RI-chromatogram was attributed to be xylan. Furthermore, since decomposition of LCCs was observed at 120 °C in the UV-chromatogram, the increase of xylan signal was caused by degradation of the LCCs.

Fig. 3 shows MwD of extracted polysaccharides and lignin at various extraction time at 80 °C. As the figure clearly shows, the extracted amount and fraction of high Mw cellulose increased as increasing the treatment time. However, compared to the effect of temperature (see Fig. 2), the extraction time affected the fraction of high Mw polysaccharides only a bit. Also the extracted amount of lignin also increased by increasing the extraction time.

The extraction process was also performed at 25 °C (see ESI, Fig. S2). As mentioned above, only low Mw polysaccharides were extracted at 25 °C for 2h, but longer extraction time (e.g. 96h) led to extraction of high Mw polysaccharides. This result strongly suggests that [C_2mim][(MeO)(H)PO_2] has an ability to extract high Mw polysaccharides even at 25 °C; however, other factors, such as viscosity of ILs, prevent the extraction of high Mw polymers. It should be noted, nevertheless, that low Mw polysaccharides were main components of extracts at 25 °C even after 96h stirring with an extracted polysaccharides yield (14 %) similar

to those obtained at 50 °C for 2h (17 %) and at 80 °C for 10min (13 %), respectively. Undoubtedly, the elevating temperature certainly accelerated the extraction of polysaccharides.

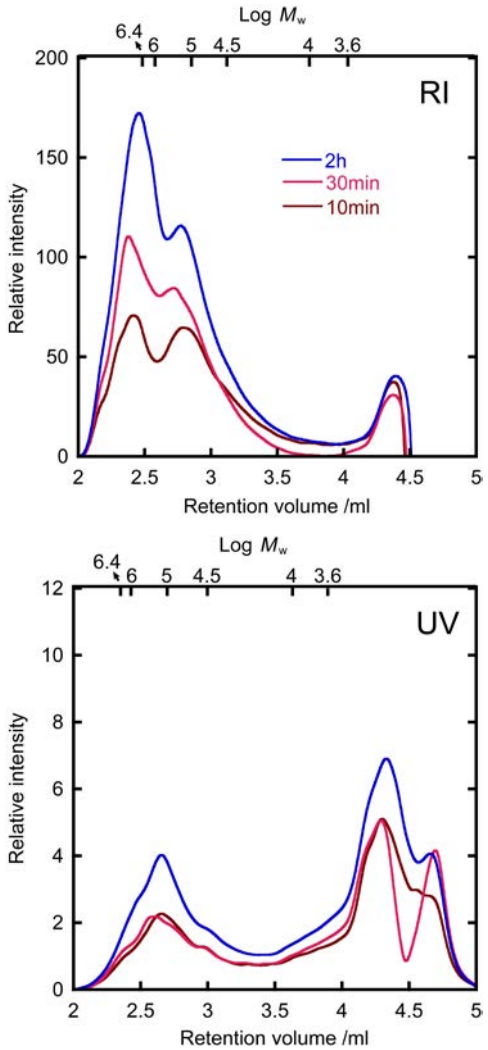
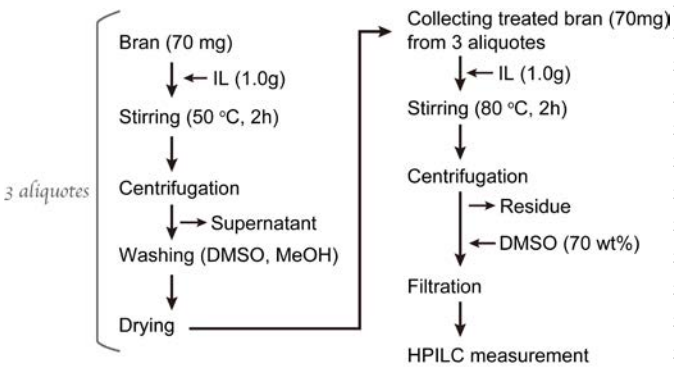


Fig. 3 Chromatograms of extracts for various extraction time from wheat bran with [C_2mim][(MeO)(H)PO_2] at 80 °C upper: with RI detector, lower: with UV detector.



Scheme 1 Extraction at 80 °C from bran treated with [C_2mim][(MeO)(H)PO_2] at 50 °C.

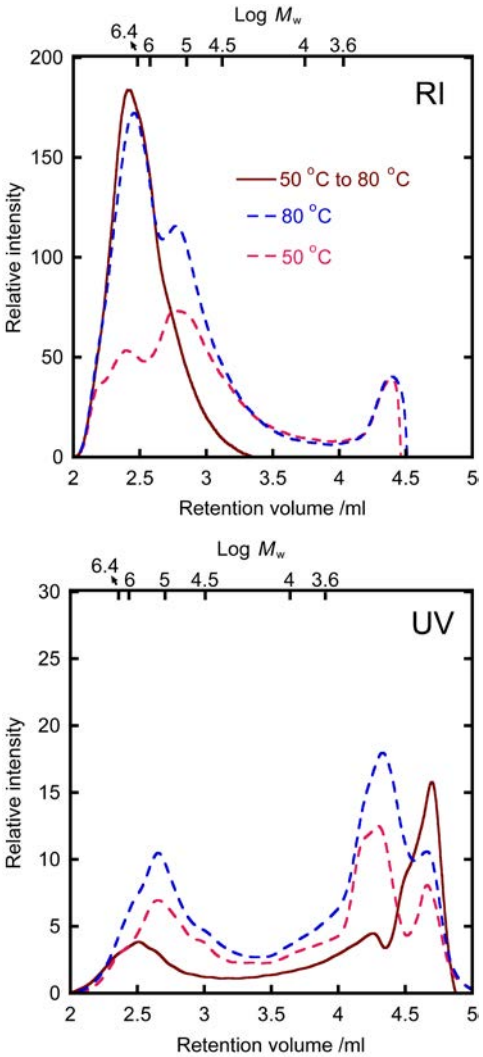


Fig. 4 Chromatograms of the extract from wheat bran (80 °C) after treatment with [C_2mim][(MeO)(H)PO_2] at 50 °C upper: with RI detector, lower: with UV detector.

Our results in fact show that the fraction of high Mw polysaccharides in extracts was affected by the extraction temperature more than extraction time. Therefore, we expected that only high Mw polysaccharides could be obtained from the bran pre-treated at lower temperature. Bran was first stirred at 50 °C for 2h, and successively the treated bran was immersed in fresh IL at 80 °C (summarised in Scheme 1). As shown in Fig. 4, only high Mw polysaccharides were successfully extracted. Furthermore, lignin content in the extract from IL-treated bran was found to be low. These results show that extracts predominantly composed of high Mw polysaccharides were successfully obtained. This is the first report on the control of MwD of the extracted polysaccharides with single and pure IL just by varying temperature. This fact should be helpful to improve problems concerning industrial processes such as removing excess co-solvents.

HPILC analysis of extracts from wood powder

We extracted polysaccharides from pine (*Picea jezoensis (Sieb. et Zucc.) Carr.*) and from oak (*Quercus crispula* Blume) as a typical examples of softwood and hardwood, respectively. The extracts were analysed and compared as shown in Fig. 5. As well known, it is more difficult to extract polysaccharides from woody biomass than that from herbaceous species, and this trend was also observed in the present experiments; extraction yield from both pine and oak was 3 % at 80 °C (around 1/5 of that from bran). Additionally, Mw of the extracted lignin from woody biomass was larger than that from bran extracted under the same condition.

When we extracted polysaccharides from two different woody biomasses at 80 °C for 2h, a bimodal distribution in MwD was observed in the RI-chromatograms (Fig. 5). The peaks at smaller and larger retention volume in the RI-chromatogram should be attributed to polysaccharides and lignin, considering the profile of UV-chromatograms. Extracts from pine contained low Mw polysaccharides and low Mw lignin, compared to oak. The broad MwD of polysaccharides and narrow MwD of lignin observed in pine were also seen in the case of cedar (Softwood, *Cyptomeria japonica D. Don*) as shown in Fig. S3 in ESI. Thus, these data can be concur to clarify the characteristics of softwood (the structures of lignin in softwood and hardwood are different[25]). All this considering, we may state that our HPILC analysis should contribute to elucidate the presently unsolved discussion on the relation between properties of components from various wood samples and their lignin structures.

Compared with the data extracted at 80 °C, the extracted amount of polysaccharides was a little increased when the oak powder was treated at 120 °C (yield: 6 %, this value includes both polysaccharides and lignin). Increase in the extracted yield of lignin was also found. In the extracts from pine, increase of extracted yield of polysaccharides was also confirmed (yield: 5 %), but the extracted amount of lignin was not changed. This indicates that in the case of pine, polysaccharides can be preferentially obtained at higher temperature. From the viewpoint of MwD, fraction of high Mw polysaccharides was found to increase by changing the treatment temperature from 80 °C to 120 °C.

Figure 6 compares the MwD of extracts after 2h and 6h, respectively at 80 °C (Fig. 6). In the case of pine, no change was observed in the RI- and UV- chromatograms. It strongly suggested that extractable components in pine powder were

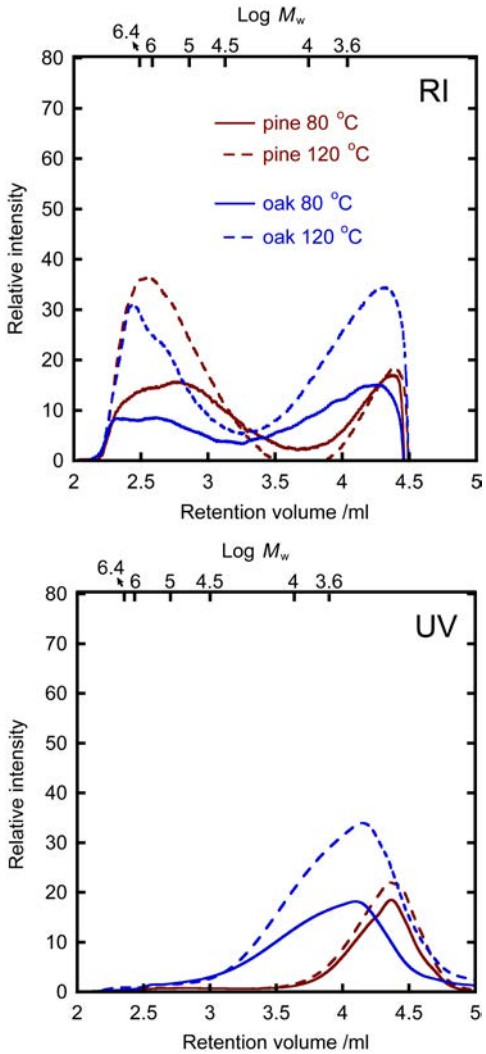


Fig. 5 Chromatograms of extracts at 80 °C and 120 °C from woody biomass with $[C_2mim][(MeO)(H)PO_2]$ upper: with RI detector, lower: with UV detector.

sufficiently extracted within 2h at 80 °C. In the case of oak, the relative intensity of both the RI- and UV-detected chromatograms was found to increase by longer treatment time. This might be due to the different assembled structure of LCCs. We however do not have any supporting data to confirm this hypothesis, hence, a more complete analysis of these biomasses should be carried out to clarify this point.

HPILC analysis of extracts from different parts of *Prunus* × *yedoensis 'Somei-yoshino'*

For efficient use of plant biomass, various regions of plant biomass such as twigs and leaves should be utilized. Since they are intrinsically different tissues, the extracted polysaccharides are expected to have a different MwD and therefore, a different profile of the extraction. Analysis of MwD of component polymers in different tissues in plants is of another great interest in plant biology. We have examined leaves, petals, and twigs of *Prunus* × *yedoensis 'Somei-yoshino'* as typical biomass in Japan in spite of hardness of the woody part of cherry tree.

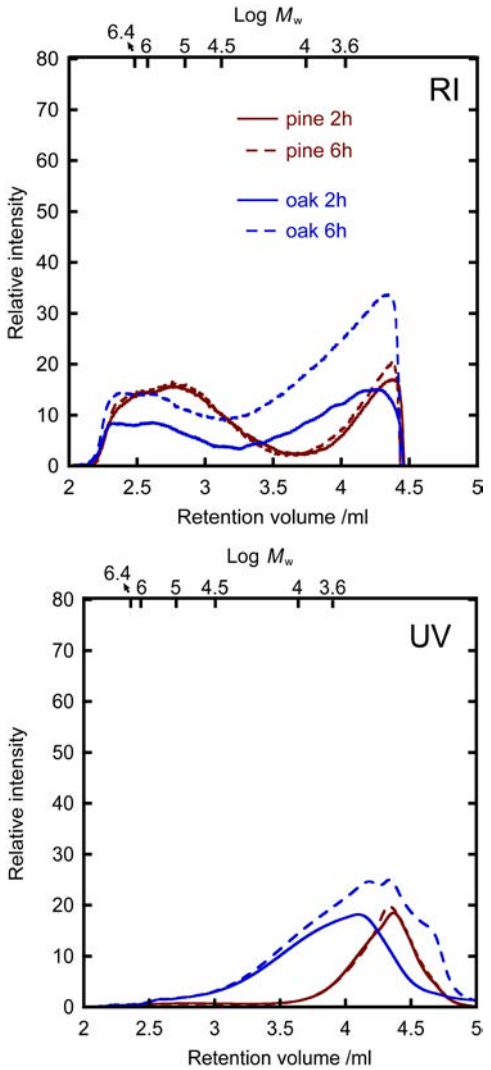


Fig. 6 Chromatograms of extracts for 2h and 6h from woody biomass with [C_2mim][(MeO)(H)PO_2] (load amount: 70 mg, IL amount: 1.0 g extraction temperature: 80 °C, upper: with RI detector, lower: with UV detector).

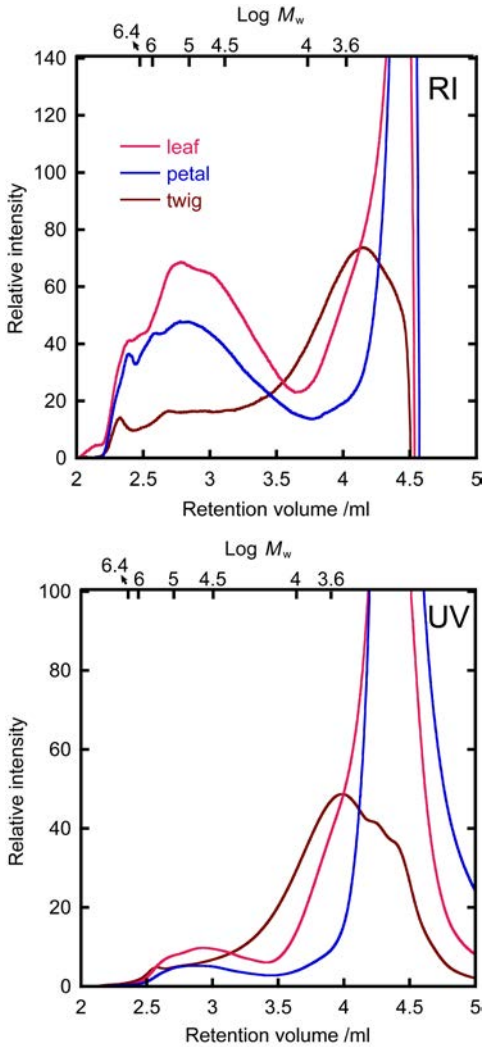


Fig. 7 Chromatograms of extracts from different parts of *Prunus* × *yedoensis 'Somei-yoshino'* with [C_2mim][(MeO)(H)PO_2] at 120 °C upper: with RI detector, lower: with UV detector.

These were added into [C_2mim][(MeO)(H)PO_2] and stirred at 120 °C for 2h. A large amount of polysaccharides was extracted from leaves compared to that from twigs (Fig. 7). In terms of leaves, relatively low Mw polysaccharides were mainly extracted, while high Mw as well as low Mw polysaccharides were extracted from twigs. MwD of polysaccharides extracted from petals was almost similar to that extracted from leaves in spite that the extracted amount was somewhat lower than that obtained from petals. This may be related to the different role and life-span of the tissues. Lignin extracted from twigs showed the largest Mw among them. This result is fairly comprehensible from the similar viewpoint as mentioned above. It should be noted here that UV-chromatograms of leaf and petal may include other low Mw aromatic compounds such as flavonoids and chlorophyll. Analysis of these aromatic compounds will be the further task, and it is expected to be carried out with multi-wavelength spectrophotometers.

We also performed the extraction experiments at 80 °C for 2h and analysed them (Fig. 8 and Fig. S4 in ESI). Except for increase of extracted amount from twigs, significant change was not observed. This indicates that heating at 80 °C was enough to extract polysaccharides from leaves and petals. On the other hand, higher temperature (> 120 °C) might be preferred for efficient extraction from twigs. These reflect different composition of lignin in different parts of plants.

HPILC was successfully applied to direct analysis of extracts from woody biomass. It is noted here that some improvements lead to more convenient and precise measurement. At present, one measurement needs 8h due to slow feeding based on high viscosity of ILs. Elevating column temperature or/and using pressure-durable column are expected to be potential solutions. Additionally, the void volume of 2.2 ml is another critical point because the analysis of super high Mw might not be precise. Seeking a column applicable to super high Mw should be needed for the improvement.

In the present paper, HPILC was successfully examined to analyse extracted both polysaccharides and lignin. This methodology is also useful to get a clue to extract only high or low Mw cellulose. MWD of cellulosic materials is very important when their physico-chemical properties are controlled.

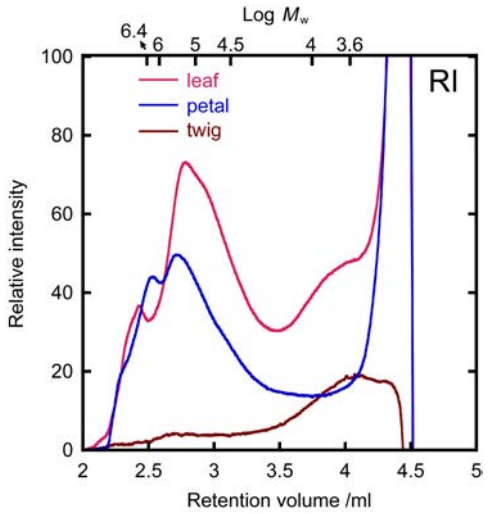


Fig. 8 Chromatograms of extracts from different parts of *Prunus* × *yedoensis* *'Somei-yoshino'* with [C_2mim][(MeO)(H)PO_2] at 80 °C with RI detector.

Conclusions

We have examined the extraction power of a polar IL from a viewpoint of MwD of polysaccharides and lignin in extracts using HPILC. Higher extraction temperature led to increase of the fraction of high Mw polysaccharides and extracted amount. Whereas longer extraction temperature also improved the extraction yield, it was less effective than that by temperature change. Considering these, we have tried to extracted polysaccharides with desired Mw, showing that only high Mw polysaccharides were extracted at 80 °C from bran pre-treated at 50 °C with the same IL. Extracts from woody biomass were also investigated to find similar effect of temperature to that in case of bran. In a viewpoint of wood types, the broad MwD of polysaccharides and narrow MwD of lignin were seen in the case of softwood compared to hardwood. The findings can be a clue to establish efficient biorefinery against each of wood species. Polysaccharides extracted from different parts of *Prunus* × *yedoensis* were also analysed. It was observed that polysaccharides extracted from leaf were materials having relatively low Mw in respect to those extracted from twig. It was also found that treatment at 80 °C was appropriate to extract polysaccharides in the case of leaves and petals, providing a clue for the extraction of other valuable molecules from plants without partial decomposition.

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Notes and references

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† Electronic Supplementary Information (ESI) available: a UV-*vis* spectrum of lignin/[C_2mim][(MeO)(H)PO_2] solution; RI- and UV-chromatograms of extracts at 25 °C for various extraction time; chromatograms of cedar extracted at 80 and 120 °C; chromatograms of extracts from three parts of *P.* × *yedoensis* at 80 °C. See DOI: 10.1039/b000000x/

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