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

Supporting Information

^{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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Experimental Section

To confirm that there are no possible anionic impurities in the synthesis of the ILs (I^- and OH^-), we investigated anion/cation ratio with NMR measurement. It is noted here that anion/cation ratio was 0.99 concerning [C_1mim][MeCOO], which is an only IL synthesized through [C_1mim]I and [C_1mim]OH.

Results and Discussion

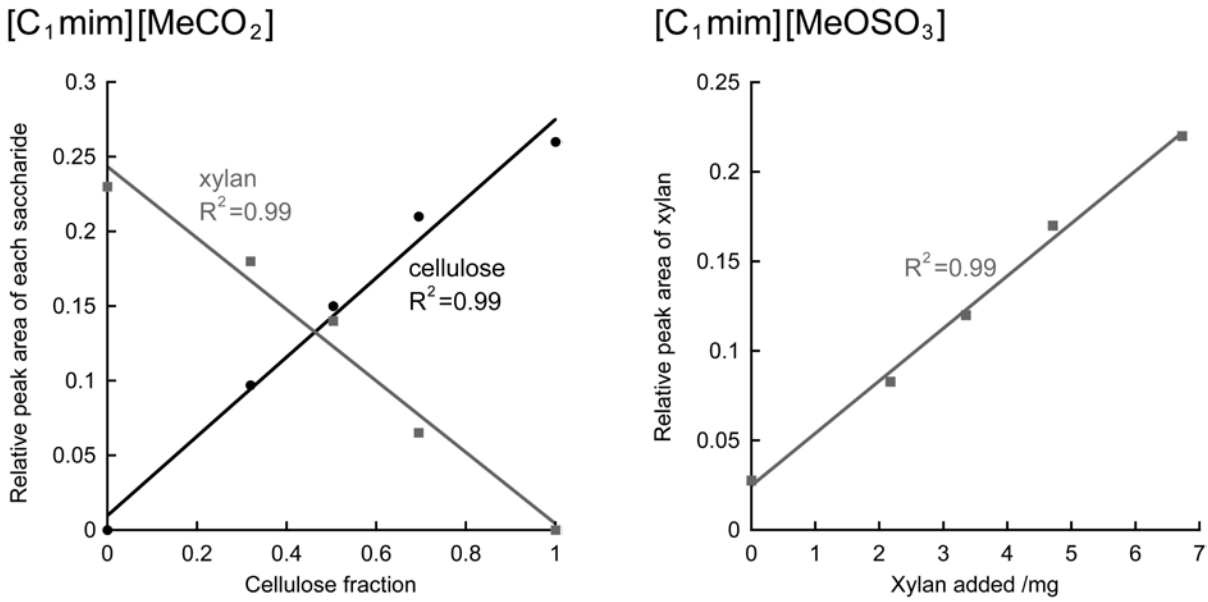


Figure S1 Relation between cellulose fraction of the mixed sample and relative peak area of xylan and cellulose in $[C_1mim][MeCO_2]$ and $[C_1mim][MeOSO_3]$.

Relation concerning cellulose and xylan in $[C_1mim][MeCO_2]$ was linear (R^2 values $\geq$0.99). Relation of xylan in $[C_1mim][MeOSO_3]$ was also linear (R^2 values $\geq$0.99). In $[C_1mim][MeOSO_3]$, the peak area was not zero when xylan was not added. This is attributed to an impurity of $[C_1mim][MeOSO_3]$ at the same chemical shift. These results indicate that cellulose and xylan in mixed samples can be quantified with ^1H NMR.

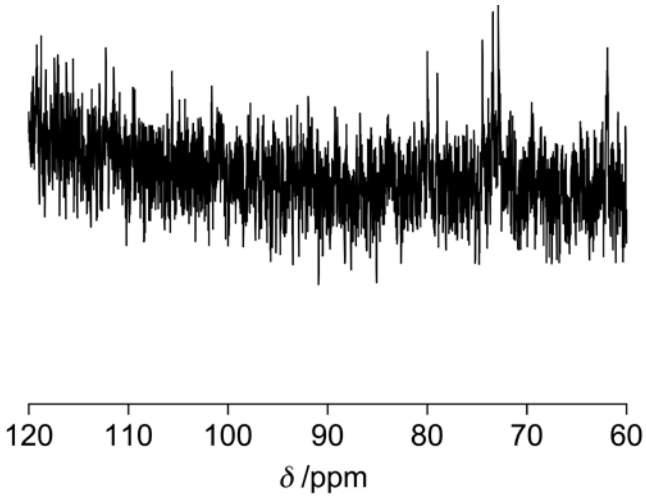


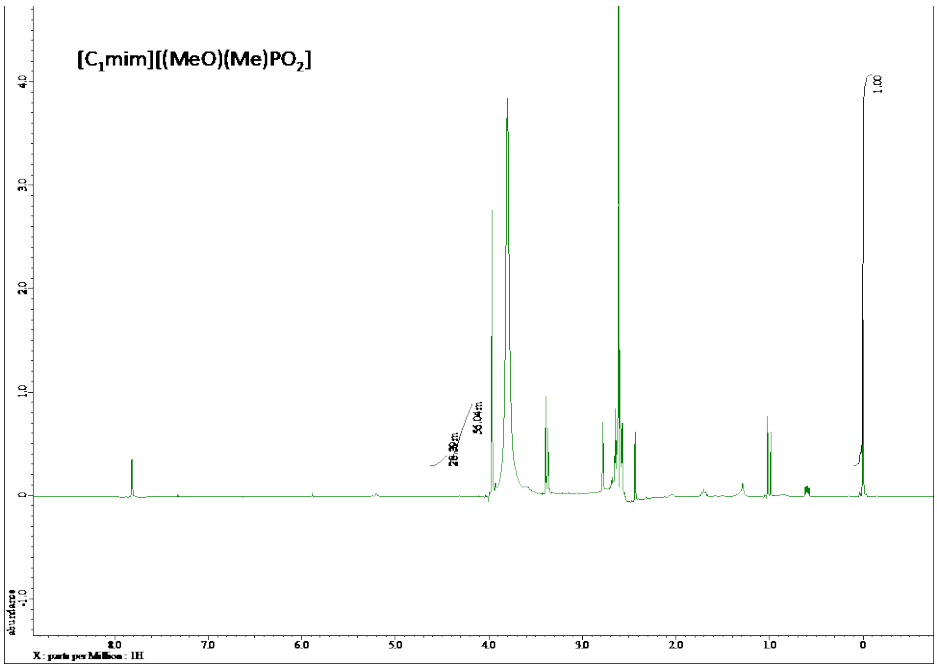
Figure S2 ^{13}C NMR spectrum of the extract from bran with $[C_1mim][(MeO)(Me)PO_2]$.

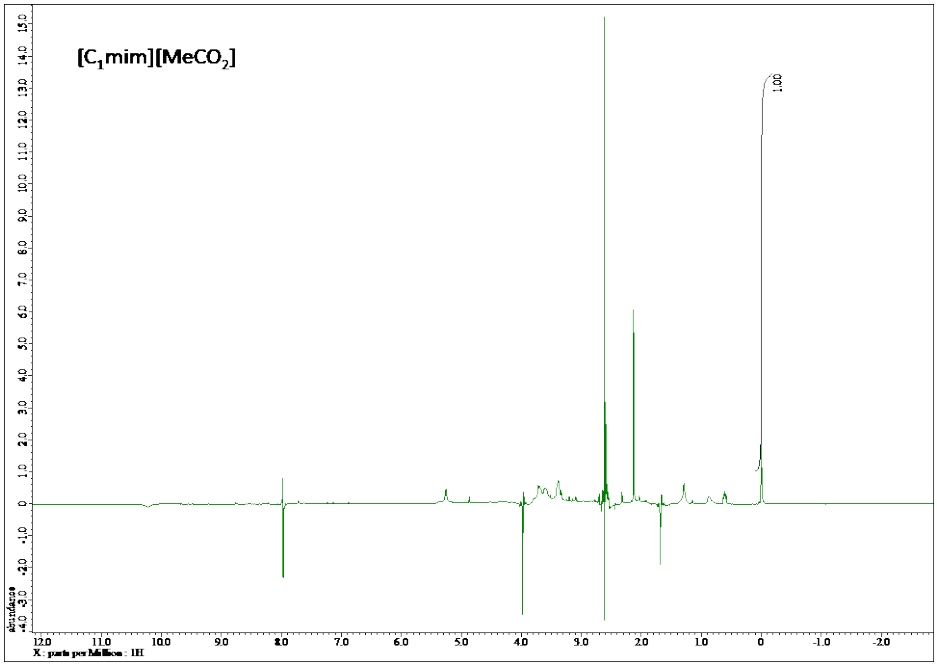
^{13}C NMR analysis of cellulose in ILs has been reported by Rogers *et al.*[1)] It is also a potential candidate for analysis of polysaccharides extracted by ILs. Then the extracted sample at 80 ^{o}C for 2h with $[C_1mim][(MeO)(Me)PO_2]$ (the same sample shown in Figure 6a) was analyzed by ^{13}C NMR at 100 ^{o}C with the accumulation of 5000 scans (about 4h). No signals of polysaccharides were detected in the spectrum while it is known that cellulose shows 6 signals from 60 to 110 ppm in polar ILs. This result was caused by lower sensitivity of ^{13}C NMR compared to that of ^{1}H NMR (about 1/100). In addition, concentration of polysaccharides of this sample (1.1 wt%) was lower than that Rogers *et al.* used (5 wt%). We think that signals can be observed with a great number of accumulations but ^{1}H NMR surely has advantage in this measurement because quantification needs high signal to noise ratio.

1) 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.

Comparison of ^{1}H NMR analysis and usual method (weighing re-precipitated solid) for quantification of polysaccharides

We compared the quantity of polysaccharides analyzed by ^{1}H NMR and weight of re-precipitated solid. Amount of polysaccharides measured by ^{1}H NMR was 11.3 mg, and that of re-precipitated solid was 25 mg. As mentioned in the article, re-precipitated solid includes other components such as ash and then we performed thermogravimetric analysis. After heating up to 500 ^{o}C, about 55 wt% of the extract was lost, and native cellulose and xylan burned out at 500 ^{o}C. Additionally, about 5 wt% of extracts was assigned to water. From these results, the ratio of organic compounds including polysaccharides in the extract was 50 % (12.5 mg). Considering lignin and other materials included in the organic compound fraction of the re-precipitated solid, these values (11.3 mg and 12.5 mg) were reasonably similar.


[C1mim][(MeO)(Me)PO2]
1.00
55.04
26.30
Abundance
X : parts per Million : 1H


[C1mim][MeCO2]
1.00
Abundance
X : parts per Million : 1H

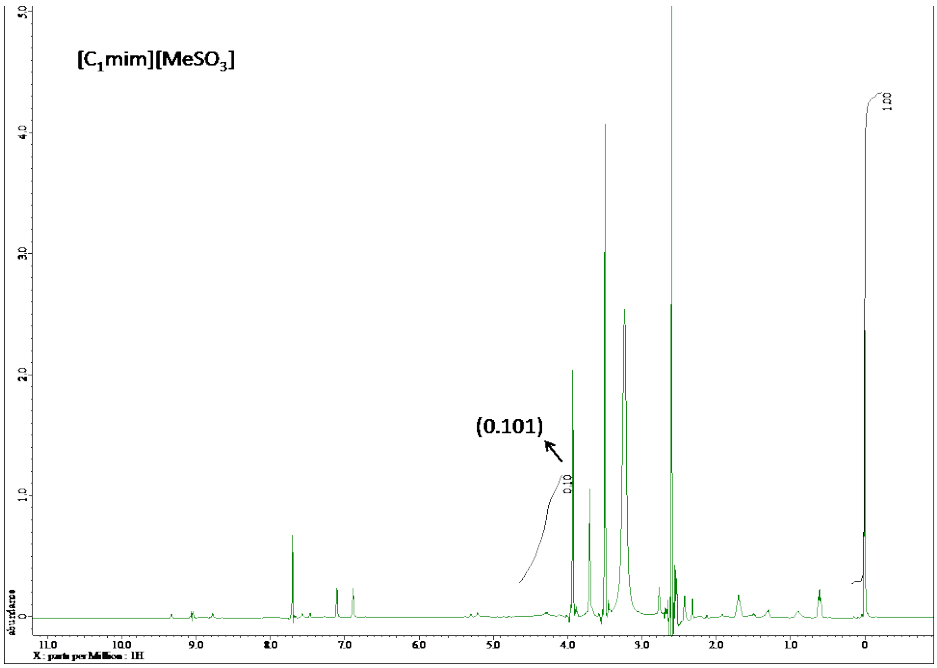


Figure S3 Full spectra of extract with (top) $[C_1mim][(MeO)(Me)PO_2]$, (middle) $[C_1mim][MeCO_2]$, (bottom) $[C_1mim][MeSO_3]$.

Data processing was performed Delta ver. 5.0.2. In the case of $[C_1mim][(MeO)(Me)PO_2]$ and $[C_1mim][MeOSO_3]$, we calculated integration from baseline as usual. In the case of $[C_1mim][MeCO_2]$, we applied deconvolution of the spectra as Lorentzian lineshapes using grams/386. The integration value for cellulose and xylan was 0.0482 and 0.122, respectively. Baseline was simply adjusted to be straight between 0 and 10 ppm (especially 0 and 5 ppm) by adjusting ϕ parameters.

Confirmation of existence of polysaccharides in the bran treated with $[C_1mim][(MeO)(Me)PO_2]$ twice

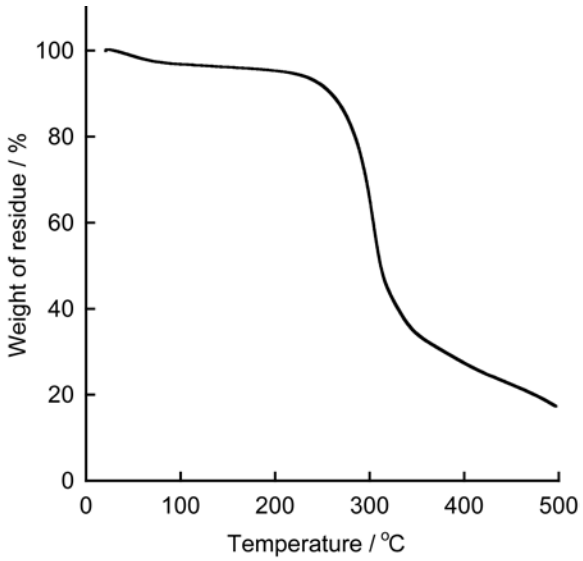


Figure S4 TGA curve of the bran treated with $[C_1mim][(MeO)(Me)PO_2]$ twice.

Table S1 Absorbance of 2,6-dichlorophenolindophenol sodium salt at 600 nm.

Sample	Absorbance at 600 nm
Control (buffer)	0.72
Hydrolyzed sample (hydrolyzed bran after treatment with $[C_1mim][(MeO)(Me)PO_2]$ twice)	0.32

To confirm that there is cellulose in the bran treated with $[C_1mim][(MeO)(Me)PO_2]$ twice, it was analyzed by thermogravimetric analysis (Figure S4). After heating up to 500 ^oC, about 80 % was lost. This result indicates that the IL-treated bran presumably contains polysaccharides whereas only little amount of polysaccharides was extracted in 2nd extraction. To confirm it more precisely, we performed hydrolysis of cellulose included in the bran treated with $[C_1mim][(MeO)(Me)PO_2]$ twice, and then generated glucose was detected (Table S1). The IL-treated bran was hydrolyzed with cellulase and the resulting solution was added into 2,6-dichlorophenolindophenol sodium salt (DCIP) solution (detailed procedure is described below). Absorbance of DCIP decreased by addition of the hydrolyzed sample because electron was transferred from glucose to DCIP. It clearly shows that cellulose was included in the IL-treated bran. These results claim that extraction of polysaccharides is limited despite existence of polysaccharides.

Detailed procedure

Hydrolysis

The bran treated with $[C_1mim][(MeO)(Me)PO_2]$ twice (3.0 mg) and 10 mg of cellulase (celluclast, from Novozyme) were added into 0.40 ml of 100 mM acetate buffer (pH 5.0). The sample was stirred at 60 ^oC for 3h and the supernatant was obtained after centrifugation. Cellulase was used after purification with ultrafiltration.

Detection of glucose

Glucose oxidase (Toyobo Co., Ltd.) and DCIP (Wako Pure Chemical Industries, Ltd.) were dissolved into 100 mM acetate buffer (pH 6.0) to be 10mg/ml and 1 mM, respectively. We mixed 120 μl of the hydrolyzed sample, 20 μl of the glucose oxidase solution, and 100 μl of the DCIP solution and stirred the sample at room temperature. Absorbance of the resulting solution at 600 nm was measured with UV-*vis* spectrometry using quartz cells with 1 cm light-path length. As a control, a sample with 120 μl of acetate buffer as an alternative of the hydrolyzed sample solution was measured and compared.

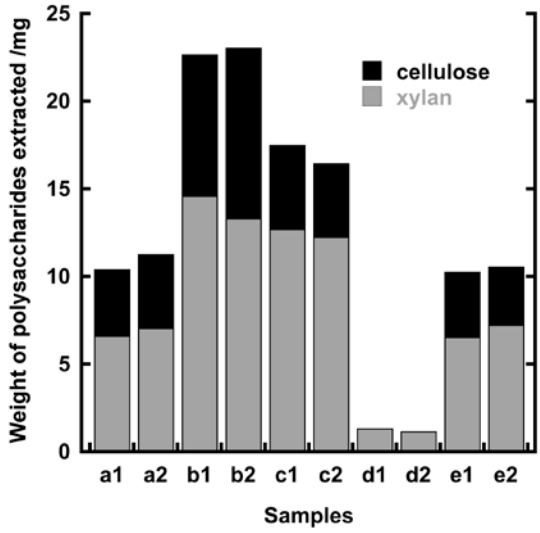


Figure S5 Extraction degree of cellulose and xylan from wheat bran. The extraction was carried out twice.

Experiments are based on Figure 9 in the manuscript. There was little difference between them. Since the error in NMR experiments was shown to be little considering the result of Figure 5 of the manuscript ($R^2 > 0.99$), the error seems to be attributed to the extraction procedure due to heterogeneous process.