

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

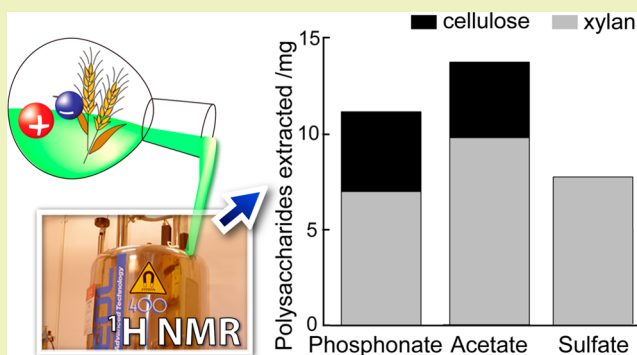
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S Supporting Information

ABSTRACT: Cellulose and xylan, extracted from wheat bran with polar ionic liquids (ILs), were quantified using ^1H NMR spectroscopy. Both No-D NMR and solvent suppression techniques were applied to realize direct analysis of extracts in nondeuterated ILs. As models of extracts, mixtures of cellulose and xylan dissolved in ILs were measured with ^1H 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. A relation between extracted amount and extraction conditions was obtained.

KEYWORDS: Ionic liquid, Cellulose, Xylan, Biomass, ^1H NMR



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 ^1H NMR analysis of cellulose in nondeuterated 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 nondeuterated 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 nondeuterated 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 physicochemical properties, in order to exhibit the effectiveness of the present method.

EXPERIMENTAL SECTION

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)-

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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.). ^1H - 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 ($[\text{C}_1\text{mim}][(\text{MeO})(\text{Me})\text{PO}_2]$) 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 °C for 48 h. 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 °C. ^1H NMR (400 MHz; CDCl_3 ; Me_4Si) δ = 1.26 (3H, d, J = 15.6 Hz, POCH_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, NCHCHN), 10.73 (1H, s, NCHN). ^{13}C NMR (100 MHz; CDCl_3 ; Me_4Si) δ = 11.47, 12.79 (POCH_3), 36.06 (NCH_3), 51.18 (POCH_3), 123.26 (NCHCHN), 139.79 (NCHN).

1,3-Dimethylimidazolium acetate ($[\text{C}_1\text{mim}][\text{MeCO}_2]$) 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 °C. The reaction mixture was stirred at room temperature for 24 h. 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 48 h for removing excess amount of acid. After filtration, resulting liquid was dried *in vacuo* at 80 °C. ^1H NMR (400 MHz; $\text{DMSO}-d_6$; Me_4Si) δ = 1.61 (3H, s, CH_3CO), 3.89 (6H, s, NCH_3), 7.84 (2H, d, J = 1.6 Hz, NCHCHN), 10.02 (1H, s, NCHN). ^{13}C NMR (100 MHz; $\text{DMSO}-d_6$; Me_4Si) δ = 28.80 (CH_3CO), 38.12 (NCH_3), 51.18 (POCH_3), 126.14 (NCHCHN), 141.00 (NCHN), 176.25 (CH_3CO).

1,3-Dimethylimidazolium methyl sulfate ($[\text{C}_1\text{mim}][\text{MeOSO}_3]$) 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 °C for 24 h. 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 °C. ^1H NMR (400 MHz; $\text{DMSO}-d_6$; Me_4Si) δ = 3.40 (3H, s, SOCH_3), 3.86 (6H, s, NCH_3), 7.69 (2H, d, J = 1.6 Hz, NCHCHN), 9.04 (1H, s, NCHN). ^{13}C NMR (100 MHz; $\text{DMSO}-d_6$; Me_4Si) δ = 36.15 (NCH_3), 53.44 (SOCH_3), 123.95 (NCHCHN), 137.63 (NCHN).

The water content of these ILs was confirmed to be less than 2000 ppm.

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 no. 33, RD33, 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 °C for 3 h). These IL solutions were placed into quartz cells with 1 mm light-path length. In the case of $[\text{C}_1\text{mim}][\text{MeCO}_2]$, 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:

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

$$E_{\text{T}}(30) = 0.9986(28592/\lambda_{\text{max}(\text{RD33})}) - 8.6878$$

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

$$\alpha = 0.0649E_{\text{T}}(30) - 2.03 - 0.72\pi^*$$

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

Analysis of Mixtures Composed of Cellulose and Xylan by

^1H 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 homogeneous. 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 ^1H 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 (0.5–2 h, 25–120 °C). The resulting solutions were centrifuged at 14 800 rpm (16 200g) 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 °C for 3 min. After filtration with a 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 ^1H NMR at 100 °C with 240 scans. The yield of polysaccharides was calculated from the following equation:

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

When polysaccharides were extracted from IL-treated bran, the IL/bran solution remaining after the first extraction (extraction temperature 80 °C, extraction time 2 h, feed bran 70 mg, $[\text{C}_1\text{mim}][(\text{MeO})(\text{Me})\text{PO}_2]$ 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 1 min, to strip away any dissolved substances adsorbed or trapped within the solid texture. The solution was centrifuged (16 200g), and the supernatant was removed. For further washing, 30 mL of methanol was added and the solution was mixed with a vortex mixer for 1 min. The solution was centrifuged (16 200g), 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 $[\text{C}_1\text{mim}][(\text{MeO})(\text{Me})\text{PO}_2]$ with stirring (200 rpm) at 80 °C for 2 h.

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

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

^{13}C NMR analysis was performed at 100 °C with 5000 scans, using the same sample employed for ^1H NMR analysis.

Measurement of Weight of Extracted Polysaccharides through Reprecipitation. We passed the extract/[C₁mim][(MeO)(Me)PO₂] sample (80 °C, 2 h) through a glass filter. The solution was then stirred with excess methanol to reprecipitate dissolved materials. The reprecipitated 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 [C₁mim][(MeO)(Me)PO₂] and stirred at 80 °C for 2 h. After centrifuging and separation, excess methanol was added to the supernatant so as to reprecipitate polysaccharides. The precipitate was washed repeatedly with methanol and dried in vacuo in order to collect reprecipitated solids. The weight of the reprecipitated solid was 25 mg, with a degree of extraction of 36 wt %. Thermogravimetric analysis was performed at a heating rate of 10 °C/min (Figure 1). Upon heating to 500 °C,

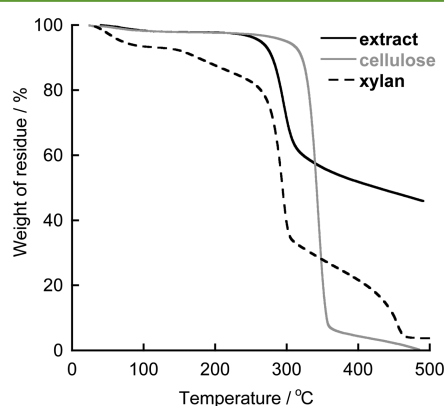


Figure 1. TGA curve of the extract from wheat bran with [C₁mim][(MeO)(Me)PO₂], cellulose, and xylan.

about 45 wt % of solid materials remained intact. Cellulose and xylan were both fully decomposed up to 500 °C, suggesting that the undecomposed material was inorganic. This finding indicates that weighing reprecipitated 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 reprecipitated 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 SiO₂.

Application of ¹H NMR for Analysis of Polysaccharides Extracted with ILs. As a preliminary study, cellulose and xylan were individually analyzed by ¹H NMR. As their solvents, we prepared three different ILs, composed of the 1,3-dimethylimidazolium cation and the [(MeO)(Me)PO₂] or [MeCO₂] or [MeOSO₃] anion. The structure of these is shown in Figure 2. [C₁mim][(MeO)(Me)PO₂] and [C₁mim]-[MeCO₂] both dissolve cellulose and xylan, because of their high hydrogen bond basicity (large β value in Table 1).

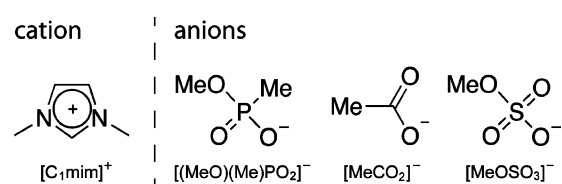


Figure 2. Structure of ILs used in this study.

Table 1. Kamlet–Taft Parameters of ILs

ILs	α	β	π*
[C ₁ mim][(MeO)(Me)PO ₂]	0.53	1.10	1.05
[C ₁ mim][MeCO ₂]	0.54 ^a	1.08 ^a	1.07 ^a
[C ₁ mim][MeOSO ₃]	0.56	0.61	1.12

^aMeasured at 60 °C.

[C₁mim][MeOSO₃] dissolves only xylan, because of its relatively low hydrogen bond basicity (β = 0.61 in Table 1). Since these ILs are highly viscous, DMSO was added to the IL solutions to facilitate the NMR measurements. In [C₁mim]-[(MeO)(Me)PO₂], 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 [C₁mim][MeCO₂], proton signals were observed at almost identical chemical shifts (Figure 3b, top and bottom). In [C₁mim][MeOSO₃], 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 groups²⁴ and their proton signals were found to shift to their lower magnetic field side in the ILs.¹⁹

Acting as models of extracted major polysaccharides, mixtures of cellulose and xylan were dissolved in [C₁mim]-[(MeO)(Me)PO₂], 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 [C₁mim][MeCO₂] the signals (at around 4.4 ppm) partly overlapped, but the components could be distinguished (Figure 3b). In [C₁mim][MeOSO₃] the signal for xylan was detected clearly at around 4.3 ppm (Figure 3c). The peak area changed with the sample concentration in both [C₁mim][MeCO₂] and [C₁mim][MeOSO₃]. 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 [C₁mim][(MeO)(Me)PO₂]. It was possible to determine the proportions of cellulose and xylan in

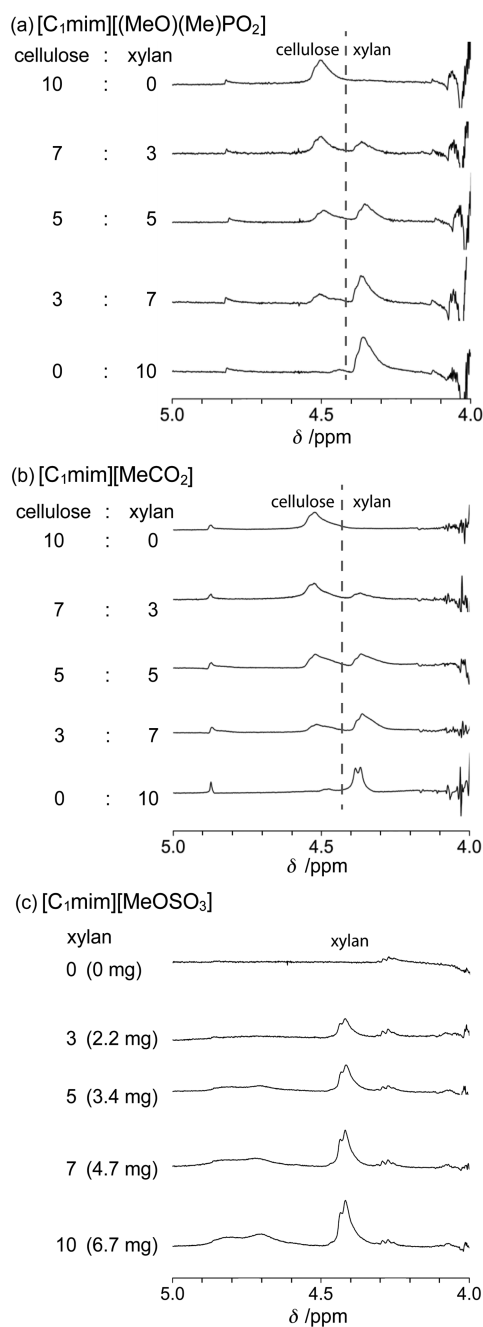


Figure 3. ^1H NMR spectra of cellulose/xylan mixtures (0:10–10:0 by molar ratio) in three different ILs.

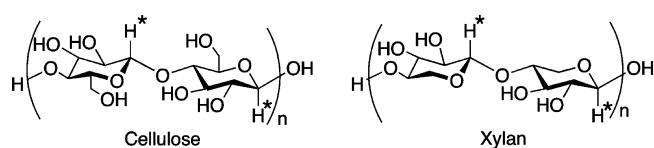


Figure 4. Structure of cellulose and xylan: (*) 1-position.

the mixed samples. As the signals from cellulose and xylan in $[\text{C}_1\text{mim}][\text{MeCO}_2]$ 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 $[\text{C}_1\text{mim}][\text{MeCO}_2]$ and $[\text{C}_1\text{mim}][\text{MeOSO}_3]$ also proved to be linear, with a value of

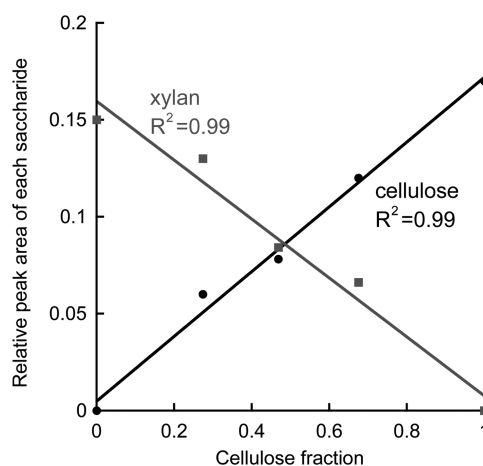


Figure 5. Relation between cellulose fraction of the mixed sample and relative peak area of xylan and cellulose in $[\text{C}_1\text{mim}][(\text{MeO})(\text{Me})\text{PO}_2]$.

R^2 of more than 0.99 (see Supporting Information (SI) Figure S1). It is therefore possible to quantify the amounts of cellulose and xylan dissolved in various ILs using ^1H NMR.

Based on these results, we analyzed the components extracted from biomass. Figure 6 shows ^1H NMR spectra of

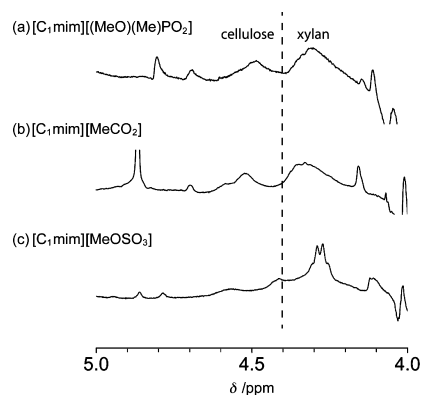


Figure 6. ^1H NMR spectra of extracts from bran treated with ILs.

the component of bran extracted with ILs. Polysaccharides were extracted from bran using these ILs at $80\text{ }^\circ\text{C}$ for 2 h. As expected, signals of cellulose and xylan were both detected in each IL, $[\text{C}_1\text{mim}][(\text{MeO})(\text{Me})\text{PO}_2]$ and $[\text{C}_1\text{mim}][\text{MeCO}_2]$, between 4.2 and 4.7 ppm. Only the xylan signal was detected in the treated solution of $[\text{C}_1\text{mim}][\text{MeOSO}_3]$, 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 $80\text{ }^\circ\text{C}$ for 2 h (the same sample shown in Figure 6a) was analyzed with ^{13}C NMR at $100\text{ }^\circ\text{C}$ using an accumulation of 5000 scans (see SI Figure S2), but no signals were detected in the spectrum.

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 Figure 5 and the S1. Polysaccharide was extracted from wheat bran, with 16.1% yield when bran was immersed in $[\text{C}_1\text{mim}][(\text{MeO})(\text{Me})\text{PO}_2]$ at $80\text{ }^\circ\text{C}$ for 2 h. The weights of cellulose and xylan extracted with $[\text{C}_1\text{mim}][(\text{MeO})(\text{Me})\text{PO}_2]$ were 4.2 and 7.1

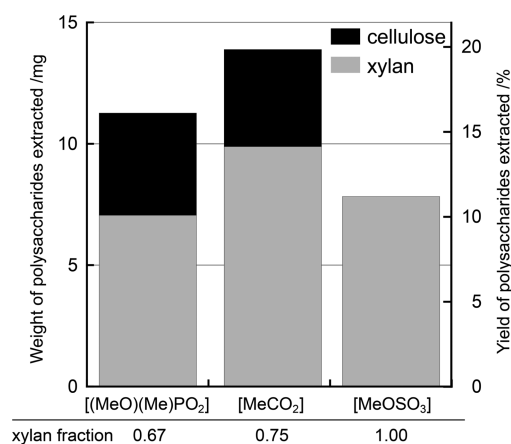


Figure 7. Weight and yield of the cellulose and xylan extracted with ILs at 80 °C for 2 h.

mg, respectively. To consider this value further (11.3 mg; 4.2 + 7.1 mg), we compared it to the weight of reprecipitated solid from the IL solution used for extraction. As mentioned above, the reprecipitated solid weighed 25 mg, and its organic compound fraction was about 50% (12.5 mg) according to thermogravimetric analysis (see Figure 1 and the 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.

The weights of cellulose and xylan extracted with [C₁mim]-[MeCO₂] were 4.0 and 9.8 mg, respectively. The xylan fraction was 0.67 and 0.75 for [C₁mim][(MeO)(Me)PO₂] and [C₁mim][MeCO₂], respectively. [C₁mim][MeOSO₃] extracted xylan selectively; its extraction ability was somewhat greater than that of [C₁mim][(MeO)(Me)PO₂]. These three ILs have almost the same α value (see Table 1), but β is lower for [C₁mim][MeOSO₃] 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 [C₁mim][(MeO)(Me)PO₂] to it. Figure 8 shows the amount of extracted polysaccharides from bran (first extraction) and from IL-treated bran (second extraction).

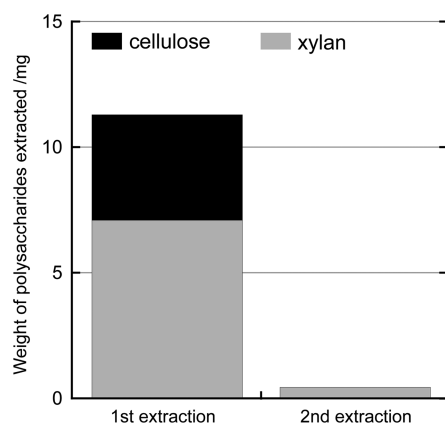


Figure 8. Weight of extracted polysaccharides from 70 mg of bran at 80 °C for 2 h (first extraction) and from the IL-treated bran at 80 °C for 2 h (second extraction) with [C₁mim][(MeO)(Me)PO₂], calculated from NMR signals.

Both extractions were undertaken at 80 °C for 2 h with fresh [C₁mim][(MeO)(Me)PO₂]. To our surprise, almost no polysaccharides were extracted from IL-treated bran. We then analyzed the component of bran treated with [C₁mim]-[(MeO)(Me)PO₂] twice and found that cellulose was still included (SI 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

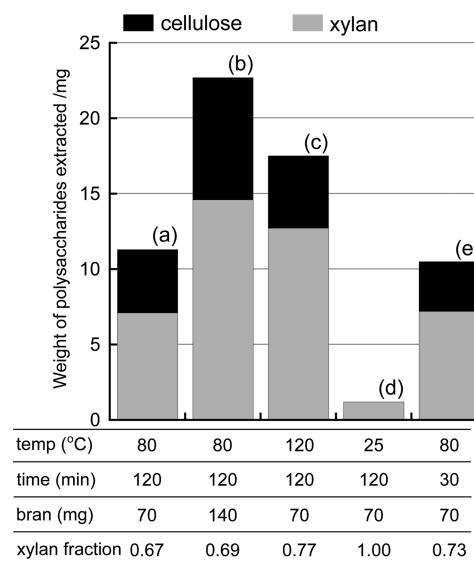


Figure 9. Weight of polysaccharides extracted under various conditions using [C₁mim][(MeO)(Me)PO₂], calculated from NMR signals.

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 °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 liquefies²⁹ 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 and 120 °C (difference between c and a) forms LCCs, but the xylan extracted at 25 °C does not. It is not easy to conclude the different extraction degree at 80 and 25 °C to be partial decomposition of LCC at 80 °C. As for the effect of the duration of extraction, the amount of polysaccharides extracted

in 30 min and 2 h was almost the same (see Figure 9a and e). This shows that almost all polysaccharides extractable at 80 °C were extracted within 30 min. In other words, no LCC was strongly suggested to be decomposed at 80 °C.

The composition of extracts in ILs was easily obtained using ¹H 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 ¹H NMR. Both No-D NMR and the WET technique were used for direct detection of extracted polysaccharides with nondeuterated ILs. This enables rapid analysis without troublesome pretreatments. 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 30 min with stirring at 80 °C.

ASSOCIATED CONTENT

Supporting Information

Experimental section concerning anion/cation ratio analyzed with ¹H NMR; relations between relative peak areas of polysaccharides and cellulose fraction of mixed samples in [C₁mim][MeCO₂] and [C₁mim][MeOSO₃]; ¹³C NMR spectrum of the extract from bran using [C₁mim][(MeO)-(Me)PO₂]; full spectra of the spectra shown in Figure 6; comparison between quantity of polysaccharides analyzed by ¹H NMR and weight of reprecipitated solid; TGA curve of the bran treated with [C₁mim][(MeO)(Me)PO₂] twice; UV–vis absorbance of 2,6-dichlorophenolindophenol sodium salt in the solution of the hydrolysate of the bran treated with [C₁mim]-[(MeO)(Me)PO₂] 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>.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

ILs, ionic liquids; ¹H NMR, proton nuclear magnetic resonance; No-D NMR, no-deuterium NMR; [C₁mim]-

[(MeO)(Me)PO₂], 1,3-dimethylimidazolium methyl methylphosphonate; [C₁mim][MeCO₂], 1,3-dimethylimidazolium acetate; [C₁mim][MeOSO₃], 1,3-dimethylimidazolium methyl sulfate; DMSO, dimethyl sulfoxide; TMS salt, 3-(trimethylsilyl)-propanesulfonic acid sodium salt; LCC, lignin-carbohydrate complex

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