1H NMR Evaluation of Polar and Nondeuterated Ionic Liquids for Selective Extraction of Cellulose and Xylan from Wheat Bran

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^{1}H NMR evaluation of polar and non-deuterated ionic liquids for selective extraction of cellulose and xylan from wheat bran

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ABSTRACT: Cellulose and xylan, extracted from wheat bran with polar ionic liquids (ILs), were quantified using ^{1}H NMR spectroscopy. Both No-D NMR and solvent suppression technique were applied to realize direct analysis of extracts in non-deuterated ILs. As models of extracts, mixtures of cellulose and xylan dissolved in ILs were measured with ^{1}H NMR spectroscopy. There was a linear relation between mixing ratio and specific peak area of each polysaccharide. Extracts from bran in ILs were analyzed with the obtained calibration curve. This NMR analysis

was confirmed to be applicable to three representative ILs used for extraction of polysaccharides. Relation between extracted amount and extraction conditions was obtained.

Introduction

Ionic liquids (ILs)[1] are a recently developed family of solvents that dissolve polymers which are insoluble in conventional solvents.[2-4] Precisely designed polar ILs dissolve cellulose and hemicellulose under mild conditions,[5-6] and ILs have accordingly been used as media for extracting those polysaccharides from plant biomass.[7-12] Cellulose and hemicellulose should be extracted selectively due to their distinct physicochemical properties. Only few papers identify the components of polysaccharides, however; most report only entire weights of the extracts. A convenient method for quantifying these polysaccharides would be highly desirable.

The composition of extracts from biomass using ILs has been analyzed via indirect methods.[13-14] Unfortunately, this method involves complex pretreatments before analysis, including precipitation of polysaccharides with alcohol or water, washing, drying, and hydrolyzing the polysaccharides with sulfonic acid. The data obtained often contain considerable errors due to incomplete precipitation of all the extracts. The sum of the amount of precipitates and that of undissolved residues seldom reached 100 % of the untreated biomass.

In any direct analysis of IL extracts, new methods must be developed with ILs as solvents. ILs which can dissolve cellulose have recently been applied for some analyses.[15-17] As an example, ILs were used as eluents for high performance liquid chromatography so as to directly analyze depolymerization of cellulose in ILs with ultrasonication.[18] We have also reported the ^{1}H NMR analysis of cellulose in non-deuterated ILs.[19] This measurement was made with the aid of a no-deuterium (No-D) NMR technique[20-21] and a solvent suppression technique (water

suppression enhanced through T_1 effect: WET). These techniques enable the detection of cellulose in a variety of non-deuterated ILs. Compared to previously reported studies on the NMR measurements with fully deuterated ILs (in spite of only few deuterated ILs were reported)[22-23], these techniques greatly facilitate direct NMR measurements of target materials in many non-deuterated ILs.

In this study we chose three typical ILs for treatment of wheat bran, and determined their capability at extracting cellulose and xylan (the main hemicellulose contained in bran) together with their physico-chemical properties, in order to exhibit the effectiveness of the present method.

Experimental

Materials and Instruments

1-Methylimidazole was purchased from Kanto Chemical Co., Inc., and dried with KOH and distilled before use. Dimethyl methylphosphonate and dimethyl sulfate were purchased from Tokyo Chemical Ind. Co., Ltd. and distilled before use. Acetic acid and toluene were purchased from Kanto Chemical Co., Inc., and used as received. Microcrystalline cellulose powder (cellulose powder C) was purchased from Advantec Toyo Co. and used after drying under reduced pressure. Xylan was purchased from Tokyo Chemical Ind. Co., Ltd. It was dissolved in dimethyl sulfoxide (DMSO) and precipitated by water. The precipitation was washed with excess amount of water and dried before use. 3-(Trimethylsilyl)-propanesulfonic acid sodium salt (TMS salt) was purchased from Merck KGaA and used as received. The amount of water of IL samples was confirmed by Karl Fischer coulometric titration (Kyoto Electronics; MKC-510N). Thermogravimetric analysis was performed on a SII TG/DTA 7200 (Seiko Instruments

Inc.). ^{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.).

Preparation of ILs

1,3-Dimethylimidazolium methyl methylphosphonate ([C_1mim][(MeO)(Me)PO_2]) was synthesized as follows. Dimethyl methylphosphonate (50 g; 0.40 mol) and 1-methylimidazole (36.4 g; 0.44 mol) were added into 100 ml of toluene under an argon gas atmosphere at room temperature. The reaction mixture was stirred at 120 ^{o}C for 48h. The resulting liquid was dried *in vacuo* and 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 ^{o}C. ^{1}H NMR (400 MHz; $CDCl_3$; Me_4Si) δ = 1.26 (3H, d, J = 15.6 Hz, PCH_3), 3.57 (3H, d, J = 10.1 Hz, POCH_3), 4.05 (6H, s, NCH_3), 7.57 (2H, d, J = 1.8 Hz, NC*H*C*H*N), 10.73 (1H, s, NC*H*N). ^{13}C NMR (100 MHz; $CDCl_3$; Me_4Si) δ = 11.47, 12.79 (P*C*H_3), 36.06 (N*C*H_3), 51.18 (PO*C*H_3), 123.26 (NCH*C*HN), 139.79 (N*C*HN).

1,3-Dimethylimidazolium acetate ([C_1mim][$MeCO_2$]) was synthesized as follows. Methyl iodide (30 g; 0.21 mol) and 1-methylimidazole (18 g; 0.22 mol) were added into 240 ml of tetrahydrofuran under an argon gas atmosphere at 0 ^{o}C. The reaction mixture was stirred at room temperature for 24h. The resulting solid was washed with excess of dehydrated diethyl ether and dried *in vacuo*. Iodide anion was converted into hydroxide by passing an aqueous solution of the iodide salt through a column filled with anion exchange resin (Amberlite IRN 78A). This aqueous hydroxide solution was then neutralized with small excess of acetic acid, and weak base anion exchange resin (Amberlite IRA 67) was added and stirred for 48h for removing excess amount of acid. After filtration, resulting liquid was dried *in vacuo* at 80 ^{o}C. ^{1}H NMR (400

MHz; DMSO-d_6; Me_4Si) δ = 1.61 (3H, s, CH_3CO), 3.89 (6H, s, NCH_3), 7.84 (2H, d, J = 1.6 Hz, NC*H*C*H*N), 10.02 (1H, s, NC*H*N). ^{13}C NMR (100 MHz; DMSO-d_6; Me_4Si) δ = 28.80 (*C*H_3CO), 38.12 (N*C*H_3), 51.18 (PO*C*H_3), 126.14 (NCH*C*HN), 141.00 (N*C*HN), 176.25 (CH_3*C*O).

1,3-Dimethylimidazolium methyl sulfate ([C_1mim][$MeOSO_3$]) was synthesized as follows. Dimethyl sulfate was washed with 3.6 % Na_2CO_3 aq. and dried with excess of $CaCl_2$. After distillation, dimethyl sulfate (10 g; 0.12 mol) and 1-methylimidazole (16.1 g; 0.13 mol) were added into 60 ml of tetrahydrofuran under an argon gas atmosphere at room temperature. The reaction mixture was stirred at 70 ^{o}C for 24h. The resulting liquid was dried *in vacuo* and 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 ^{o}C. ^{1}H NMR (400 MHz; DMSO-d_6; Me_4Si) δ =3.40 (3H, s, SOCH_3), 3.86 (6H, s, NCH_3), 7.69 (2H, d, J = 1.6 Hz, NC*H*C*H*N), 9.04 (1H, s, NC*H*N). ^{13}C NMR (100 MHz; DMSO-d_6; Me_4Si) δ =36.15 (N*C*H_3), 53.44 (SO*C*H_3), 123.95 (NCH*C*HN), 137.63 (N*C*HN).

Water content of these ILs was confirmed to be less than 200ppm.

Measurement of Kamlet-Taft parameters of ILs

Measurement of the Kamlet-Taft parameters of a series of ILs was carried out as follows. The solvatochromic dyes, (2,6-dichloro-4-(2,4,6-triphenyl-1-pyridinio)phenolate (Reichardt's dye #33, from Fluka), 4-nitroaniline (from Tokyo Chemical Industries Co., Ltd), and *N*,*N*-diethyl-4-nitroaniline (from Kanto Chemical Co., Inc.), were used as received. Methanol solutions containing dye molecules (0.01 ml) were added to 0.25 g of ILs. The methanol was then carefully removed by vacuum drying (under 2 mmHg, at 25 ^{o}C for 3h). These IL solutions were placed into quartz cells with 1 mm light-path length. In the case of [C_1mim][$MeCO_2$],

temperature of the sample was maintained at 60 °C by water circulation because it was solid below 60 °C. From the wavelength at the maximum absorption (λ_{max}) determined, the α, β and π^* values were calculated by use of the following equations:

$$\nu(\text{dye}) = 1/(\lambda_{\max(\text{dye})}10^{-4})$$

$$E_T(30) = 0.9986\ (28\ 592/\lambda_{\max\ (\text{Reichardt's dye \#33})}) - 8.6878$$

$$\pi^* = 0.314(27.52 - \nu_{(N,N\text{-diethyl-4-nitroaniline})})$$

$$\alpha = 0.0649E_T(30) - 2.03 - 0.72\ \pi^*$$

$$\beta = (1.035\nu_{(N,N\text{-diethyl-4-nitroaniline})} + 2.64 - \nu_{(4\text{-nitroaniline})})/2.80$$

Analysis of mixtures composed of cellulose and xylan by ^{1}H NMR

TMS salt was added to 20 g of DMSO to a final concentration of 0.5 wt%. A 0.70 g aliquot of the resulting DMSO/TMS salt solution was mixed with 0.30 g of ILs and stirred until the solutions were homogenous. Cellulose and xylan were added to the solutions and the resulting solutions were stirred until they had become clear and homogeneous. The samples were then transferred into 5 mm NMR tubes. The sample tubes were capped with plastic lids and the tops were wrapped in parafilm. The samples were analyzed at 100 °C with 12 scans.

WET method: measurements were performed based on the standard pulse sequence specified by JEOL Ltd. For suppression of ^{13}C satellite peaks, MPF8 was applied as a decoupling sequence.

Analysis of extracts from wheat bran with ILs by ^{1}H NMR

Wheat bran was dried under reduced pressure before use (the water content was 4.5 wt%, so that the final water concentration of IL/bran solutions was about 0.51 wt%). The dried bran (70 mg, 42 - 50 mesh size) was added to 1.0 g of dried ILs and stirred at 200 rpm in an oil bath (0.5 - 2h, 25 - 120 °C). The resulting solutions were centrifuged at 14,800 rpm (16,200 G) for 10 to 60

min in order to remove residue. The supernatants were mixed with 70 wt% of the DMSO/TMS salt solutions mentioned above, and the resulting solutions were stirred at 80 ^{o}C for 3min. After filtration with glass filter under reduced pressure, the samples were transferred to 5 mm NMR tubes. The sample tubes were capped with plastic lids, and the tops were wrapped in parafilm. The samples were analyzed by ^{1}H NMR at 100 ^{o}C with 240 scans. The yield of polysaccharides was calculated from the following equation:

$$\text{yield (\%)} = \frac{weight\ of\ polysaccharides\ from\ NMR\ (mg)}{70\ (mg)} \times 100 \quad (1)$$

When polysaccharides were extracted from IL-treated bran, the IL/bran solution remaining after the first extraction (extraction temperature: 80 ^{o}C, extraction time: 2h, feed bran: 70 mg, [C_1mim][(MeO)(Me)PO_2]: 1.0 g, stirring: 200 rpm) was centrifuged, and the precipitate was collected. The precipitate was dispersed into 10 ml of DMSO and mixed with vortex mixer for 1min, to strip away any dissolved substances adsorbed or trapped within the solid texture. The solution was centrifuged (16,200 G) and the supernatant was removed. For further washing, 30 ml of methanol was added and the solution was mixed with vortex mixer for 1min. The solution was centrifuged (16,200 G) and the supernatant was removed. This washing process with methanol was repeated twice. After drying under reduced pressure at room temperature, the IL-treated bran was added to 1.0 g of fresh [C_1mim][(MeO)(Me)PO_2] with stirring (200 rpm) at 80 ^{o}C for 2h.

In the selective extraction pocedure, the xylan fraction of extracts was calculated according to the following equation:

$$\text{xylan fraction (\%)} = \frac{\text{xylan extracted (mol)}}{\text{cellulose extracted (mol)+xylan extracted (mol)}} \times 100 \quad (2)$$

^{13}C NMR analysis was performed at 100 ^{o}C with 5,000 scans, using the same sample employed for ^{1}H NMR analysis.

Measurement of weight of extracted polysaccharides through re-precipitation

We passed the extract/[C_1mim][(MeO)(Me)PO_2] sample (80 ^{o}C, 2h) through a glass filter. The solution was then stirred with excess methanol to re-precipitate dissolved materials. The re-precipitated solid was repeatedly washed with methanol to remove residual ILs. This solid was collected by filtration and dried under reduced pressure. It was then weighed. In view of the loss of sample during these treatments, we calibrated the weight of polysaccharides according to that in 1.0 g of ILs.

Results and discussion

Effect of ash on the apparent weight of the extracted materials

A proportion of inorganic material is also expected to be dissolved during treatment with polar ILs. To determine the amount, we undertook a thermogravimetric analysis. Wheat bran (70 mg) was added to 1.0 g of dried [C_1mim][(MeO)(Me)PO_2] and stirred at 80 ^{o}C for 2h. After centrifuging and separation, excess methanol was added to the supernatant so as to re-precipitate polysaccharides. The precipitate was washed repeatedly with methanol and dried *in vacuo* in order to collect re-precipitated solids. The weight of the re-precipitated solid was 25 mg, with a degree of extraction of 36 wt%. Thermogravimetric analysis was performed at a heating rate of 10 ^{o}C/min (Figure 1). Upon heating to 500 ^{o}C, about 45 wt% of solid materials remained intact. Cellulose and xylan were both fully decomposed up to 500 ^{o}C, suggesting that the undecomposed material was inorganic. This finding indicates that weighing re-precipitated solid is not recommended when studying extracted polysaccharides. For further analysis of the main

components of the inorganic material, the sample was washed with water. An excess of milli-Q water was added to the dried extract, and the sample was stirred for 48 h. The resulting solution was filtered and the residue was dried and weighed. During this process 38 wt% of the re-precipitated solid was washed out, suggesting that these components were water-soluble inorganic salts (84 wt% of inorganic materials). The rest is believed to be mainly SiO_2.

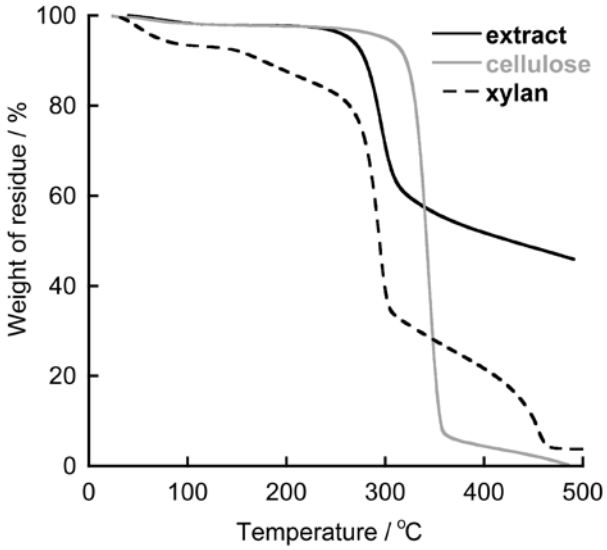


Figure 1. TGA curve of the extract from wheat bran with $[C_1mim][(MeO)(Me)PO_2]$, cellulose, and xylan.

Application of ^{1}H NMR for analysis of polysaccharides extracted with ILs

As a preliminary study, cellulose and xylan were individually analyzed by ^{1}H NMR. As their solvents, we prepared three different ILs, composed of the 1,3-dimethylimidazolium cation and the $[(MeO)(Me)PO_2]$ or $[MeCO_2]$ or $[MeOSO_3]$ anion. The structure of these is shown in Figure 2. $[C_1mim][(MeO)(Me)PO_2]$ and $[C_1mim][MeCO_2]$ both dissolve cellulose and xylan, because of their high hydrogen bond basicity (large β value in Table 1). $[C_1mim][MeOSO_3]$ dissolves only xylan, because of its relatively low hydrogen bond basicity ($\beta = 0.61$ in Table 1). Since these ILs are highly viscous, DMSO was added to the IL solutions to facilitate the NMR

measurements. In $[C_1mim][(MeO)(Me)PO_2]$, proton signals from cellulose were observed at 3.0 - 3.1, 3.6 - 3.7, and 4.45 - 4.55 ppm. Proton signals from xylan were observed at 3.0 - 3.1, 3.5 - 3.6, and 4.3 - 4.4 ppm. These data suggest that signals from cellulose and xylan detected around 4.4 ppm should be observed even in arbitrary mixtures (Figure 3a, top and bottom). These signals are assigned to protons at the 1-position of cellulose and xylan (as shown in Figure 4), because the ^1H signal at the 1-position of cello-oligosaccharides is detected at almost the same chemical shift in conventional polar solvents.[24-25] In the case of $[C_1mim][MeCO_2]$, proton signals were observed at almost identical chemical shifts (Figure 3b, top and bottom). In $[C_1mim][MeOSO_3]$, the proton signal of xylan was also detected at around 4.3 ppm (Figure 3c bottom). It was confirmed that the peaks were observed at almost the same chemical shifts in these three ILs in spite of their different polarities. It is known that ILs interact strongly with hydroxyl groups[24] and their proton signals were found to shift to their lower magnetic field side in the ILs.[19]

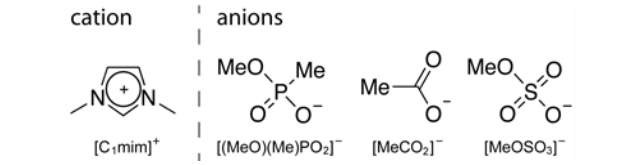


Figure 2. Structure of ILs used in this study.

Table 1. Kamlet-Taft parameters of ILs.

ILs	α	β	π^*
$[C_1mim][(MeO)(Me)PO_2]$	0.53	1.10	1.05
$[C_1mim][MeCO_2]$	0.54[a]	1.08[a]	1.07[a]
$[C_1mim][MeOSO_3]$	0.56	0.61	1.12

[a] measured at 60 °C.

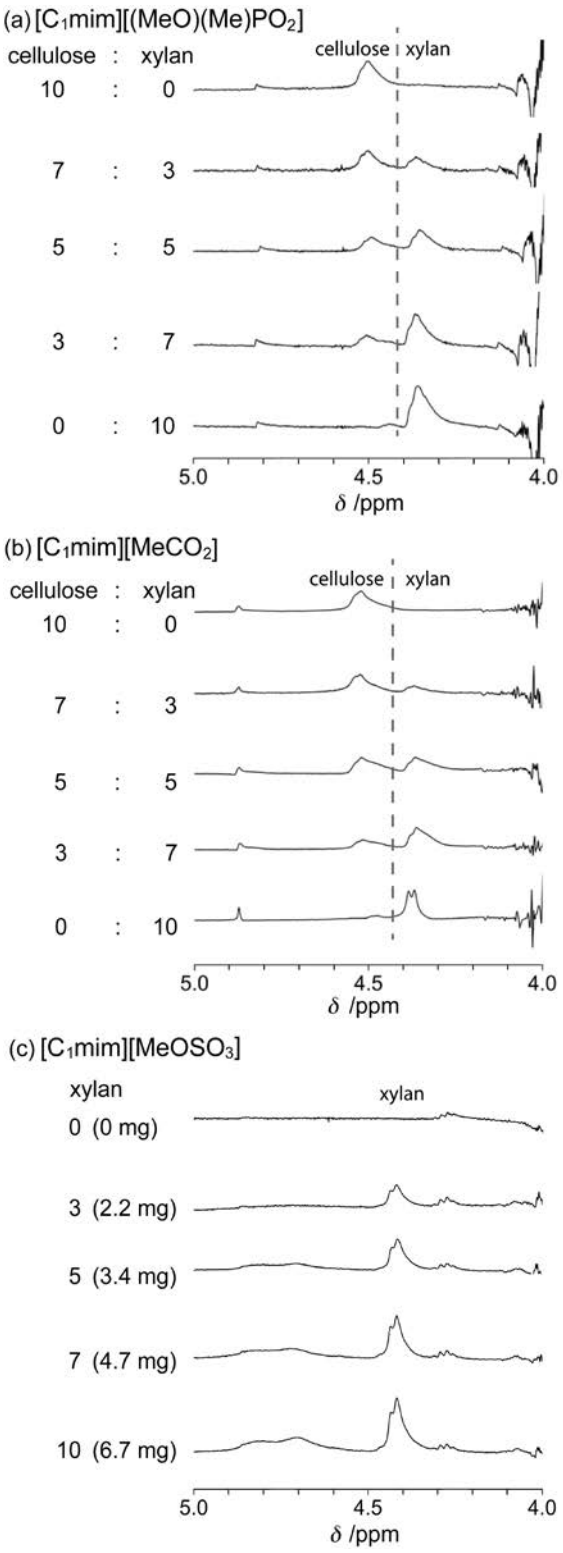


Figure 3. ^{1}H NMR spectra of cellulose/xylan mixtures (0:10 - 10:0 by molar ratio) in three different ILs.

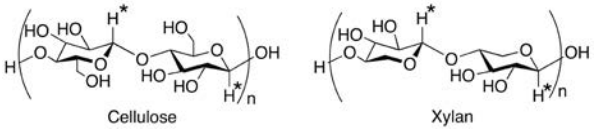


Figure 4. Structure of cellulose and xylan. *1-position

Acting as models of extracted major polysaccharides, mixtures of cellulose and xylan were dissolved in $[C_1mim][(MeO)(Me)PO_2]$, and their NMR signals were detected (Figure 3a). The signals for cellulose and xylan were observed independently at around 4.3 and around 4.5 ppm respectively, as expected. The peak area increased with increasing fraction of each polysaccharide. In $[C_1mim][MeCO_2]$ the signals (at around 4.4 ppm) partly overlapped, but the components could be distinguished (Figure 3b). In $[C_1mim][MeOSO_3]$ the signal for xylan was detected clearly at around 4.3 ppm (Figure 3c). The peak area changed with the sample concentration in both $[C_1mim][MeCO_2]$ and $[C_1mim][MeOSO_3]$. Signals from both cellulose and xylan, which have very similar chemical structures, were resolved separately regardless of IL species.

Figure 5 shows the relation observed between cellulose fraction and relative peak area. In the case of NMR, the peak area in different spectra cannot be compared directly without a common standard. We therefore used TMS salt as a standard. The peak area of polysaccharides was calculated relative to the TMS salt signal. Figure 5 reveals a linear relation between the relative peak area of polysaccharides and the cellulose fraction of the mixed samples in $[C_1mim][(MeO)(Me)PO_2]$. It was possible to determine the proportions of cellulose and xylan in the mixed samples. As the signals from cellulose and xylan in $[C_1mim][MeCO_2]$ partly overlap, we analyzed deconvolution of the spectra as Lorentzian line shapes using grams/386 ver. 3.04 (Galactic Industrial Corporation). The relation between the cellulose fraction in a cellulose/xylan mixture and the peak area of cellulose and xylan in $[C_1mim][MeCO_2]$ and

[C_1mim][$MeOSO_3$] also proved to be linear, with a value of R^2 of more than 0.99 (see Figure S1). It is therefore possible to quantify the amounts of cellulose and xylan dissolved in various ILs using ^{1}H NMR.

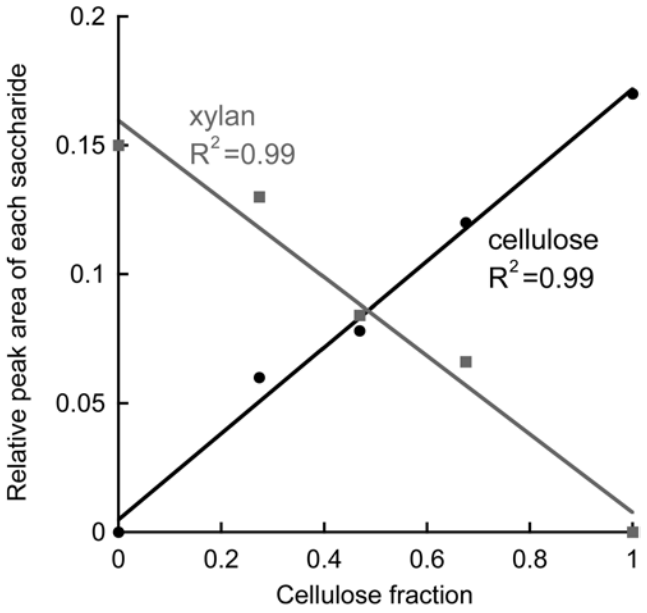


Figure 5. Relation between cellulose fraction of the mixed sample and relative peak area of xylan and cellulose in [C_1mim][(MeO)(Me)PO_2].

Based on these results, we analyzed the components extracted from biomass. Figure 6 shows ^{1}H NMR spectra of the component of bran extracted with ILs. Polysaccharides were extracted from bran using these ILs at 80 ^{o}C for 2h. As expected, signals of cellulose and xylan were both detected in each IL, [C_1mim][(MeO)(Me)PO_2] and [C_1mim][$MeCO_2$], between 4.2 and 4.7 ppm. Only the xylan signal was detected in the treated solution of [C_1mim][$MeOSO_3$], at 4.4 ppm. Furthermore, lignin was not detected in these spectra, whereas lignin is observed at 6 - 8 ppm in polar IL solutions.[22] The sample extracted at 80 ^{o}C for 2h (the same sample shown in Figure 6a) was analyzed with ^{13}C NMR at 100 ^{o}C using an accumulation of 5,000 scans (see SI, Figure S2), but no signals were detected in the spectrum.

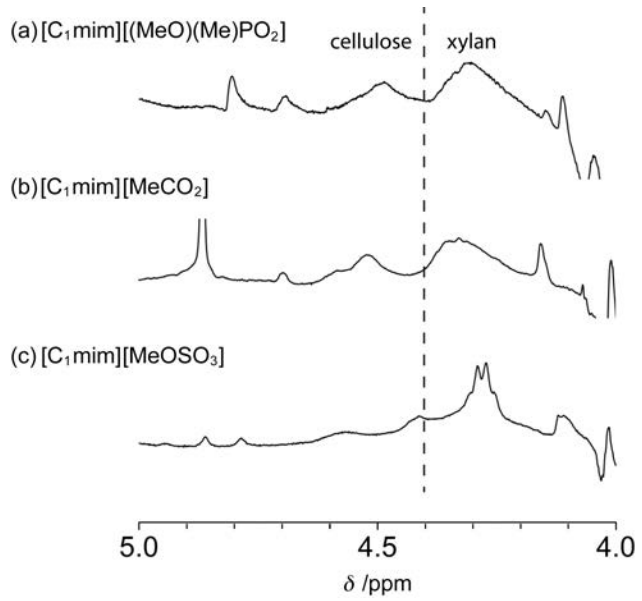


Figure 6. ^{1}H NMR spectra of extracts from bran treated with ILs.

Estimation of the ability of ILs to extract polysaccharides from wheat bran

Figure 7 shows the amount and yield of cellulose and xylan extracted from wheat bran using three different ILs. We have quantified the amount of extracted polysaccharides using the standard relations shown in Figures 5 and S1. Polysaccharide was extracted from wheat bran, with 16.1% yield when bran was immersed in $[C_1mim][(MeO)(Me)PO_2]$ at 80 ^{o}C for 2h. The weights of cellulose and xylan extracted with $[C_1mim][(MeO)(Me)PO_2]$ were 4.2 and 7.1 mg, respectively. To consider this value further (11.3 mg; 4.2 + 7.1 mg), we compared it to the weight of re-precipitated solid from the IL solution used for extraction. As mentioned above, the re-precipitated solid weighed 25 mg, and its organic compound fraction was about 50 % (12.5 mg) according to thermogravimetric analysis (see Figures 1 and SI). Thus, the values obtained (11.3 mg and 12.5 mg) were reasonably similar. The difference between the values can be explained by the presence of other organic compounds.

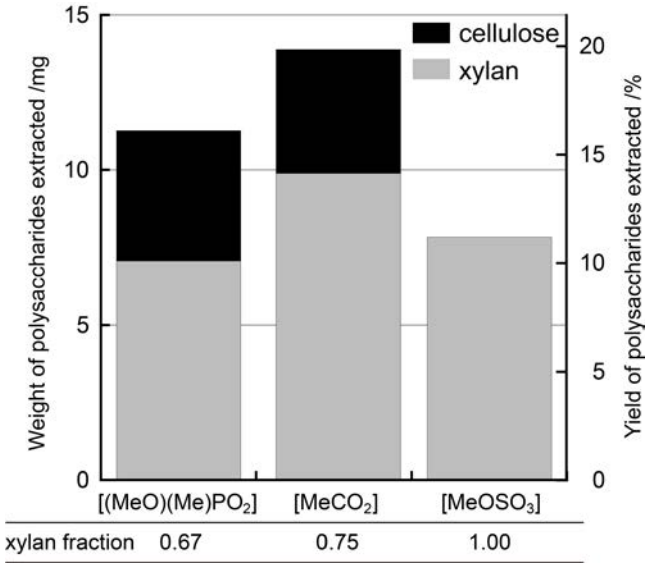


Figure 7. Weight and yield of the cellulose and xylan extracted with ILs at 80 ^{o}C for 2h.

The weights of cellulose and xylan extracted with $[C_1mim][MeCO_2]$ were 4.0 and 9.8 mg, respectively. The xylan fraction was 0.67 and 0.75 for $[C_1mim][(MeO)(Me)PO_2]$ and $[C_1mim][MeCO_2]$, respectively. $[C_1mim][MeOSO_3]$ extracted xylan selectively; its extraction ability was somewhat greater than that of $[C_1mim][(MeO)(Me)PO_2]$. These three ILs have almost the same α value (see Table 1), but β is lower for $[C_1mim][MeOSO_3]$ than for the other two ILs (Table 1). Dissolution of cellulose depends strongly on the β value, but the optimal properties of the ILs for dissolving xylan are not known.

We sought to extract further cellulose from the IL-treated bran, by adding fresh $[C_1mim][(MeO)(Me)PO_2]$ to it. Figure 8 shows the amount of extracted polysaccharides from bran (1st extraction) and from IL-treated bran (2nd extraction). Both extractions were undertaken at 80 ^{o}C for 2h with fresh $[C_1mim][(MeO)(Me)PO_2]$. To our surprise, almost no polysaccharides were extracted from IL-treated bran. We then analyzed the component of bran treated with $[C_1mim][(MeO)(Me)PO_2]$ twice, and found that cellulose was still included (Figure S4 and Table S1). These observations suggest that 6 and 10 wt%, for cellulose and xylan

respectively, will be the upper limit of extraction under these conditions. It is confirmed that this limit is not due to the solubility limit of polysaccharides or the increase of viscosity caused by dissolution of polysaccharides.

To investigate this limit further, we varied the amount of feed bran and the temperature (Figure 9). When we used double the quantity of bran (12.3 wt%), the amount of cellulose and xylan extracted also doubled (Figure 9b) despite a considerable increase in viscosity. This result also supports that the limit is not caused by solubility of polysaccharides nor increase of viscosity. The treatment temperature is known to affect the amount of polysaccharides extracted from bran or other biomass. As shown in Figure 9c, more was extracted at higher temperature (120 ^{o}C). In detail, more xylan was obtained (7.1 to 12.7 mg), but the amount of cellulose extracted did not increase. This increase in the amount of xylan extracted can be explained by the effect of lignin-carbohydrate complexes (LCCs). LCCs are composed of covalently bound hemicellulose and lignin, and they are known to suppress the solubilization of plant biomass.[26] Lignin partially decomposes[27-28] and liquefies[29] at high temperature in polar ILs. The increase in xylan extracted is therefore attributable to partial decomposition and liquefaction of LCC. Furthermore, xylan was extracted at room temperature (1.2 mg, see Figure 9d). The xylan extracted was considered not to form LCCs at room temperature. We infer that the extra xylan extracted at 80 ^{o}C and 120 ^{o}C (difference between c and a) forms LCCs, but the xylan extracted at 25 ^{o}C does not. It is not easy to conclude the different extraction degree at 80 ^{o}C and 25 ^{o}C to be partial decomposition of LCC at 80 ^{o}C. As for the effect of the duration of extraction, the amount of polysaccharides extracted in 30min and 2h was almost the same (see Figure 9, a and e). This shows that almost all polysaccharides extractable at 80 ^{o}C were extracted within 30min. In other words, no LCC was strongly suggested to be decomposed at 80 ^{o}C.

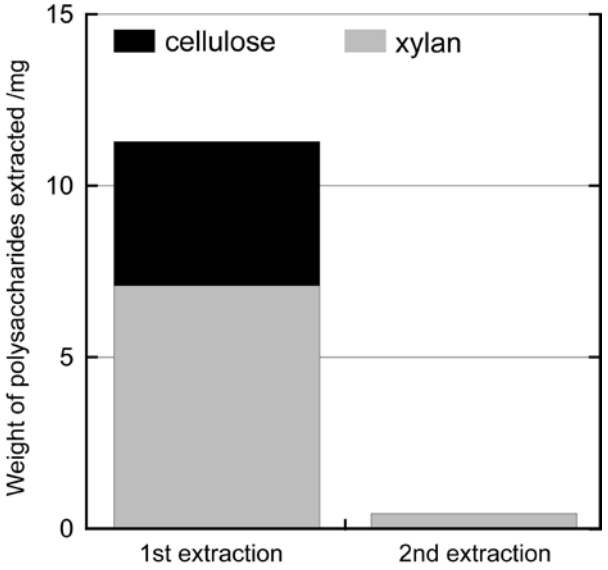
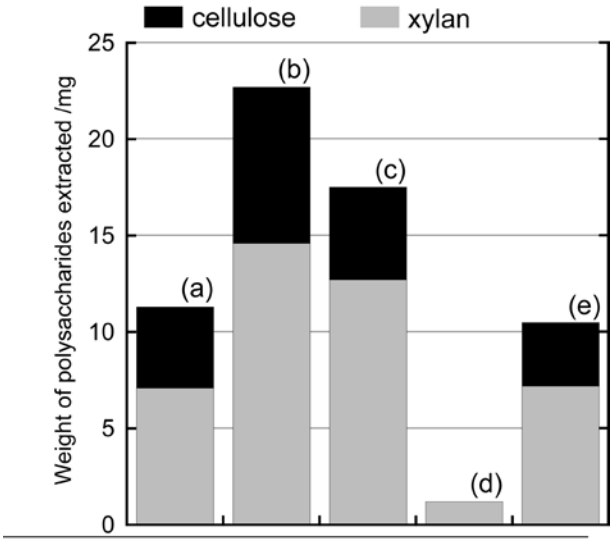


Figure 8. Weight of extracted polysaccharides from 70 mg of bran at 80 ^{o}C for 2h (1st extraction) and from the IL-treated bran at 80 ^{o}C for 2h (2nd extraction) with $[C_1mim][(MeO)(Me)PO_2]$, calculated from NMR signals.



temp (^{o}C)	80	80	120	25	80
time (min)	120	120	120	120	30
bran (mg)	70	140	70	70	70
xylan fraction	0.67	0.69	0.77	1.00	0.73

Figure 9. Weight of polysaccharides extracted under various conditions using $[C_1mim][(MeO)(Me)PO_2]$, calculated from NMR signals.

The composition of extracts in ILs was easily obtained using ^{1}H NMR with No-D NMR and the WET technique. The results obtained will lead to real-time monitoring of extraction with polar ILs.

Conclusions

We have analyzed the performance of ILs in extracting polysaccharides from wheat bran, using ^{1}H NMR. Both No-D NMR and the WET technique were used for direct detection of extracted polysaccharides with non-deuterated ILs. This enables rapid analysis without troublesome pre-treatments. This analysis was unaffected by the presence of ash in crude samples. We confirmed that this method was applicable to three representative ILs for extraction of polysaccharides. The effect of LCCs on extraction with ILs was confirmed. Almost all of the extractable polysaccharides were obtained within 30min with stirring at 80 ^{o}C.

ASSOCIATED CONTENT

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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Notes

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Supporting Information

Experimental section concerning anion/cation ratio analyzed with ^{1}H NMR; Relations between relative peak area of polysaccharides and cellulose fraction of mixed samples in $[C_1mim][MeCO_2]$ and $[C_1mim][MeOSO_3]$; ^{13}C NMR spectrum of the extract from bran using $[C_1mim][(MeO)(Me)PO_2]$; full spectra of the spectra shown in Fugure 6; comparison between quantity of polysaccharides analyzed by ^{1}H NMR and weight of re-precipitated solid; A TGA

curve of the bran treated with [C_1mim][(MeO)(Me)PO_2] twice; UV-*vis* absorbance of 2,6-dichlorophenolindophenol sodium salt in the solution of the hydrolysate of the bran treated with [C_1mim][(MeO)(Me)PO_2] twice; Comparison of extraction degree from measurement twice to clarify experimental error. This material is available free of charge via the Internet at http://pubs.acs.org.

ABBREVIATIONS

ILs, ionic liquids; ^{1}H NMR, proton nuclear magnetic resonance; No-D NMR, no-deuterium NMR; [C_1mim][(MeO)(Me)PO_2], 1,3-dimethylimidazolium methyl methylphosphonate; [C_1mim][$MeCO_2$], 1,3-dimethylimidazolium acetate; [C_1mim][$MeOSO_3$], 1,3-dimethylimidazolium methyl sulfate; DMSO, dimethyl sulfoxide; TMS salt, 3-(trimethylsilyl)-propanesulfonic acid sodium salt; LCC, lignin-carbohydrate complex

REFERENCES

(1) Welton, T., Room-temperature ionic liquids. Solvents for synthesis and catalysis. *Chem. Rev.* **1999,** *99*, 2071-2083.

(2) Swatloski, R. P.; Spear, S. K.; Holbrey, J. D.; Rogers, R. D., Dissolution of Cellose with Ionic Liquids. *J. Am. Chem. Soc.,* **2002,** *124*, 4974-4975.

(3) Phillips, D. M.; Drummy, L. F.; Conrady, D. G.; Fox, D. M.; Naik, R. R.; Stone, M. O.; Trulove, P. C.; De Long, H. C.; Mantz, R. A., Dissolution and regeneration of Bombyx mori Silk fibroin using ionic liquids. *J. Am. Chem. Soc.,* **2004,** *126*, 14350-14351.

(4) Xie, H.; Li, S.; Zhang, S., Ionic liquids as novel solvents for the dissolution and blending of wool keratin fibers. *Green Chem.* **2005,** *7*, 606-608.

(5) Abe, M.; Fukaya, Y.; Ohno, H., Extraction of polysaccharides from bran with phosphonate or phosphinate-derived ionic liquids under short mixing time and low temperature. *Green Chem.* **2010,** *12*, 1274-1280.

(6) Fukaya, Y.; Hayashi, K.; Wada, M.; Ohno, H., Cellulose dissolution with polar ionic liquids under mild conditions: required factors for anions. *Green Chem.* **2008,** *10*, 44-46.

(7) Sun, N.; Rodríguez, H.; Rahman, M.; Rogers, R. D., Where are ionic liquid strategies most suited in the pursuit of chemicals and energy from lignocellulosic biomass? *Chem. Commun.* **2011,** *47*, 1405-1421.

(8) Ohno, H.; Fukaya, Y., Task Specific Ionic Liquids for Cellulose Technology. *Chem. Lett.* **2009,** *38*, 2-7.

(9) Armand, M.; Endres, F.; MacFarlane, D. R.; Ohno, H.; Scrosati, B., Ionic-liquid materials for the electrochemical challenges of the future. *Nat. Mater.* **2009,** *8*, 621-629.

(10) Kilpeläinen, I.; Xie, H.; King, A.; Granstrom, M.; Heikkinen, S.; Argyropoulos, D. S., Dissolution of wood in ionic liquids. *J. Agric. Food Chem.* **2007,** *55*, 9142-9148.

(11) Brandt, A.; Gräsvik, J.; Hallett, J. P.; Welton, T., Deconstruction of lignocellulosic biomass with ionic liquids. *Green Chem.* **2013,** *15*, 550-583.

(12) Tan, S. S. Y.; MacFarlane, D. R., *Ionic Liquids in Biomass Processing*. 1st ed.; Springer: Berlin Heidelberg, 2009; Vol. 290, p 29.

(13) Froschauer, C.; Hummel, M.; Iakovlev, M.; Roselli, A.; Schottenberger, H.; Sixta, H., Separation of hemicellulose and cellulose from wood pulp by means of ionic liquid/cosolvent systems. *Biomacromolecules* **2013,** *14*, 1741-1750.

(14) Qiu, Z.; Aita, G. M.; Walker, M. S., Effect of ionic liquid pretreatment on the chemical composition, structure and enzymatic hydrolysis of energy cane bagasse. *Bioresour. Technol.* **2012,** *117*, 251-256.

(15) Fukaya, Y.; Tsukamoto, A.; Kuroda, K.; Ohno, H., High performance "ionic liquid" chromatography. *Chem Commun.* **2011,** *47*, 1994-1996.

(16) Moulthrop, J. S.; Swatloski, R. P.; Moyna, G.; Rogers, R. D., High-resolution 13C NMR studies of cellulose and cellulose oligomers in ionic liquid solutions. *Chem Commun.* **2005**, 1557-1559.

(17) Froschauer, C.; Hummel, M.; Laus, G.; Schottenberger, H.; Sixta, H.; Weber, H. K.; Zuckerstätter, G., Dialkyl phosphate-related ionic liquids as selective solvents for xylan. *Biomacromolecules* **2012,** *13*, 1973-1980.

(18) Kuroda, K.; Fukaya, Y.; Ohno, H., Direct HPILC analysis of cellulose depolymerisation in ionic liquids. *Anal. Methods* **2013,** *5*, 3172-3176.

(19) Kuroda, K.; Kunimura, H.; Fukaya, Y.; Ohno, H., 1H NMR analysis of cellulose dissolved in non-deuterated ionic liquids. *Cellulose* **2014**, 21, 2199-2206.

(20) Hoye, T. R.; Eklov, B. M.; Voloshin, M., No-D NMR spectroscopy as a convenient method for titering organolithium (RLi), RMgX, and LDA solutions. *Org. Lett.* **2004,** *6*, 2567-2570.

(21) Hoye, T. R.; Eklov, B. M.; Ryba, T. D.; Voloshin, M.; Yao, L. J., No-D NMR (no-deuterium proton NMR) spectroscopy: a simple yet powerful method for analyzing reaction and reagent solutions. *Org. Lett.* **2004,** *6*, 953-956.

(22) Jiang, N.; Pu, Y. Q.; Samuel, R.; Ragauskas, A. J., Perdeuterated pyridinium molten salt (ionic liquid) for direct dissolution and NMR analysis of plant cell walls. *Green Chem.* **2009,** *11*, 1762-1766.

(23) Hardacre, C.; Holbrey, J. D.; McMath, S. E. J., highly efficient synthetic procedure for deuteriating imidazoles and imidazolium salts. *Chem. Commun.* **2001**, 367-368.

(24) Zhang, J.; Zhang, H.; Wu, J.; Zhang, J.; He, J.; Xiang, J., NMR spectroscopic studies of cellobiose solvation in EmimAc aimed to understand the dissolution mechanism of cellulose in ionic liquids. *Phys. Chem. Chem. Phys.* **2010,** *12*, 1941-1947.

(25) Isogai, A., NMR analysis of cellulose dissolved in aqueous NaOH solutions. *Cellulose* **1997,** *4*, 99-107.

(26) Leskinen, T.; King, A. W. T.; Kilpeläinen, I.; Argyropoulos, D. S., Fractionation of Lignocellulosic Materials Using Ionic Liquids: Part 2. Effect of Particle Size on the Mechanisms of Fractionation. *Ind. Eng. Chem. Res.* **2013,** *52*, 3958-3966.

(27) Kim, J. Y.; Shin, E. J.; Eom, I. Y.; Won, K.; Kim, Y. H.; Choi, D.; Choi, I. G.; Choi, J. W., Structural features of lignin macromolecules extracted with ionic liquid from poplar wood. *Bioresour. Technol.* **2011,** *102*, 9020-9025.

(28) Wen, J.-L.; Yuan, T.-Q.; Sun, S.-L.; Xu, F.; Sun, R.-C., Understanding the chemical transformations of lignin during ionic liquid pretreatment. *Green Chem.* **2014,** *16*, 181-190.

(29) Li, W. Y.; Sun, N.; Stoner, B.; Jiang, X. Y.; Lu, X. M.; Rogers, R. D., Rapid dissolution of lignocellulosic biomass in ionic liquids using temperatures above the glass transition of lignin. *Green Chem.* **2011,** *13*, 2038-2047.

Table of Contents; For Table of Contents Use Only

^{1}H NMR evaluation of polar and non-deuterated ionic liquids for selective extraction of cellulose and xylan from wheat bran

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SYNOPSIS

Cellulose and xylan extracted from bran with polar ionic liquids were quantified with the aid of ^{1}H NMR. Avioding deuteration of ionic liquids and complex pretreatments made direct measurement of these extracts possible.