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Fractional Isolation and Structural Characterization of Hemicelluloses from Soybean Hull

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Hemicellulosic fractions were extracted from soybean hull with various concentrations of NaOH at 50 °C for 5 h. The chemical compositions and physicochemical properties were determined by high performance anion exchange chromatography (HPAEC), Fourier transform infrared spectroscopy (FT-IR), thermogravimetric analyzer (TGA), and ^{13}C and 2D nuclear magnetic resonance (NMR) analysis. The crystallinity of soybean hulls treated and untreated by alkali liquid was examined by X-ray diffraction (XRD). The sugar analysis results indicated that all of the hemicellulosic preparations were heteropolysaccharides containing arabinose, galactose, glucose, xylose, and mannose. According to the spectral analysis, hemicelluloses from soybean hull were assumed to be L-arabino-4-O-methyl-D-glucurono-D-xylan. Xylose was the predominant monosaccharide in the hemicellulose fraction 1 (H1), and it ranged between 40.1% and 48.8% of the total neutral sugars. It was found that hemicellulose fraction 3 (H3) had the highest thermal stability and H1 had the lowest thermal stability. The crystallinity index (CrI) was found to be about 20.8%, 14.3%, 10.9%, and 4.7% for soybean hulls, untreated and treated with 1.5, 2.0, and 2.5 M NaOH, respectively.

INTRODUCTION

Soybean is not only a traditional leguminous plant used worldwide, but also an important source of oil and protein for humans and animals. Soybean hull is usually regarded as an industrial waste because of its low protein content (Nagata *et al*. 2001), and it is generally separated and applied as animal feed. Soybean hull constitutes about 8% of the whole seed (Agela *et al*. 2008), and the carbohydrate fraction contains 16 to 18% hemicelluloses, 46 to 51% celluloses, 30% pectin, and only 1.4 to 2% lignin (Yoo *et al*. 2011). Therefore, soybean hull is a potentially inexpensive commercial source of hemicelluloses.

Hemicelluloses are types of plant cell wall heteropolysaccharides, generally defined as polysaccharides that can be extracted by water or aqueous alkali from plant tissue (Peng *et al*. 2012). Unlike cellulose, which consists of a single monomer, hemicelluloses are heteroglycans arranged in various proportions and with various sugar units (Sun *et al*. 2005a). The difference from cellulose is the branchings' or side chains' positions of hemicelluloses at C-2 and C-3; the branched polymers have low molecular weights with degrees of polymerization of only 80 to 200 (Ren *et al*. 2008a). They are called pentosans and hexosans, and their general formulas are $(C_5H_8O_4)n$ and $(C_6H_{10}O_5)n$, respectively (Fang *et al*. 1999). Hemicelluloses, which are present in trees and the majority of terrestrial plants, are the world's second most abundant renewable polymers (Sun *et al*.

2005b). The application of hemicelluloses has been studied in recent years, and estimated data show that the annual production of hemicelluloses worldwide reached about 60 billion tons (Xu *et al*. 2006b). Examples of potential future applications of hemicelluloses are as health foods, bioethanol, and barrier films (Persson *et al*. 2009). Hemicelluloses also have the ability to serve as emulsifiers, adhesives, thickeners, and stabilizers in health foods or decorative paints (Fang *et al*. 1999).

Hemicelluloses are usually associated with lignin, cellulose, and protein in plant cell walls (Peng *et al.* 2012). To improve the accessibility of hemicelluloses, it is necessary to remove the lignin. In the plant kingdom, xylans are the most abundant hemicellulose-type polysaccharide constituents (Xu *et al*. 2008). It is difficult to liberate xylan components from grasses, cereal straws, and woods, primarily because of the linkage of ester and ester lignin-hemicelluloses (Xu *et al.* 2006a). Therefore, the first step to isolate hemicelluloses from lignocelluloses is to remove lignin with sodium chlorite in acetic acid solutions; alternatively, the residual lignin can be removed from isolated hemicelluloses (Sun *et al*. 1998, 2000). However, these methods may modify the hemicelluloses and could cause serious environmental problems. Soybean hull only contains 1.4 to 2.0% lignin (Yoo *et al*. 2011); therefore, it does not require delignification prior to isolation of hemicelluloses as compared with other lignocellulosic biomass.

To investigate the potential value of soybean hull hemicelluloses, a thorough study and characterization of alkali-soluble polysaccharides are necessary. The purpose of the present work was to isolate the hemicellulosic fractions from soybean hull and characterize them with various analytical techniques. X-ray diffraction (XRD) was employed to characterize the soybean hull and residues of soybean hull treated by aqueous alkali.

EXPERIMENTAL

Materials

Soybean hull was provided by Henan Sunshine Oils and Fats Group (Zhengzhou, China). The soybean hull was smashed to ensure it could pass through 40-mesh screen. The soybean hull was first extracted with benzene-ethanol (2:1, v/v) in a Soxhlet apparatus for 5 h. The residues were dried in an oven at 50 °C for 15 h and stored in a desiccator before used. The protein content was analyzed by Kjeldahl technique (FOSS Kjeltec^{TM} 8400, Sweden) according to Baboli and Kordi (2010). The ash of soybean hull was analyzed by heating to 600 °C for 6 h in a muffle furnace, and the moisture was analyzed by drying at 130 °C for 3 h in a forced-draft oven. The results showed that the composition of the soybean hull included 12.0% protein, 5.6% ash, and 6.8% moisture. All used chemicals were of analytical or reagent grade.

Extraction of Alkali-soluble Hemicelluloses

Alkali-soluble hemicelluloses were extracted with 1.5, 2.0, or 2.5 M NaOH at 50 °C for 5 h and with an extraction ratio of 1:20 (g/mL), respectively. The liquid was filtered, and the solid material was washed extensively with water. The filtered liquid was neutralized to pH 5.5 with 6 M hydrochloric acid solution, and the precipitate was considered alkali-soluble hemicellulosic fraction H1. The neutralized supernatant fluid was poured into three volumes of 95% ethanol and stirred sufficiently for 3 h. The solid material obtained was thoroughly washed with 70% ethanol. After filtration, the precipitate was placed into dialysis bags, dialyzed in water for 3 days, and the precipitate was named alkali-

soluble hemicellulosic fraction H2. The alkali-soluble hemicellulosic fraction H3 was recovered from the ethanol liquid by rotary evaporation at 45 °C; the ethanol was recycled, and the residues were dialyzed for 3 days. H1, H2, and H3 were all freeze-dried and stored in a desiccator before use.

Characterization

The neutral sugar composition of the hemicelluloses was determined by hydrolysis with 3% H_2SO_4 for 2.5 h at 105 °C. The liberated neutral sugars were analyzed by high-performance anion exchange chromatography (Dionex ICS-3000, Sunnyvale, CA) using a carbopac PA-1 ion exchange column (4×250 mm).

Fourier transform infrared (FT-IR) spectra were obtained using a Nicolet iN10 FT-IR spectroscope (Thermo Nicolet Corporation, Madison, WI) equipped with a liquid nitrogen-cooled mercury cadmium telluride (MCT) detector. The experiments were carried out by scanning in the range of 4000 to 750 cm^{-1} with a resolution of 8 cm^{-1} and a total of 64 scans for each sample.

The solution-state NMR spectrum of fraction H2 was obtained on a Bruker AVIII 400 MHz spectrometer at 25 °C. The hemicellulosic sample (80 mg for ^{13}C NMR and 20 mg for HSQC NMR) was dissolved in 1 mL of D_2O. Solution state ^{13}C and HSQC NMR spectra were recorded according to the literatures (Sun *et al*. 2013).

Thermal analyses of hemicellulosic fractions were performed with a simultaneous thermal analyzer (SDT Q600, TA instrument). A continuous measurement of sample weight was provided by this apparatus at a range of temperatures between room temperature and 600 °C in a nitrogen environment. Hemicellulosic fractions weighing between 8 and 12 mg were heated in a platinum crucible to 600 °C at the rate of 10 °C per min.

XRD patterns of the treated and un-treated soybean hulls were obtained with a PANalytical X'pert PRO diffractometer (PANalytical, Netherlands) at a generator voltage of 40 kV and diffraction angle of 10° to 30° 2θ angle. The crystallinity index (CrI) of the treated and un-treated soybean hulls was calculated from XRD data by the Segal method (Segal *et al*. 1959) as follows,

$$CrI = [(I_{002} - I_{am}) / I_{002}] \times 100\% \qquad (1)$$

where I_{002} is the peak for both crystalline and amorphous regions and I_{am} assumes only the amorphous part. It should be noted in this study that I_{002} is the intensity of the 002 crystal orientation at 2θ = 21°, and I_{am} is the intensity of diffraction at 2θ = 17.4°.

RESULTS AND DISCUSSION

Yield and Sugar Composition of Hemicelluloses

Table 1 gives the yield and the compositions of the isolated hemicelluloses. The yield of the total hemicelluloses (H1+H2+H3) increased from 12.3 to 16.0% with an increase in the concentration of alkali liquor from 1.5 to 2.5 M. The content of fraction H1 increased from 2.6 to 4.8% when the concentration of alkali liquor increased from 2.0 to 2.5 M. Fraction H2 was the main product during the process of isolating the hemicelluloses. Compared with extraction using 1.5 M NaOH, the production of H2 increased from 5.5 to 9.0% when the concentration increased to 2.5 M. Fraction H3 was the second component, and the content decreased dramatically from 4.7 to 2.3% when the solvent concentration

increased from 2.0 to 2.5 M. To characterize the hemicellulose content in soybean hull, the nine fractions were prepared, and their substituent sugars were determined. The results of sugar analysis of the nine isolated hemicellulosic fractions are shown in Table 1. Xylose is present as a predominant sugar component in fraction H1, comprising 40.1 to 48.8% of the total sugars. It should be noted that all three H1 fractions also contained high amount of protein (19.1-19.7%). These results maybe suggest that the present of xylose binging protein structure in the H1 fractions. A small amount of mannose (1.1-3.5%), arabinose (1.6-2.1%), glucose (0.4-0.6%), and uronic acids (0.7-1.2%) were also verified in the three H1 preparations. When the concentration of alkali liquor increased from 1.5 to 2.5 M, the xylose concentration increased from 40.1% to 48.8%. It has been reported that xylan-rich hemicelluloses could inhibit the growth rate of sarcoma-180 and other tumors (Sun *et al.* 2002). In fraction H2, arabinose and glucose appeared as the major sugar constituents, and arabinose was the dominant constituent of fraction H3. It is believed that arabinoxylans have the ability to retain gas in dough and have the useful functional property of protecting protein foam against thermal disruption (Sun *et al.* 2002). Comparing fractions H1, H2, and H3 extracted with 1.5, 2.0, and 2.5 M NaOH, the results demonstrated that the concentration of alkali liquor did not result in an obvious change in the composition of sugar. However, it is also believed that increasing the concentration of alkali liquor could increase the content of xylose and decrease the content of mannose. The contents of uronic acid were 7.5, 7.8 , and 8.9% in H2 obtained by treatment with 1.5, 2.0, and 2.5 M NaOH, respectively, suggesting that alkali extraction soybean hull favored the isolation of the hemicelluloses enriched in uronic acids.

Table 1. Yield and Components (relative % hemicellulosic sample, w/w) in Isolated Hemicellulosic Preparations Obtained with 1.5, 2.0, and 2.5 M NaOH for 5 h from Soybean Hull

Yield/ components (Wt %)	1.5 M NaOH			2.0 M NaOH			2.5 M NaOH		
	H1	H2	H3	H1	H2	H3	H1	H2	H3
Yield	2.2	5.5	4.6	2.6	6.3	4.7	4.8	9.0	2.3
Protein	19.7	9.1	15.7	19.1	9.6	11.4	19.1	8.5	10.1
Arabinose	2.1	15.8	10.6	1.6	16.1	9.2	1.8	17.8	10.9
Galactose	0.6	13.7	1.2	0.4	12.5	1.2	0.6	11.4	1.8
Glucose	4.9	2.2	0.7	4.5	3.9	1.0	4.1	3.4	1.2
Xylose	40.1	6.1	0.6	43.5	11.2	0.6	48.8	7.9	0.5
Mannose	3.5	8.6	1.0	1.1	3.6	0.9	1.5	2.3	1.1
Uronic acid	1.2	7.5	2.1	0.7	7.8	1.9	0.9	8.9	1.8

FT-IR Analysis

Figure 1 shows the FT-IR spectra of all the nine hemicellulosic fractions (H1, H2 and H3) which respectively obtained from soybean hull with 1.5, 2, and 2.5 M NaOH aqueous solutions at 50 °C for 5 h. The FT-IR spectra were used to evaluate the structural differences between the nine hemicellulosic fractions extracted using the different concentrations of NaOH. Comparing Fig. 2a, 2b, and 2c, the spectra of the above fractions isolated by the various concentrations of NaOH showed broadly similar patterns, indicating that the polymers had similar structures. A sharp band at 892 cm^{-1} can be assigned to β-glucosidic linkages between the sugar units, indicating that the xylose residues forming the backbone of the macromolecule were linked by β-form bonds (Ren *et al.* 2008b). The band

region of 1200 to 950 cm^{-1} is attributed to the signals of C-O stretching (Sun *et al.* 2002). Two shoulders at 968 and 1031 cm^{-1} are characteristic of arabinose substitution at C-3 of the xylose residues, indicating a characteristic typical of substituted arabinoxylans (Xu *et al.* 2006b). A sharp and intensive band at 1066 cm^{-1} is indicative of the C-O-C stretching that is typical for xylans (Sun *et al.* 2004). Each spectrum presents a peak at 1412 cm^{-1} that is indicative of the CH$_2$ bending and one at 1312 cm^{-1} representing O-H bending. The absorption at 1634 cm^{-1} principally indicates absorbed water. The band at 3337 cm^{-1} arises from the stretching of -OH groups, and the absorption at 2908 cm^{-1} corresponds to C-H stretching. As seen from the spectra of fraction H1, the occurrence of a low-intensity shoulder in the region of 1500 to 1200 cm^{-1} undoubtedly corresponds to the results obtained by alkaline nitrobenzene oxidation and is probably the small amounts of associated lignin in the hemicelluloses. Hemicellulose treatment by aqueous alkali below 50 °C for 5 h completely saponified these ester bonds. In contrast, the results shown in the spectra of H1, H2, and H3 correspond to the sugar analysis.
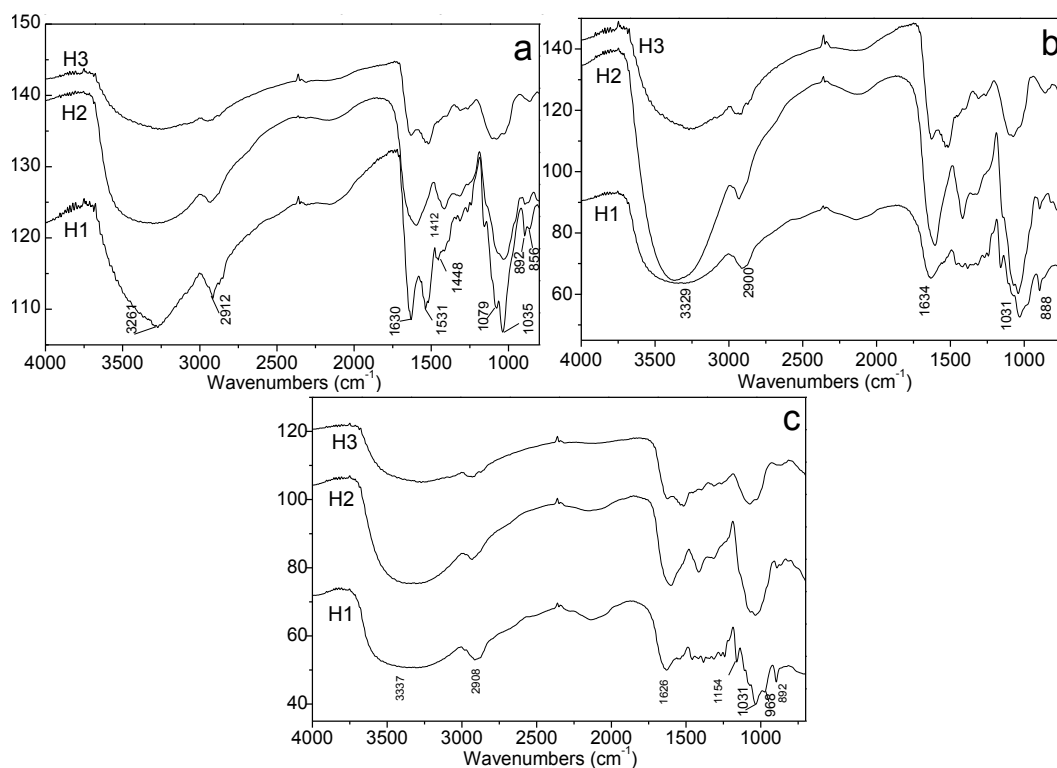

Fig. 1. FT-IR spectra of hemicellulose fractions: (a) hemicelluloses H1, H2, and H3 isolated by 1.5 M NaOH at 50 °C for 5 h; (b) hemicelluloses H1, H2, and H3 isolated by 2.0 M NaOH at 50 °C for 5 h; (c) hemicelluloses H1, H2, and H3 isolated by 2.5 M NaOH at 50 °C for 5 h

^{13}C and 2D NMR Spectra

To characterize the structural features of the isolated hemicelluloses, the hemicellulosic fraction H2 extracted by 2.0 M NaOH was analyzed by NMR spectra. Analysis by ^{13}C-NMR spectroscopy in D$_2$O can elucidate the backbone of the polymer and define the type of side-chain branching along the backbone (Sun *et al*. 1999). Clearly, Fig. 2 shows five significant signals at 108.8, 82.5, 78.3, 76.2, and 68.1 ppm, corresponding to the C-1, C-4, C-3, C-2, and C-5 positions of α-L-arabinofuranosyl units (Sun *et al*. 2013). Other, weaker signals at 98.4, 76.2, 75.6, 71.5, and 61.4 ppm can be assigned to the C-1,

C-4, C-3, C-2, and C-5 positions of β-D-xylopranosyl units (Sun *et al*. 2013). Meanwhile, small characteristic signals at 61.4 and 181.5 ppm can be ascribed to -OCH₃ (Jin *et al*. 2009).
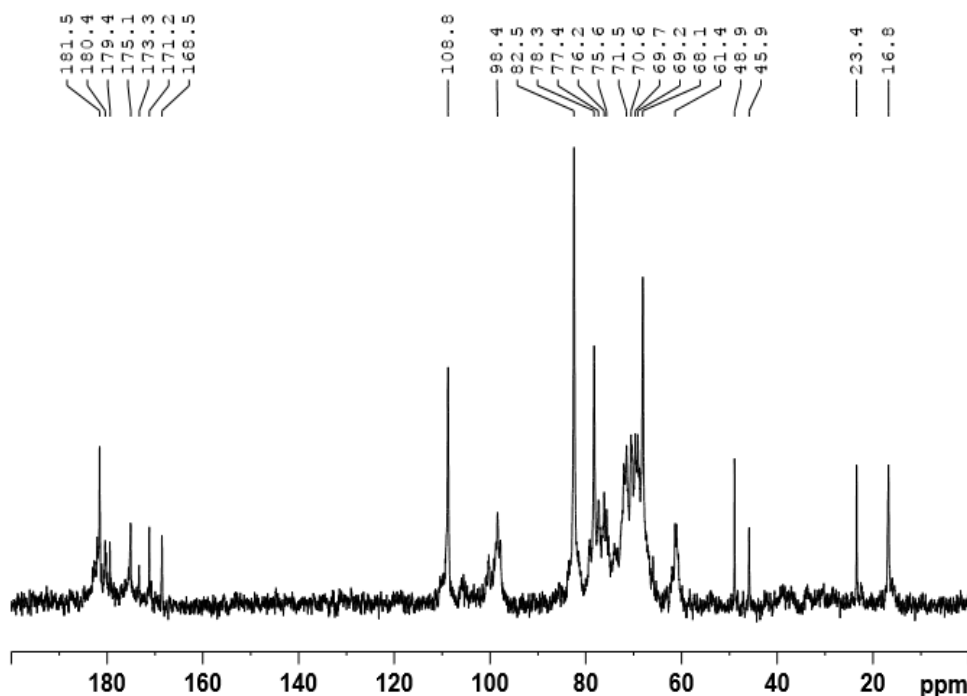
Fig. 2. ¹³C NMR spectrum (in D₂O) of the hemicellulosic fraction isolated with 2.0 M NaOH at 50 °C for 5 h
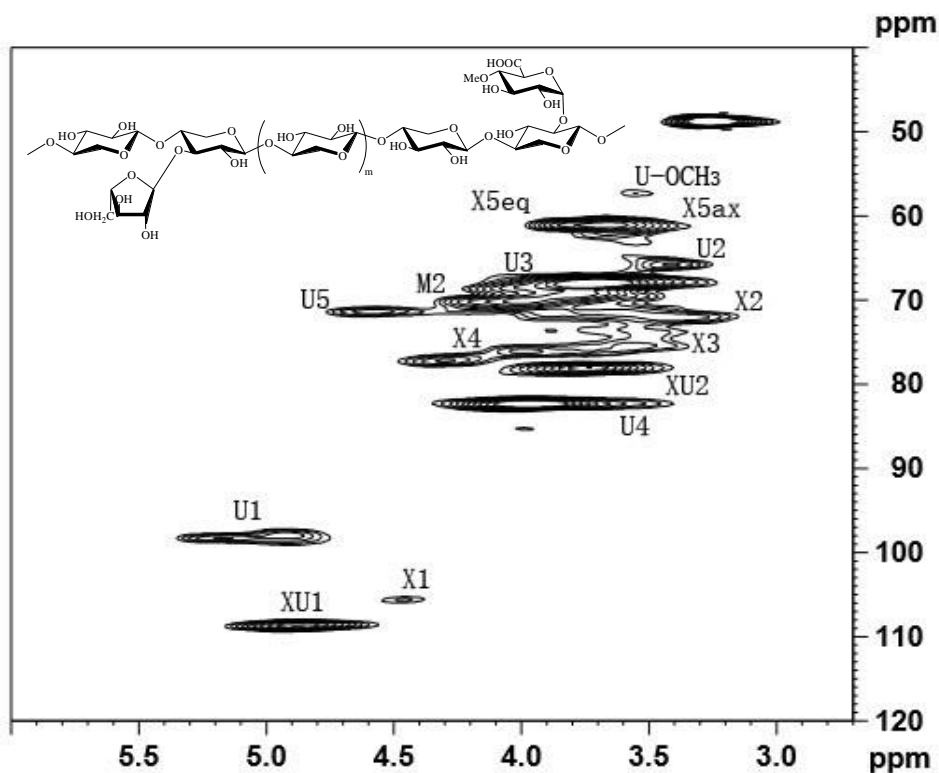
Fig. 3. 2D HSQC NMR spectrum of hemicelluloses H2 extracted by 2.0 M NaOH at 50 °C for 5 h. X: (1→4)-β-D-Xylp; UA: 4-O-Methyl-α-D-GlcpA; XU: (1→4)-β-D-Xylp-2-O-(4-oMe-D-GlcpA).

Further specific information about the structural variation of H2 was obtained by 2D HSQC NMR spectrometry (Fig. 3). Clearly, five dominant cross-peaks of α-L-arabinofuranosyl units were detected at 108.8/5.1, 82.5/4.0, 78.3/3.7, 84/4.7, and 65/3.5 ppm, which were characteristic of C1-H1, C2-H2, C3-H3, C4-H4, and C5-H5, respectively (Sun *et al*. 2013). Moreover, the signals at 105/4.5, 73.2/3.2, 75.6/3.5, 76/3.6, and 63.4/3.5 ppm correspond to C1-H1, C2-H2, C3-H3, C4-H4, and C5-H5 of β-D-xylopranosyl units (Sun *et al*. 2013). In addition, some weak cross-peaks at 97/5.1, 72/3.4, 73/3.7, 78/4.0, and 49/3.4 ppm, which can be assigned to C1-H1, C2-H2, C3-H3, C4-H4, C5-H5, and -OCH$_3$ of 4-O-Me-α-D-GlcpA units (Sun *et al*. 2014; Peng and She 2014), were also detected in the HSQC spectrum. The peaks at C1-H1 of D-GalpA residues at 97/5.1 were shifted compared to that usually reported at 101.5/5.1 (Shakhmatov *et al*. 2015). Although there was no clear evidence of protein attachment, it is suggested that there might be a connection between proteins and D-GlapA residues. In the FT-IR spectra of the fraction H2, the sharp band at 1600 cm^{-1} and between 1640 and 1560 cm^{-1} corresponds to –NH$_2$, which may be a secondary proof of this conjecture. Based on the FT-IR data and NMR spectra analysis, it is concluded that the hemicellulosic fraction H2 extracted with 2.0 M NaOH from soybean hull was primarily composed of L-arabino-4-O-methyl-D-glucurono-D-xylan.

Thermal Analysis

The thermal properties of the hemicellulosic preparations were studied by thermogravimetric analysis (TGA), which can investigate the behavior of substances as a function of temperature. In this study, the thermograms of hemicellulosic preparations H1, H2, and H3 extracted with 2.0 M NaOH at 50 °C for 5 h are shown in Fig. 4. Clearly, the thermograms were divided into three stage temperatures. In the first stage, the early minor weight loss observed for all samples was a result of the free water desorbed from the structure of hemicelluloses (Ren *et al*. 2008a). In the second stage, the weight loss that occurred was most likely because of the bound water which is more strongly associated with the sugar structure (Ren *et al*. 2008a). The weight loss in the third stage (above 400 °C) may have resulted from the degradation of the sugar structure.
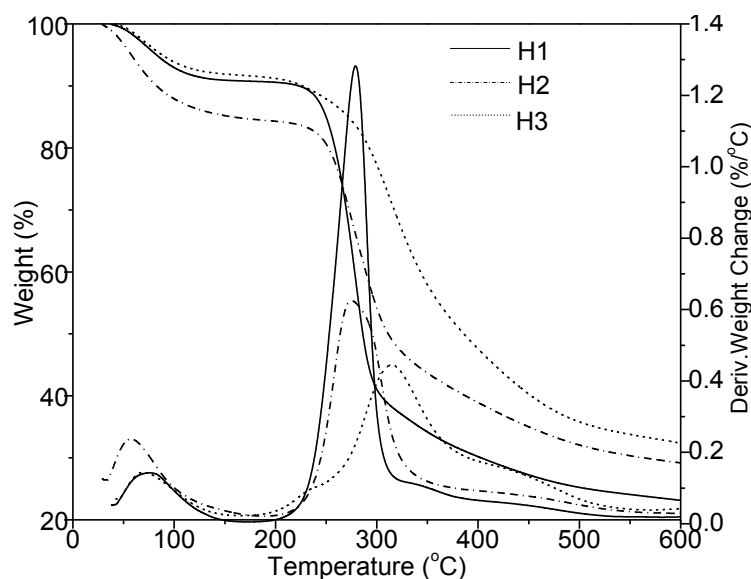

Fig. 4. Thermograms of hemicellulosic preparations H1, H2, and H3 extracted with 2.0 M NaOH at 50 °C for 5 h

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As shown in the thermograms, H2 experienced more weight loss in the early stage than fractions H2 and H3, which indicated that H2 had more absorbed water in the structure. In addition, all three samples of hemicelluloses had a sharp weight loss when the temperature was above 250 °C. At 60% weight loss, the decomposition temperature occurred at 450 °C for H3, at 375 °C for H2, and at 300 °C for H1. These decreasing trends of decomposition temperature indicated that the thermal stability of H3 was higher than that of H2 and the stability of H1 was the worst.

X-Ray Diffraction

The X-ray diffraction patterns of the treated by NaOH aqueous solutions (1.5, 2.0, and 2.5 M) and untreated soybean hull residuals are shown in Fig. 5. The crystallinity index (CrI) for all samples was calculated from the XRD data. The CrI was found to be 20.8%, 14.3%, 10.9%, and 4.7% for untreated and soybean hulls treated with 1.5, 2.0, and 2.5 M NaOH, respectively. This decrease in CrI after alkali liquid treatment for 5 h not only removed the amorphous portion of cellulose but also partly destroyed the crystalline structure. Figure 5 shows a noticeable decrease in the crystallinity of celluloses under alkaline conditions; the peak of the crystalline area narrowed and became lower when the alkali liquid concentration increased. It should be noted in this study that the first highest peak shifted from 17.4° to 16.8° after treatment with 2.0 and 2.5 M NaOH. It was due to the degradation of those amorphous region such as hemicelluloses, protein, and lignin after treatment with higher amount of NaOH.
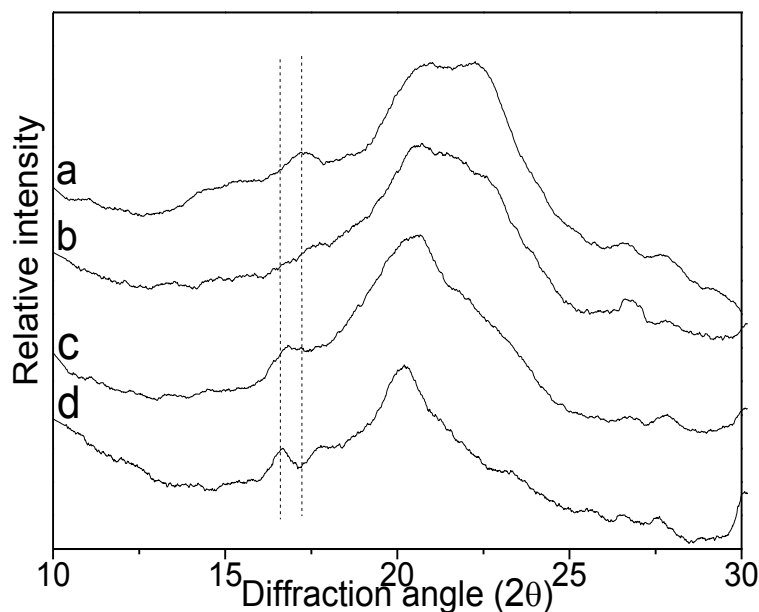
Fig. 5. X-ray diffraction patterns of (a) un-treated soybean hull and soybean hull treated with (b) 1.5, (c) 2.0, and (d) 2.5 M NaOH

CONCLUSIONS

1. The yield of the total hemicelluloses (H1+H2+H3) increased from 12.3% to 16.0% with an increase in the concentration of alkali liquor from 1.5 to 2.5 M.

2. The predominant monosaccharide in H1 fraction was xylose, ranging between 40.1% and 48.8% of the total neutral sugars. Increasing concentration of alkali liquor could increase the content of xylose and decrease the content of mannose in H1 fractions.

3. The fraction H3 extracted by 2.0 M NaOH exhibited a higher thermal stability than fraction H2.

4. The structure of hemicellulosic fraction H2 isolated from soybean hull with 2.0 M NaOH contained L-arabino-4-O-methyl-D-glucurono-D-xylan.

ACKNOWLEDGMENTS

REFERENCES CITED

Agela, C. S., Simone, H. F., Plinho, H. F., Gilvane, S. M., and Marco, A. Z. A. (2008). "Production of ethanol from soybean hull hydrolysate by osmotolerant *Candida guilliermondii* NRRL Y-2075," *Bioresour. Technol.* 99(8), 2898-2094. DOI: 10.1016/j.biortech.2007.06.042

Baboli, Z. M., and Kordi, A. A. S. (2010). "Characteristics and composition of watermelon seed oil and solvent extraction parameters effects," *J. Am. Oil Chem. Soc.* 87(6), 667-671. DOI: 10.1007/s11746-010-1546-5

Fang, J. M., Sun, R. C., Salisbury, D., Fower, P., and Tomkinson, J. (1999). "Comparative study of hemicelluloses from wheat straw by alkali and hydrogen peroxide extractions," *Polym. Degrad. Stab.* 66(3), 423-432. DOI: 10.1016/S0141-3910(99)00095-6

Jin, A. X., Ren, J. L., Peng, F., Xu, F., Zhou, G. Y., Sun, R. C., and Kennedy, J. F. (2009). "Comparative characterization of degraded and non-degradative hemicelluloses from barley straw and maize stems: Composition, structure, and thermal properties," *Carbohydr. Polym.* 78(3), 609-619. DOI: 10.1016/j.carbpol.2009.05.024

Nagata, J., Higashiuesato, Y., Maeda, G., Chinen, I., Saito, M., Wabuchi, K. I., and Onoe, K. (2001). "Effects of water-soluble hemicellulose from soybean hull on serum antibody levels and activation of macrophages in rats," *J. Agric. Food Chem.* 49(10), 4965-4970. DOI: 10.1021/jf0104883

Peng, F., Peng, P., Xu, F., and Sun, R. C. (2012). "Fractional purification and bioconversion of hemicelluloses," *Biotechnol. Adv.* 30(4), 879-903. DOI: 10.1016/j.biotechadv.2012.01.018

Peng, P., and She, D. (2014). "Isolation, structural characterization, and potential applications of hemicelluloses from bamboo: A review," *Carbohydr. Polym.* 112(11), 701-720. DOI: 10.1016/j.carbpol.2014.06.068

Persson, T., Ren, J. L., Joelsson, E., and Jönsson, A. S. (2009). "Fractionation of wheat and barley straw to access high-molecular-mass hemicelluloses prior to ethanol production," *Bioresour. Technol.* 100(17), 3906-3913. DOI: 10.1016/j.biortech.2009.02.063

Ren, J. L., Peng, F., and Sun, R. C. (2008a). "Preparation and characterization of hemicellulosic derivatives containing carbamoylethyl and carboxyethyl groups," *Carbohydr. Res.* 343(16), 2776-2782. DOI: 10.1016/j.carres.2008.05.019

Ren, J. L., Xu, F., Sun, R. C., Peng, B., and Sun, J. X. (2008b). "Studies of the lauroylation of wheat straw hemicelluloses under heating," *J. Agric. Food Chem.* 56(4), 1251-1258. DOI: 10.1021/jf072983q

Segal, L., Creely, L., Martin, A. E., and Conrad, C. M. (1959). "An empirical method for estimating the degree of crystallinity of native cellulose using the X-ray diffractometer," *Text. Res. J.* 29(10), 786-794. DOI: 10.1177/004051755902901003

Shakhmatov, E. G., Udoratina, E. V., Atukmaev, K. V., and Makarova, E. N. (2015). "Extraction and structural characteristics of pectic polysaccharides from *Abies sibirica* L," *Carbohydr. Polym.* 123(5), 228-236. DOI: 10.1016/j.carbpol.2015.01.041

Sun, J. X., Sun, X. F., Sun, R. C., and Su, Y. Q. (2004). "Fractional extraction and structural characterization of sugarcane bagasse hemicelluloses," *Carbohydr. Polym.* 56(2), 195-204. DOI: 10.1016/j.carbpol.2004.02.002

Sun, R. C., Fang, J. M., Rowlands, P., and Bolton, J. (1998). "Physicochemical and thermal characterization of wheat straw hemicelluloses and cellulose," *J. Agric. Food Chem.* 46(7), 2804-2809. DOI: 10.1021/jf971078a

Sun, R. C., Fang, J. M., and Tomkinson, J. (2000). "Characterization and esterification of hemicelluloses from rye," *J. Agric. Food Chem.* 48(4), 1247-1252. DOI: 10.1021/jf990570m

Sun, R. C., Fang, J. M., Tomkinson, J., and Jones, G. L. (1999). "Acetylation of wheat straw hemicelluloses in N,N-dimethylacetamide LiCl solvent system," *Ind. Crop. Prod.* 10(3), 209-218. DOI: 10.1016/S0926-6690(99)00025-4

Sun, R. C., Sun, X. F., and Ma, X. H. (2002). "Effect of ultrasound on the structural and physiochemical properties of organosolv soluble hemicelluloses from wheat straw," *Ultrason. Sonochem.* 9(2), 95-101. DOI: 10.1016/S1350-4177(01)00102-X

Sun, S. L., Wen, J. L., Ma, M. G., Song, X. L., and Sun, R. C. (2014). "Integrated biorefinery based on hydrothermal and alkaline treatments: Investigation of sorghum hemicelluloses," *Carbohydr. Polym.* 111(10), 663-669. DOI: 10.1016/j.carbpol.2014.04.099

Sun, S. L., Wen, J. L., Ma, M. G., and Sun, R. C. (2013). "Successive alkali extraction and structural characterization of hemicelluloses from sweet sorghum stem," *Carbohydr. Polym.* 92(2), 2224-2231. DOI: 10.1016/j.carbpol.2012.11.098

Sun, X. F., Sun, R. C., Fowler, P., and Baird, M. S. (2005a). "Extraction and characterization of original lignin and hemicelluloses from wheat straw," *J. Agric. Food Chem.* 53(4), 860-870. DOI: 10.1021/jf040456q

Sun, X. F., Xu, F., Zhao, H., Sun, R. C., Fowler, P., and Baird, M. S. (2005b). "Physicochemical characterisation of residual hemicelluloses isolated with cyanamide-activated hydrogen peroxide from organosolv pre-treated wheat straw," *Bioresour. Technol.* 96(12), 1342-1349. DOI: 10.1016/j.biortech.2004.11.018

Xu, F., Jiang, J. X., Sun, R. C., She, D., Peng, B., Sun, J. X., and Kennedy, J. F. (2008). "Rapid esterification of wheat straw hemicelluloses induced by microwave irradiation," *Carbohydr. Polym.* 73(4), 612-620. DOI: 10.1016/j.carbpol.2008.01.002

Xu, F., Liu, C. F., Geng, Z. C., Sun, J. X., Sun, R. C., Hei, B. H., Lin, L., Wu, S. B., and Je, J. (2006a). "Characterisation of degraded organosolv hemicelluloses from wheat straw," *Polym. Degrad. Stab.* 91(8), 1880-1886. DOI: 10.1016/j.polymdegradstab.2005.11.002

Xu, F., Sun, J. X., Liu, C. F., and Sun, R. C. (2006b). "Comparative study of alkali- and acidic organic solvent-soluble hemicellulosic polysaccharides from sugarcane bagasse," *Carbohydr. Res.* 341(2), 253-261. DOI: 10.1016/j.carres.2005.10.019

Yoo, J., Alavi, S., Vadlani, P., and Amanor-Boadu, V. (2011). "Thermo-mechanical extrusion pretreatment for conversion of soybean hulls to fermentable sugars," *Bioresour. Technol.* 102(16), 7583-7590. DOI: 10.1016/j.biortech.2011.04.092