DOI:10.1021/jf9033255

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Fractional Study of Alkali-Soluble Hemicelluloses Obtained by Graded Ethanol Precipitation from Sugar Cane Bagasse

Feng Peng,[†] Jun-Li Ren,[†] Feng Xu,*[,‡] Jing Bian,[‡] Pai Peng,[§] and Run-Cang Sun*[,†,‡]

[†]State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, Guangzhou, China, [‡]Institute of Biomass Chemistry and Technology, Beijing Forestry University, Beijing, China, and [§]College of Forestry, Northwest A&F University, Yangling, China

The two hemicellulosic fractions were subsequentially extracted with 5% and 8% NaOH aqueous solution at a solid to liquid ratio of 1:25 (g mL^{-1}) at 50 °C for 3 h from the water, 1 and 3% NaOH-treated sugar cane bagasse, and subfractionated into six preparations by a graded ethanol precipitation method at concentrations of 15%, 30% and 60% (v/v). Sugar composition and molecular weight analysis showed that, with an increasing concentration of ethanol, hemicellulosic subfractions with both higher Ara/Xyl ratios and higher molecular weights were obtained. In other words, with an increasing ethanol concentration from 15% to 60%, the Ara/Xyl ratios increased from 0.043 in H_1 to 0.088 in H_3 and from 0.040 in H_4 to 0.088 in H_6, and the weight-average molecular weights of hemicellulosic subfractions increased from 42 430 (H_1) to 85 510 (H_3) g mol^{-1} and from 46 130 (H_4) to 64 070 (H_6) g mol^{-1}, respectively. The results obtained by the analysis of Fourier transform infrared, sugar composition, and ^1H and ^{13}C nuclear magnetic spectroscopy showed that the alkali-soluble hemicelluloses had a backbone of xylose residues with a β-(1→4)-linkage and were branched mainly through arabinofuranosyl units at C-2 and/or C-3 of the main chain, whereas the differences may occur in the distribution of branches along the xylan backbone.

KEYWORDS: Sugar cane bagasse; arabinxylans; fractionation; sugars

INTRODUCTION

Sugar cane (*Saccharum officinarum*) is a kind of grass, which is harvested for its sucrose content. After sucrose extraction, the residual plant material is called bagasse. Sugar cane bagasse (SCB), a waste byproduct of the sugar and alcohol industries, is generated in large quantities, and about 54 million tons of bagasse being produced annually throughout the world (*1*). Some of this bagasse is burned to produce energy, but a huge amount is not used, which leads to an environmental problem. Since bagasse is mainly composed of cellulose (40−45%), hemicelluloses (30−35%) and lignin (20−30%) (*2*), the considerable research aiming at the utilization of hemicelluloses and cellulose from bagasse is increasing. Therefore, SCB, as an agroindustrial residue, can be valuable in providing transport fuels and chemical feedstock.

Lignocellulosic biomass such as SCB is primarily made up of three main types of biopolymer, namely, cellulose, lignin, and hemicelluloses, and of these, cellulose and lignin have received by far the most attention in terms of material applications. Hemicelluloses are polysaccharide polymers that are biosynthesized in large quantities by trees and terrestrial plants. An estimated annual production of hemicelluloses on the earth is in the range of 60 billion tons (*3*). According to their primary structure, four main groups of hemicelluloses may be defined: xyloglycans (xylans), mannoglycans (mannans), β-glucans, and xyloglucans (*4*). In most cases, xylans consist of a β-(1→4)-D-xylopyranose backbone with side groups on the C-2 or C-3 position. Two types of mannans exist, namely, galactomannans consisting of β-(1→4) linked D-mannopyranoses and glucomannans composed of D-mannopyranose and D-glucopyranose with β-(1→4) linkages. β-Glucans have a D-glucopyranose backbone with mixed β linkages (1→3, 1→4) in different ratios and can form highly viscous solutions and gels. Xyloglucans have a backbone of β-(1→4) linked D-glycopyranose residues with a distribution of D-xylopyranose in position 6. In grass and cereal straw, the most abundant hemicelluloses are xylans, which have the same backbone as the hardwood xylans, consisting of about 200 β-xylopyranose residues, linked together by 1,4-glycosidic bonds. They contain smaller amount of uronic acids, but are more highly branched and contain a large proportion of L-arabinofuranosyl units. The former, consisting of 4-*O*-methylglucuronic acid, are attached directly to the C-2 position of xylose, while the latter are linked mainly to the C-3 position of xylose (*5*). The hemicelluloses are potentially very useful. Studies on utilization of hemicelluloses have been demonstrated to be a potential fermentation feedstock in production of ethanol, acetone, butanol, and xylitol. Xylitol is produced by hydrolysis of xylan, crystallization of xylose and hydrogenation (*6*). Recently, there has been interest in the use of hemicelluloses as hydrogels (*7*). More often, the reported industrial

*Corresponding authors. R.-C. Sun.: State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, Guangzhou, China; e-mail, rcsun3@bjfu.edu.cn; tel, +86-10-62336972; fax, +86-10-62336972.

 Published on Web 12/16/2009

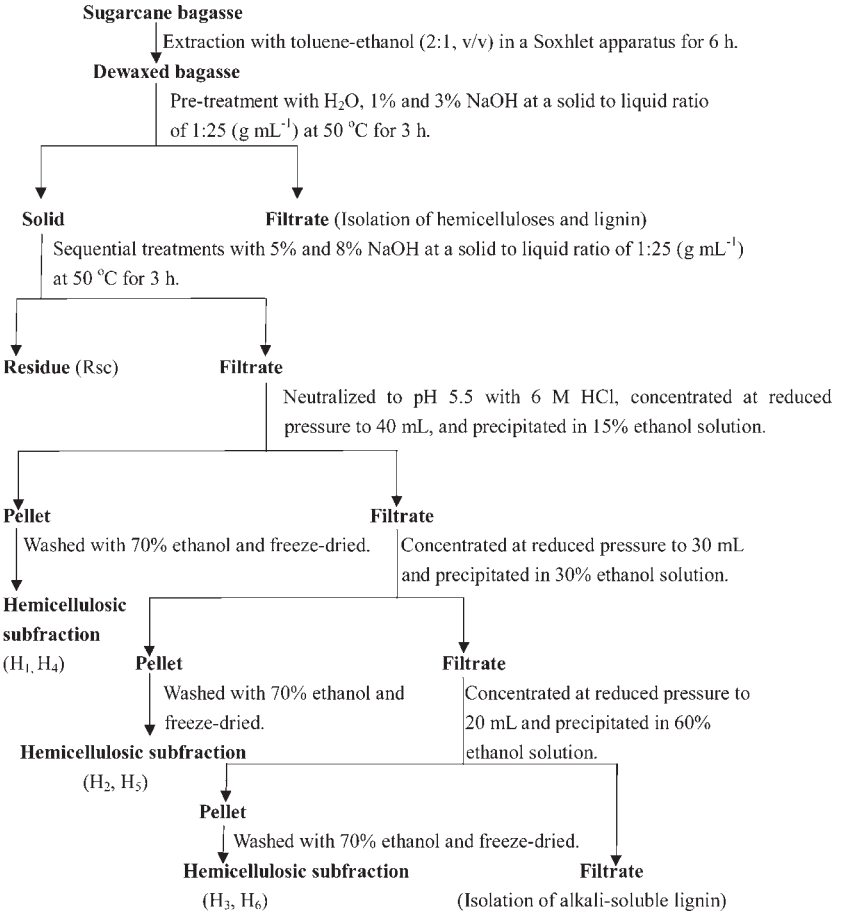

Figure 1. Scheme for subfractional isolation of hemicelluloses from sugar cane bagasse.

applications for plant hemicelluloses include their use as viscosity modifiers, gelling agents, tablet binders or wet strength additives (*8*).

Greatly increased interest in the isolation and purification of hemicelluloses from biomass has become a hot topic during recent years. Hemicelluloses are linked to cellulose and lignin via hydrogen and covalent bonds, respectively. Highly branched hemicelluloses are easily water-soluble. On the contrary, more uniform hemicelluloses (with a low degree of side-chain substitution) are tightly bound to cellulose and thus are less water-soluble (*9*). The isolation of hemicelluloses actually is a two-stage process, involving alkaline hydrolysis of ester groups, especially the ester groups present between the ferulic acid and arabinose residues of feruloylarbinoxylans, followed by their extraction into aqueous medium (*10*). It is known that high concentrations of hydroxyl ions liberated from alkali solutions cause swelling of cellulose, hydrolysis of ester linkages, and disruption of intermolecular hydrogen bonds between cellulose and hemicelluloses, thereby facilitating the extraction of hemicelluloses from the other cell wall components. Some of the methods developed for the effective extraction of hemicelluloses from cereals are extraction with (a) barium or calcium hydroxide at elevated temperatures (*11*), (b) sodium or potassium hydroxide (*12*), and (c) alkaline hydrogen peroxide (*10*). Furthermore, different fraction techniques, such as graded ethanol precipitation and anion-exchange chromatography, have been used to obtain deeper insight into the diversity of hemicelluloses (*13*−*15*). In this case, the hemicellulosic materials from the cereals or other plant cell walls are frequently fractioned to give polysaccharides having different compositions, structures, and physicochemical and functional properties. More importantly, studies of such fractionated materials have led to much structural information on molecules in those of the populations of hemicellulosic molecules recovered by the most commonly used procedures.

In a previous paper, we reported the physicochemical and thermal properties of hemicellulosic preparations, subfractionated by graded precipitation at the ethanol concentrations of 15%, 30% and 60% (v/v) from water-soluble and 1% and 3% NaOH-soluble hemicelluloses (*16*). In the present work, the insoluble residue after treatments with water, 1% and 3% NaOH, was successively extracted with 5% and 8% NaOH aqueous solution, and hemicellulosic preparations were subfractionated by precipitation with an increased levels of ethanol, and the structural features, physicochemical and thermal properties of the hemicellulosic subfractions are comparatively studied, and are compared with those hemicellulosic subfractions obtained from the water, 1% and 3% NaOH-soluble hemicelluloses of the same SCB.

MATERIALS AND METHODS

Materials. Sugar cane bagasse was obtained from a local sugar factory (Guangzhou, China). It was first dried in sunlight and then cut into small pieces (1−3 cm). The cut bagasse was ground to pass a 0.8 mm size screen and dried again in a cabinet oven with air circulation for 16 h at 60 °C. Fats, waxes and oils were removed from the cut bagasse in a Soxhlet apparatus for 6 h with 2:1 (v/v) toluene−ethanol. The composition (%, w/w) of the bagasse was cellulose 43.6%, hemicelluloses 33.5%, lignin 18.1%, ash 2.3%, and wax 0.8% on a dry weight basis (*17*), which was determined by the method for measuring the chemical composition of wheat straw described previously (*9*). All standard chemicals, such as sugars and phenolics, were analytical grade, purchased from Sigma Chemical Company (Beijing).

Isolation and Subfractionation of Hemicelluloses. In order to investigate structural differences in the hemicelluloses present in bagasse, hemicellulosic fractions were isolated by sequential extractions according to the scheme in **Figure 1**. The dewaxed sugar cane bagasse (10 g) was

successively extracted with distilled water, 1% NaOH and 3% NaOH aqueous solution at a solid to liquid ratio of 1:25 (g mL^{-1}) at 50 °C for 3 h under stirring. After filtration, the insoluble solid residue after treatments with water, 1% and 3% NaOH, was successively treated with 5% and 8% NaOH aqueous solution at a solid to liquid ratio of 1:25 (g mL^{-1}) at 50 °C for 3 h. After the treatment period, the insoluble residue (Rsc) was collected by filtration, washed with distilled water until the pH of the filtrate was neutral, and then dried at 60 °C. The pH of the filtrates was adjusted to 5.5 with 6 M HCl, and then concentrated to about 50 mL under reduced pressure. Sequentially 95% ethanol was then added to the concentrated filtrates slowly at room temperature under constant stirring until a required ethanol concentration of 15% (v/v) was reached. The precipitated hemicelluloses were recovered by filtration, washed with 70% ethanol and freeze-dried. And the filtrates were continuing to concentrate to about 30 mL at the reduced pressure, and the hemicelluloses were precipitated in the 30% ethanol by the same method mentioned above. Then the filtrates were concentrated to 20 mL, and the residual hemicelluloses were precipitated in the 60% ethanol solution. The six hemicellulosic subfractions were thoroughly washed with 70% ethanol, and then freeze-dried. All experiments were performed at least in duplicate. It should be noted that the three hemicellulosic subfractions precipitated with 15%, 30% and 60% ethanol from the hemicellulosic fraction solubilized in the 5% NaOH treatment were labeled as 5% NaOH-soluble hemicelluloses H_1, H_2 and H_3, respectively. Similarly, the precipitated hemicellulosic subfractions obtained from the hemicellulosic fraction solubilized in the 8% NaOH treatment were named as 8% NaOH-soluble hemicelluloses H_4, H_5 and H_6, respectively.

Chemical Characterization. The constituent neutral sugar in the isolated hemicellulosic subfractions was determined by high performance anion exchange chromatography (HPAEC). The neutral sugars in the hemicellulosic fractions were liberated by hydrolysis with 6% H_2SO_4 for 2.5 h at 105 °C. After hydrolysis, the sample was diluted 30-fold, filtered and injected into the HPAEC system (Dionex ISC 3000, U.S.) with amperometric detector, AS50 autosampler and a CarbopacTM PA1 column (4 × 250 mm, Dionex). Neutral sugars were separated in 18 mM NaOH (carbonate free and purged with nitrogen) with postcolumn addition of 0.3 M NaOH at a rate of 0.5 mL/min. Run time was 45 min, followed by a 10 min elution with 0.2 M NaOH to wash the column and then a 15 min elution with 0.018 M NaOH to re-equilibrate the column. The uronic acids were eluted with 0.4 M NaOH for 20 min at a rate of 1 mL/min with postcolumn addition of 0.3 M NaOH at a rate of 0.5 mL/min. Calibration was performed with standard solutions of L-arabinose, D-glucose, D-xylose, D-glucose, D-mannose, D-galactose, glucuronic and galacturonic acids. The molecular weights of the hemicellulosic subfractions were determined by gel permeation chromatography on a PL aquagel-OH 50 column (300 × 7.7 mm, Polymer Laboratories Ltd.), calibrated with PL pullulan polysaccharide standards (peak average molecular weights 783, 12 200, 100 000, 1 600 000, Polymer Laboratories Ltd.). A flow rate of 0.5 mL/min was maintained. The eluents were 0.02 N NaCl in 0.005 M sodium phosphate buffer, pH at 7.5. Detection was achieved with a Knauer differential refractometer. The column oven was kept at 30 °C. Polysaccharides were dissolved with 0.02 N NaCl in 0.005 M sodium phosphate buffer, pH 7.5 at a concentration of 0.1%. The chemical composition of phenolic acids and aldehydes liberated from alkaline nitrobenzene oxidation of the lignin associated in the hemicellulosic subfractions was determined by high-performance liquid chromatography (HPLC, Agilent, U.S.A.). The identification of the individual compounds were detected at 280 nm by computer comparison of the retention times and peak areas with the authentic phenolics. Klason lignin content in the hemicellulosic samples was determined according to Tappi method T 249 cm-85. The measurements were conducted with two parallels and the reproducibility of the values was kept within the range of 6%.

Spectroscopic and Thermal Characterization. FT-IR spectra of hemicellulosic samples were obtained on an FT-IR spectrophotometer (Nicolet 510) using a KBr disk containing 1% finely ground samples. Thirty-two scans were taken of each sample recorded from 4000 to 400 cm^{-1} at a resolution of 2 cm^{-1} in the transmission mode. The solution-state ^1H NMR spectrum was recorded on the spectrometer at 300 MHz using 15 mg of hemicelluloses in 1.0 mL of D_2O. A ^{13}C NMR spectrum was obtained on a Bruker MSL300 spectrometer at 74.5 MHz.

Table 1. Yield of Hemicelluloses Solubilized during the Successive Treatments of Dewaxed Bagasse with Distilled Water, 1%, 3%, 5% and 8% NaOH at 55 °C for 3 h

fraction	yield (% dry matter)
total solubilized hemicelluloses during the successive treatments with distilled water, 1, 3%, 5% and 8% NaOH	32.1
solubilized hemicelluloses in H_2O treatment	4.8
solubilized lignin in H_2O treatment	0.4
solubilized hemicelluloses in 1% NaOH treatment	10.9
solubilized lignin in 1% NaOH treatment	5.7
solubilized hemicelluloses in 3% NaOH treatment	9.4
solubilized lignin in 3% NaOH treatment	3.7
solubilized hemicelluloses in 5% NaOH treatment	4.2
solubilized lignin in 5% NaOH treatment	2.5
solubilized hemicelluloses in 8% NaOH treatment	2.8
solubilized lignin in 8% NaOH treatment	1.9
residue (crude cellulose)	53.7

The sample (80 mg) was dissolved in 1 mL of D_2O (99.8% D) with overnight stirring at room temperature. The spectrum was recorded at 25 °C after 30,000 scans. Chemical shifts (δ in ppm) are expressed relative to the resonance of Me_4Si ($\delta = 0$). A 60° pulse flipping angle, a 3.9 μs pulse width and a 0.85 s delay time between scans were used. Thermal behavior of the hemicelluloses was performed using thermogravimetric analysis (TGA) and differential thermal analysis (DTA) on a simultaneous thermal analyzer (DTG-60, Shimadizu, Japan). The apparatus was continually flushed with a nitrogen flow of 30 mL/min. The sample weighed between 9 and 11 mg and heated from room temperature to 550 °C at a rate of 10 °C/min.

RESULTS AND DISCUSSION

Yield of Hemicelluloses. Extraction of hemicelluloses under alkaline conditions actually involves alkaline hydrolysis of ester linkages to liberate them from the lignicellulosic matrix followed by extracting them into aqueous media (*10*). In this study, a sequential extraction of hemicelluloses with water, 1%, 3%, 5% and 8% NaOH aqueous solution was performed according to the scheme of **Figure 1**. The yields of the soluble hemicelluloses, lignin and the residues are shown in **Table 1**. In a previous paper (*16*), we showed that sequential extractions of the bagasse with water, 1% and 3% NaOH aqueous solution yielded 4.8%, 10.9% and 9.4% hemicelluloses, corresponding to release of 14.3%, 32.5% and 28.1% of the original hemicelluloses, respectively. Taken together, the three-stage treatments solubilized 25.1% hemicelluloses from bagasse, and accounted for 74.9% of the original hemicelluloses. As can be seen from **Table 1**, further treatments of the SCB residue with 5 and 8% NaOH aqueous solution released 4.2% and 2.8% hemicelluloses, corresponding to the release of 12.5%, and 8.4% of the original hemicelluloses, respectively. Meanwhile, the successive treatments also solubilized 2.5% and 1.9% lignin, corresponding to release of 7.5% and 5.7% of the original lignin, respectively. Obviously, a total yield of hemicellulosic fractions accounted for 95.8% of original hemicelluloses in the cell walls of SCB, indicating that the substantial amounts of hemicelluloses were sequentially extracted by water and sodium hydroxide with the increasing alkaline concentrations from 1% to 8% under the conditions used. These results also revealed that the sequential extractions of the bagasse were significantly effective. The highest extraction yield (10.9% w/w) was obtained with 1% NaOH, implying that the initial extraction with 1% NaOH liberated 32.5% of the total available hemicelluloses. Apparently, a minor part of hemicelluloses is loosely attached within the cell wall, while a major part of hemicelluloses are embedded firmly in the cell walls. It can be speculated that the difference in extractability of the hemicelluloses is a result of a different function of these polysaccharides in the cell walls.

Table 2. Yield of Precipitated Hemicellulosic Subfractions (Percent, 5% and 8% NaOH-Soluble Hemicelluloses) in 15%, 30% and 60% Ethanol Solution from 5% and 8% NaOH-Soluble Hemicelluloses

5% NaOH-soluble hemicelluloses			8% NaOH-soluble hemicelluloses		
H_1[a]	H_2[b]	H_3[c]	H_4[a]	H_5[b]	H_6[c]
28.6	16.7	42.9	32.1	17.9	39.3

[a] Represents the hemicellulosic subfractions obtained by precipitation in 15% ethanol. [b] Represents the hemicellulosic subfractions obtained by precipitation in 30% ethanol. [c] Represents the hemicellulosic subfractions obtained by precipitation in 60% ethanol.

Table 3. Contents of Neutral Sugars (Relative Percent Dry Hemicelluloses, w/w) and Uronic Acids (Percent Dry Matter, w/w) of Hemicellulosic Subfractions Precipitated in 15%, 30% and 60% Ethanol Solution and Residue

	hemicellulosic subfractions[a] and residue						
sugars (%)	H_1	H_2	H_3	H_4	H_5	H_6	Rsc[b]
rhamnose	nd[c]	nd	nd	nd	nd	nd	nd
arabinose	4.17	4.53	7.44	3.78	4.09	6.40	1.96
galactose	0.02	nd	1.41	0.15	nd	2.42	0.46
glucose	0.25	0.42	6.20	1.08	3.62	15.38	76.07
mannose	nd	nd	0.20	nd	nd	3.19	nd
xylose	95.56	95.05	84.75	94.99	92.29	72.61	21.51
uronic acid	2.30	1.53	1.05	1.78	1.23	0.98	nd
Ara/Xyl	0.043	0.048	0.088	0.040	0.044	0.088	0.091

[a] Corresponding to the hemicellulosic subfractions in **Table 2**. [b] Rsc, residue (crude cellulose). [c] Not detected.

In addition, the fractionation of the 5% and 8% NaOH-soluble hemicelluloses by graded ethanol precipitation at the concentrations of 15%, 30% and 60% (v/v) gave the six hemicellulosic subfractions, and their yields of precipitated hemicellulosic subfractions are shown in **Table 2**. As can be seen, the yield of the subfractions recovered by the graded ethanol precipitations was 28.6%, 16.7% and 42.9% of the 5% NaOH-soluble hemicellulosic fraction at the ethanol concentrations of 15%, 30% and 60% (v/v), respectively. Similarly, it was also found that 32.1%, 17.9% and 39.3% hemicellulosic subfractions of the 8% NaOH-soluble hemicellulosic fraction were obtained. Evidently, the major precipitation of the hemicellulosic subfractions was obtained at the ethanol concentration of 60%. At this concentration, 42.9% and 39.3% hemicellulosic subfractions of 5% and 8% NaOH-soluble hemicelluloses were precipitated, respectively. The second major fraction was obtained at the ethanol concentration of 15%. Similar results were also observed at water, 1 and 3% NaOH-soluble hemicelluloses (*16*). Clearly, the total yield of six hemicellulosic subfractions accounted for 88.8% of the total dissolved hemicelluloses during the successive treatments with 5% and 8% NaOH, indicating that 11.2% hemicelluloses, mainly degraded substances such as oligosaccharides, were not precipitated by 15%, 30% and 60% ethanol solution. Furthermore, the dark brown color of these fractions was regarded as an indication for the presence of lignin-like materials.

Sugar Composition. The composition of neutral sugars and the content of uronic acids of six hemicellulosic subfractions and crude cellulose are given in **Table 3**. Obviously, xylose was the predominant sugar component in the six hemicellulosic subfractions obtained from the 5% and 8% NaOH-soluble hemicelluloses, comprising 72.6−95.6% of the total sugars, with arabinose and glucose present in smaller amounts. Galactose and mannose were observed as minor sugar constituents. The content of uronic acids, mainly glucuronic acid or 4-*O*-methyl-D-glucuronic acid (MeGlcA), ranged between 1.0% and 2.3%. These data indicated that the six hemicellulosic subfractions of the alkaline-soluble hemicelluloses probably consist mainly of glucuronoarabinoxylans or L-arabino-(4-*O*-methyl-glucurono)-D-xylans.

It should be noted that in the hemicellulosic subfractions H_1, H_2 and H_3 obtained from 5% NaOH-soluble hemicelluloses, an increase in ethanol concentration from 15% to 60% resulted in a decrease of xylose from 95.6% (H_1) to 84.8% (H_3), and uronic acids from 2.3% (H_1) to 1.1% (H_3), but an increase of arabinose from 4.2% (H_1) to 7.4% (H_3), and glucose from 0.3% (H_1) to 6.2% (H_3). Similar results were observed in the three hemicellulosic subfractions from 8% NaOH-soluble hemicelluloses. In addition, the main differences were found in the ratio of arabinose to xylose (Ara/Xyl), in the relative proportions and sequence of various linkages between these two sugars, and in the presence of other substituents. From the ratio of arabinose to xylose (0.04−0.09) in the six subfractions (**Table 3**) obtained from 5% and 8% NaOH-soluble hemicelluloses, it can be concluded that 5% and 8% sodium hydroxide extractable sugar cane bagasse (glucurono)arabinoxylans consist of a very lowly substituted population. The lower degree of substitution indicated a higher proportion of unsubstituted xylose residues and a lower proportion of disubstituted xylose units (*18*). Furthermore, the Ara/Xyl ratio of the precipitated hemicellulosic subfractions also increased from 0.04 (H_1, H_4) to 0.09 (H_3, H_6) with an increasing ethanol concentration from 15% to 60%. Similar results were also found in the precipitated hemicellulosic subfractions obtained from the water, 1% and 3% NaOH-soluble hemicelluloses reported in our previous studies (*16*). This implied that the Ara/Xyl ratios were increased by increasing concentration of the ethanol, and the results were in agreement with the conclusions found by Gruppen and Delcour from the wheat arabinoxylans (*18*, *19*). Therefore, using the procedure of the graded ethanol precipitation, subfractions were obtained with varying Ara/Xyl ratios, ranging from 0.04 to 0.09, indicating that there was a variation in arabinoxylan structure between the different subfractions.

Analysis of the residual fraction showed that all the hemicelluloses in sugar cane bagasse were not completely soluble by the three sequential treatments. The residue still contained a noticeable amount of hemicelluloses as shown by xylose (21.5%), and a minor quantity of arabinose (2.0%). These further verified that the hemicelluloses are strongly bound to the cell wall component, cellulose (*16*).

Content of Bound Lignin. Alkaline nitrobenzene oxidation has been widely used for assaying and identifying the structure of the lignin (*20*). The presence of lignin−hemicellulose linkages was studied in detail for straw, grass, and wood samples (*20*, *21*). To verify the presence of lignin, nitrobenzene oxidation of six hemicellulosic subfractions and crude cellulose was performed. **Table 4** gives the contents of associated lignin and its phenolic composition obtained by nitrobenzene oxidation of bound lignin. As expected, a substantial cleavage of α-ether linkages between lignin and hemicelluloses occurred during the 5% and 8% NaOH treatments as shown by relatively low amounts of bound lignin (1.4−3.0%) in the precipitated hemicellulosic polymers. Interestingly, compared to the lignin content (2.2−7.8%) (data not shown) of the water, 1% and 3% NaOH-soluble hemicelluloses in the previous study (*16*), the six hemicellulosic subfractions obtained from the 5% and 8% NaOH-soluble hemicelluloses had much lower content of bound lignin, indicating that the relative higher concentrations of alkaline solution can significantly break the ether bonds between lignin and polysaccharides from the sugar cane bagasse. This also suggested that the treatment with the mild alkaline solution was an efficient method for both delignification and dissolution of hemicelluloses. Additionally, with the increase in the concentration of ethanol from 15% to 60%, the hemicellulosic subfractions with the lower lignin content

Table 4. Yield (Percent Hemicellulosic Sample, w/w) of Phenolic Acids and Aldehydes from Alkaline Nitrobenzene Oxidation of Associated Lignins in the Hemicellulosic Subfractions and Residue

phenolic acids and aldehydes	hemicellulosic subfractions[a] and residue						
	H_1	H_2	H_3	H_4	H_5	H_6	Rsc[b]
p-hydroxybenzoic acid	0.049	0.042	0.035	0.038	0.044	0.032	0.051
p-hydroxybenzaldehyde	0.055	0.052	0.040	0.037	0.026	0.023	0.095
vanillic acid	0.030	0.028	0.036	0.041	0.038	0.029	0.047
syringic acid	0.033	0.009	0.008	0.027	0.003	0.002	0.029
vanillin	0.53	0.44	0.29	0.39	0.34	0.24	0.42
syringaldehyde	0.56	0.49	0.33	0.43	0.35	0.27	0.54
p-coumaric acid	0.057	0.043	0.041	0.045	0.037	0.029	0.051
acetovanillone	0.013	0.005	0.004	0.016	0.003	0.003	0.023
acetosyringone	0.053	0.065	0.015	0.054	0.069	0.049	0.066
ferulic acid	0.046	0.035	0.028	0.038	0.045	0.022	0.034
total	1.43	1.21	0.82	1.12	0.96	0.70	1.36
content of klason lignin	2.95	2.36	1.54	2.17	1.85	1.35	2.58

[a] Corresponding to the hemicellulosic subfractions in **Table 2**. [b] Rsc, residue (crude cellulose).

Table 5. Weight-Average (M_w) and Number-Average (M_n) Molecular Weights and Polydispersities (M_w/M_n) of the Hemicellulosic Subfractions

	hemicellulosic subfractions[a]					
	H_1	H_2	H_3	H_4	H_5	H_6
M_w	42 430	74 430	85 510	46 130	60 760	64 070
M_n	27 650	37 390	32 970	31 100	28 930	34 610
M_w/M_n	1.53	1.99	2.59	1.48	2.10	1.85

[a] Corresponding to the hemicellulosic subfractions in **Table 2**.

were obtained, and this result is consistent with the previous research results (*16*). Analysis of the residue showed that the crude cellulose (Rsc) contained a small amount of bound lignins (2.6%), resulting from the residual lignin linked to the residual hemicelluloses in the cellulose preparations.

It is important to note that the major products obtained from the alkaline nitrobenzene oxidation were identified to be vanillin and syringaldehyde, which ranged from 34.3% to 37.1% and 36.4% to 40.5% of total phenolic monomers, respectively. This suggested that the bound lignin in the hemicellulosic subfractions contained roughly equal amount of noncondensed guaiacyl (G) and syringyl (S) units, which was in good agreement with the results obtained from the pure lignin polymers solubilized during the alkali treatment of sugar cane bagasse (*22*). A noticeable amount of *p*-hydroxybenzaldehyde, *p*-hydroxybenzoic acid, vanillic acid, *p*-coumaric acid, acetosyringone, ferulic acid and traces of syringic acid and acetovanillone were also found to be present in the nitrobenzene oxidation mixtures from the associated lignin in the hemicellulosic subfractions.

Molecular Weight. In this study, the molecular weights of the hemicellulosic polymers were determined by gel permeation chromatography (GPC). The values of the weight-average (M_w) and number-average (M_n) molecular weights and polydispersity (M_w/M_n) of the six hemicellulosic polymers are given in **Table 5**. It is clear that the first three hemicellulosic subfractions H_1, H_2 and H_3 obtained from the 5% NaOH-soluble hemicelluloses showed a relatively higher degree of polymerization with M_w between 42 430 and 85 510 g mol^{-1} than those of the last three hemicellulosic subfractions H_4, H_5 and H_6 obtained from the 8% NaOH-soluble hemicelluloses (M_w = 46130−64070 g mol^{-1}). This implied that the treatment with 8% NaOH aqueous solution probably resulted in a slight degradation of macromolecular structure of hemicelluloses. In addition, the molecular weight distributions of six hemicellulosic subfractions are also shown in **Figure 2**. The fractions (H_3 and H_6) precipitated at highest ethanol concentration (60%) had a slight higher proportion of high molecular weight components compared with the subfractions precipitated at lower ethanol concentrations, which is in accordance with the result in **Table 5**. In other words, with increasing ethanol concentrations, the average molecular weights of the hemicellulosic subfractions increased. This was consistent with the observation the M_w of water-unextractable arabinoxylans from wheat flour increased when the ethanol concentration increased (*19*). From this observation and the noticed increase in the Ara/Xyl ratio with increasing ethanol concentration, it could be also concluded that hemicellulosic polymers with a low molecular weight and a low Ara/Xyl ratio preferentially precipitated in a lower ethanol concentration and that, with increasing ethanol concentration, the molecular weight and the Ara/Xyl ratio increased. Similar results were found in the precipitated hemicellulosic subfractions obtained from the water, 1% and 3% NaOH-soluble hemicelluloses in our previous studies (*16*). Furthermore, the polydispersity of the subfractions are shown in the molecular weight distributions (**Figure 3** and **Table 5**). All of the subfractions had a relatively low index of polydispersity (1.5−2.6), which indicated that the six hemicellulosic subfractions obtained by the means of graded ethanol precipitation had a chemical and structural homogeneity.

FT-IR Spectra. Infrared spectroscopy is one of the most often used spectroscopic tools for the study of polymers. The method is rapid and sensitive, with a great variety of sampling techniques, and yet the instrumentation can still be considered inexpensive (*23*). In this study, FT-IR spectroscopy was used to evaluate the structural differences among the hemicellulosic subfractions. The spectra of the hemicellulosic subfractions H_1 (spectrum a), H_2 (spectrum b) and H_3 (spectrum c) by precipitation in 15%, 30% and 60% ethanol solutions from 5% NaOH-soluble hemicelluloses are shown in **Figure 3**. The spectral profiles and relative intensities of the bands were similar, indicating similar structure of the hemicellulosic subfractions obtained in different concentrations of the aqueous ethanol solution. The absorption at 3423 cm^{-1} relates to the stretching of H-bonded OH groups, and two bands at 2921 and 2979 cm^{-1} to the C−H stretching. The band at 1639 cm^{-1} is attributed to the bending mode of absorbed water (*24*). The prominent band at 1044 cm^{-1} is assigned to the C−O, C−C stretching or C−OH bending in xylans, indicating a dominant xylan of the fractionated hemicelluloses (*25*). The band 1162 cm^{-1} is assigned to C−O and C−O−C stretching with some contribution of OH bending mode; this band reflects changes in conformation as well as in the hydration properties of superstructure. The low intensity of the bands at 1162 and 988 cm^{-1} indicates the presence of arabinosyl units, which have been reported to be attached the xylopyranosyl constituents (*25*). The band at 1091 cm^{-1} relates to the C−OH bending, which is strongly influenced by the degree of branching. The sharp band 892 cm^{-1}, which corresponds to the C_1 group frequency, is characteristic of β-glycosidic linkages between the sugar units (*26*). The bands at 1465, 1423, 1383, and 1245 cm^{-1} represent C−H, OH or CH_2 bending vibrations. In addition, the occurrence of a very small band at 1510 cm^{-1} in all the spectra is presumed due to the presence of small amount of associated lignin in the hemicelluloses, while its rather weak presence in spectrum c, indicating only a minor amount of bound lignin in this hemicellulosic subfraction, precipitated with 60% ethanol solution, which corresponded to the results obtained by alkaline nitrobenzene oxidation. A similar phenomenon was also observed in the other hemicellulosic subfractions from the 1% and 3% NaOH-soluble hemicellulosic in the previous study (*16*).

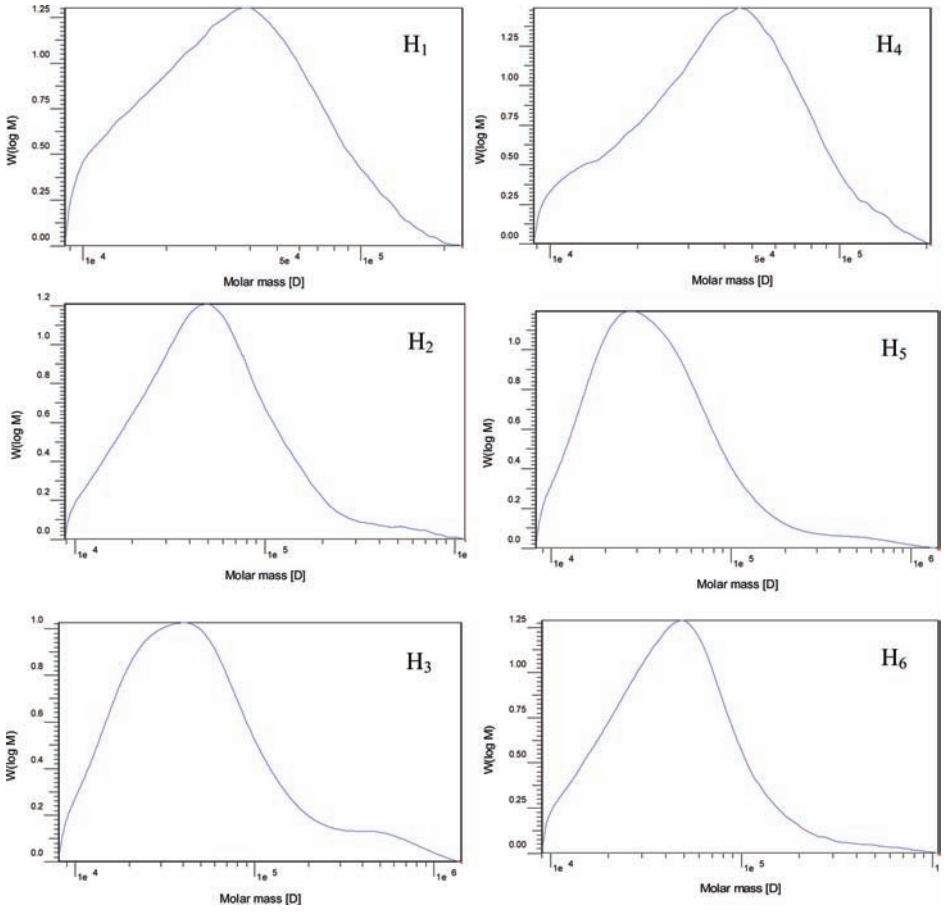

Figure 2. Molecular weight distributions of six hemicellulosic subfractions.

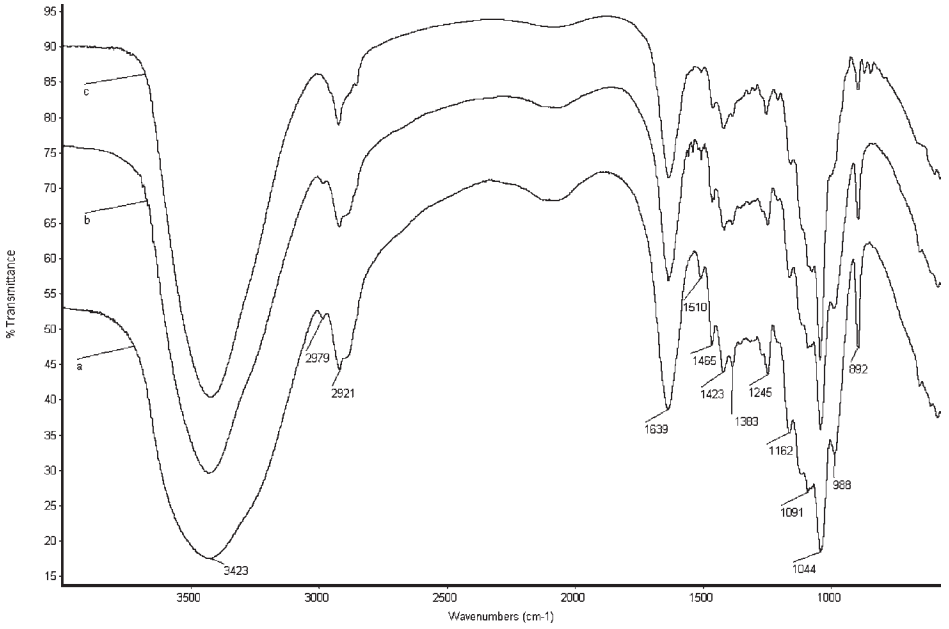

Figure 3. FT-IR spectra of the hemicellulosic subfractions H_1 (spectrum a), H_2 (spectrum b) and H_3 (spectrum c).

The FT-IR spectra of the hemicellulosic subfractions H_4 (spectrum a), H_5 (spectrum b), H_6 (spectrum c) are illustrated in **Figure 4**. As expected, the three spectral profiles and relative intensities of most of the bands were rather similar, indicating a similar structure of the three hemicellulosic subfractions. The most obvious feature is the similarity of these spectra. The absorbances at 1466, 1419, 1382, 1162, 1075, 1043, 985, and 894 cm^{-1} of the three spectra are associated with the typical values of hemicelluloses. All the spectra have an intense absorbed water-related absorbance at 1635 cm^{-1}. Obviously, a weak absorption at 1509 cm^{-1} in the three spectra in **Figure 4** is characterized by aromatic skeleton vibrations belonging to lignin. This phenomenon indicated that the hemicellulosic fractions, extracted with 8% aqueous NaOH solution, contained noticeable amounts of lignin.

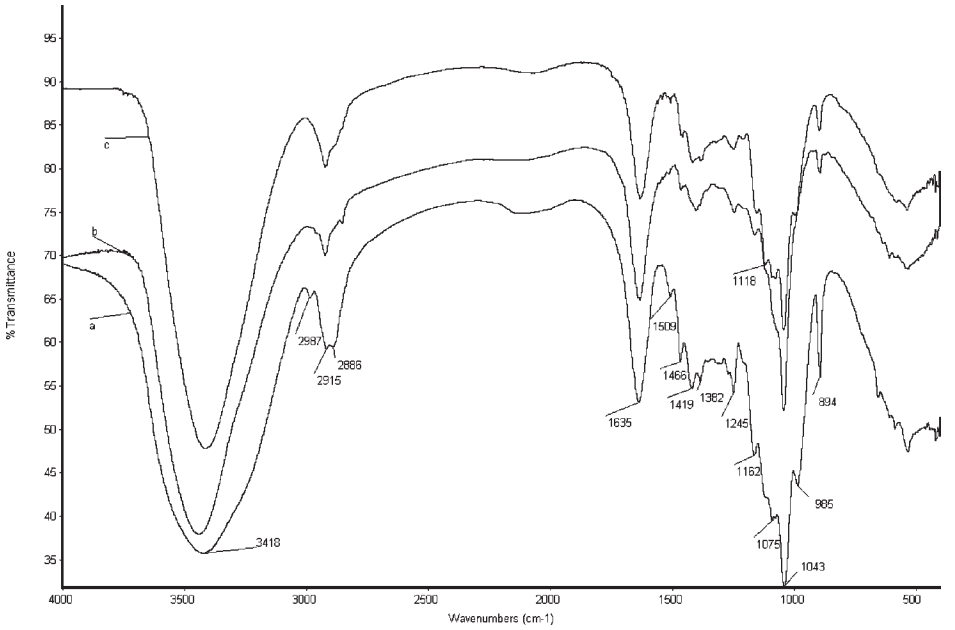

Figure 4. FT-IR spectra of the hemicellulosic subfractions H_4 (spectrum a), H_5 (spectrum b) and H_6 (spectrum c).

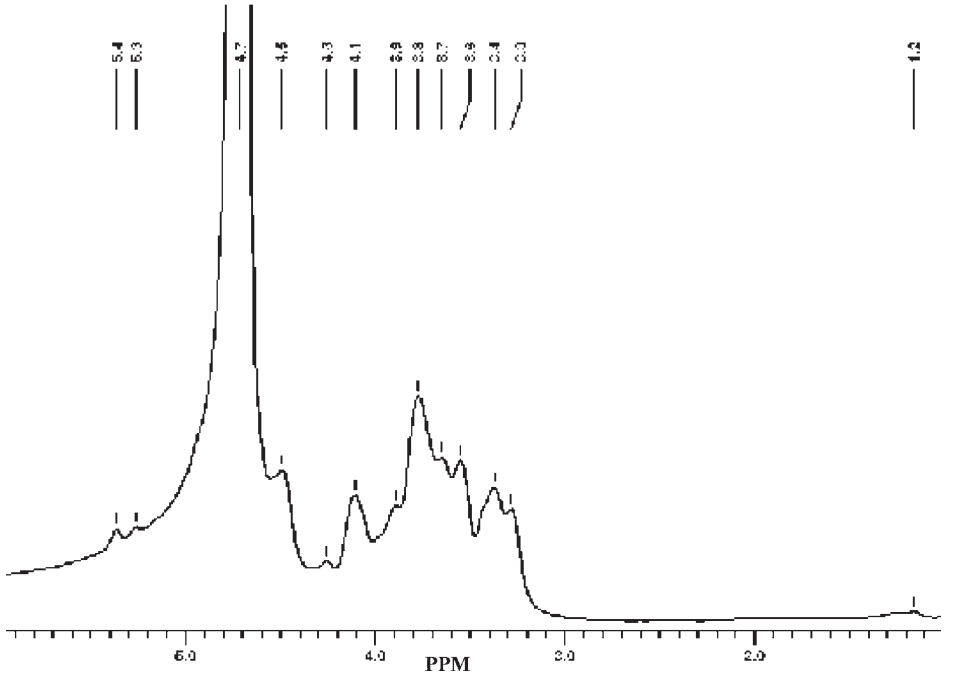

Figure 5. ^{1}H NMR spectrum of hemicellulosic subfraction H_6.

^{1}H and ^{13}C NMR Spectra. Nuclear magnetic resonance (NMR) spectroscopy was used to obtain structural information on high molecular weight materials and their building blocks. This nondestructive method provides direct information on the chemical structure of polysaccharide (*27*). ^{1}H NMR analysis of hemicellulosic subfraction H_6 (**Figure 5**) revealed signals for anomeric protons of terminal α-L-arabinfuranosyl residues at 5.3 and 5.4 ppm and of β-D-xylopyranosyl residues at 4.5 ppm (*28*). The signal at 5.4 ppm was assigned to the terminal arabinofuranosyl residues linked to O-3 of the branched xylopyranosyl residues, and the signal at 5.3 ppm represents the anomeric protons of α-L-arabinfuranosyl linked to O-2 and O-3 of the xylopyranosyl residues (*28*, *29*). As can be seen, the strong signal at 4.7 ppm is indicative of the residual solvent (HDO). The signals at 3.2−4.3 ppm are originated from the equatorial proton and other protons of the anhydroxylose residues (*30*). The methyl protons of small amounts of 4-*O*-methyl-D-glucuronic acid give weak peaks at 1.2 ppm.

To confirm the structural features, the hemicellulosic subfraction H_6 was also characterized by ^{13}C NMR, as shown in **Figure 6**. The resonances around 63.3, 73.3, 74.8, 76.0, and 102.3 ppm were assigned to C-5, C-3, C-2, C-4 and C-1 positions of the main 1,4-linked β-D-xylose units. The signals at 86.5, 79.8, 79.0, and 60.8 ppm (data not shown) are originated from C-4, C-2, C-3 and C-5 of α-L-arabinfuranosyl residues linked to β-D-xylans, respectively. These signals of arabinose groups are typical of arabinoxylan isolated from the sugar cane bagasse (*1*). Therefore, the alkali-soluble bagasse hemicelluloses had a classical structure, with a backbone of β-(1→4)-linked xylosyl residue substituted with arabinose at C-2 and/or C-3 of the main chain.

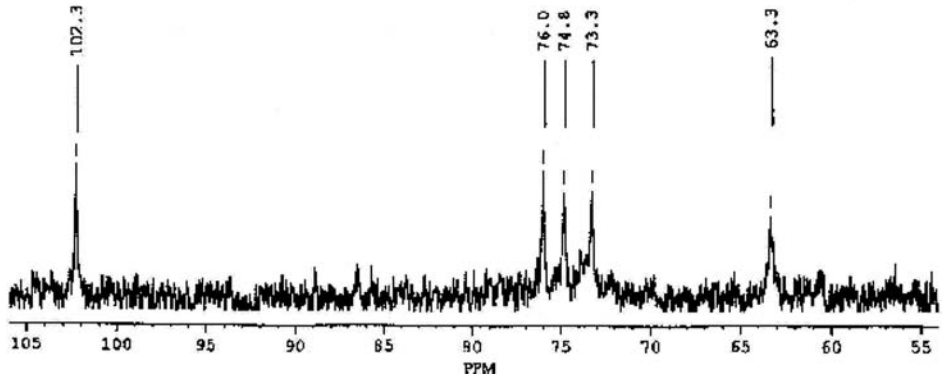

Figure 6. ^{13}C NMR spectrum of hemicellulosic subfraction H_6.

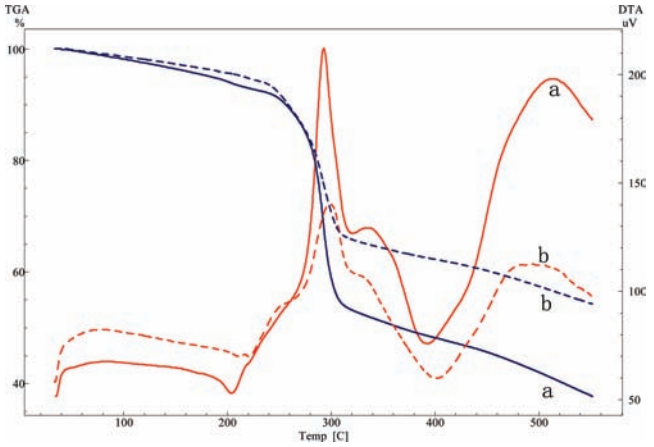

Figure 7. Thermograms of hemicellulosic subfractions H_1 (curve a) and H_2 (curve b).

Thermal Analysis. Thermal analysis of polymers is an important study covering a broad field of applications and a method used for understanding the structure−property relation and mastering the technology for industrial production of different polymeric materials (*31*, *32*). Thermal degradability is affected by the chemical composition of material. **Figure 7** shows typical TGA/DTA curves of hemicellulosic subfractions H_1 (curve a) and H_2 (curve b) precipitated at 15% and 30% ethanol solution from 5% NaOH-soluble hemicelluloses. In thermogravimetry, the losses of weight are due to evolution of water, carbon monoxide, carbon dioxide, and evaporation of other pyrolysis products, and collectively measured as a percentage of original weight. As illustrated in **Figure 7**, the TGA curves of two hemicellulosic subfractions, H_1 and H_2, start to decompose at 235 and 243 °C, respectively. At 10% weight loss, the decomposing temperature of the two hemicellulosic subfractions occurred at 256 and 258 °C, respectively. These data indicated that the thermal stability of the hemicellulosic subfractions H_1 and H_2, precipitated with 15% and 30% ethanol solution, was very similar.

Figure 7 depicts DTA curves for hemicellulosic samples H_1 and H_2 and exhibits a single small endothermic and two exothermic reactions. The exothermic peaks, which represent heat released from the hemicellulosic subfractions, were observed at 293 and 514 °C for H_1 precipitated from the 15% ethanol solution, and at 300 and 495 °C for H_2 precipitated from the 30% ethanol solution. Thus, DTA showed two degradation peaks: the first is at lower temperatures, related to initial hemicellulose loss, and the second related the organic material oxidation (*33*). It should be noted that the overlapping first (and second) exothermic effects were not resolved, but were regarded as one effect, due to the small temperature range covered by such reactions.

In summary, this study has shown that substantial amounts of hemicelluloses were extracted sequentially by water and sodium hydroxide with increasing alkaline concentrations from 1% to 8% under a solid to liquid ratio of 1:25 (g mL^{-1}) at 50 °C for 3 h. For the hemicellulosic subfractions, noticeable differences in the chemical composition and molecular weights were observed. It was found that the subfractions with higher Ara/Xyl ratios were precipitated at higher ethanol concentrations. Another factor that could affect hemicellulosic properties by precipitation in ethanol is the molecular size of the polysaccharides. The hemicelluloses with higher Ara/Xyl ratios had higher molecular weights. FT-IR, sugar composition and ^1H and ^{13}C NMR analysis showed that the alkali-soluble bagasse hemicelluloses had a classical structure, with a backbone of β-(1→4)-linked xylosyl residue substituted with arabinose at C-2 and/or C-3 of the main chain, whereas a difference may occur in the distribution of branches along the xylan backbone. The thermal stability of the hemicelluloses was found to increase slightly with increasing molecular weight.

LITERATURE CITED

(1) Sun, J. X.; Sun, X. F.; Sun, R. C.; Su, Y. Q. Fractional extraction and structural characterization of sugarcane bagasse hemicelluloses. *Carbohydr. Polym.* **2004**, *56*, 195–204.

(2) Xu, F.; Sun, R. C.; Sun, J. X.; Liu, C. F.; He, B. H.; Fan, J. S. Determination of cell wall ferulic and *p*-coumaric acids in sugarcane bagasse. *Anal. Chim. Acta* **2005**, *552*, 207–217.

(3) Geng, Z. C.; Sun, J. X.; Liang, S. F.; Zhang, Y. Y.; Xu, F.; Sun, R. C. Characterization of water- and alkali-soluble hemicellulosic polymers from sugarcane bagasse. *Int. J. Polym. Anal. Charact.* **2006**, *11*, 209–226.

(4) Ebringerova, A.; Hromadkova, Z.; Heinze, T. Hemicellulose. *Adv. Polym. Sci.* **2005**, *186*, 1–67.

(5) Sun, R. C.; Sun, X. F. Fractional and structural characterization of hemicelluloses isolated by alkali and alkaline peroxide from barley straw. *Carbohydr. Polym.* **2002**, *49*, 415–423.

(6) Silva, C. J. S. M.; Roberto, I. C. Improvement of xylitol production by Candida guilliermondii FTI 20037 previously adapted to rice straw hemicellulosic hydroglysate. *Lett. Appl. Microbiol.* **2001**, *32*, 248–252.

(7) Lindblad, M. S.; Ranucci, E.; Albertsson, A. C. Biodegradable polymers from renewable sources. New hemicellulose-based hydrogels. *Macromol. Rapid Commun.* **2001**, *22*, 962–967.

(8) Watson, S. A. Corn hull gum, in industrial gums. In *Polysaccharides and Their Derivatives*; Whistler, R. L., BeMiller, J. N., Eds.; Academic Press: New York, 1959; pp 299−306.

(9) Lawther, J. M.; Sun, R. C.; Banks, W. B. Extraction, fractionation and characterization of structural polysaccharides from wheat straw. *J. Agric. Food Chem.* **1995**, *43*, 667–675.

(10) Donar, L. W.; Hicks, K. Isolation of hemicelluloses from corn fiber by alkaline hydrogen peroxide extraction. *Cereal Chem*. **1997**, *74*, 176–181.
(11) Bergmans, M. E. F.; Beldman, G.; Gruppen, H.; Voragen, A. G. J. Optimization of the selective extraction of (glucurono)-arabinoxylans from wheat bran: use of barium and calcium hydroxide solution at elevated temperatures. *J. Cereal Sci*. **1996**, *23*, 235–245.
(12) Dupont, M. S.; Selvendran, R. R. Hemicellulose polymers from the cell walls of beeswing wheat bran: part 1, polymers solubilised by alkali at 2. *Carbohydr. Res*. **1987**, *163*, 99–113.
(13) Brillouet, J. M.; Joseleau, J. P.; Utille, J. P.; Lelièvre, D. Isolation, purification, and characterization of a complex heteroxylan from industrial wheat bran. *J. Agric. Food Chem*. **1982**, *30*, 488–494.
(14) Brillouet, J. M.; Joseleau, J. P. Investigation of the structure of a heteroxylan from the outer pericarp (beeswing bran) of wheat kernel. *Carbohydr. Res*. **1987**, *159*, 109–126.
(15) D′Appolonia, B. L.; MacArthur, L. A. Comparison of starch, pentosans and sugars of some conventional height and semidwarf hard red spring wheat flours. *Cereal Chem*. **1975**, *53*, 230–239.
(16) Peng, F.; Ren, J. L.; Xu, F.; Bian, J.; Peng, P.; Sun, R. C. Comparative study of hemicelluloses obtained by graded ethanol precipitation from sugarcane bagasse. *J. Agric. Food Chem*. **2009**, *57*, 6305–6317.
(17) Sun, J. X.; Sun, X. F.; Zhao, H.; Sun, R. C. Isolation and characterization of cellulose from sugarcane bagasse. *Polym. Degrad. Stab*. **2004**, *84*, 331–339.
(18) Delcour, J. A.; Van Win, H.; Grobet, P. J. Distribution and structural variation of arabinoxylans in common wheat mill streams. *J. Agric. Food Chem*. **1999**, *47*, 271–275.
(19) Gruppen, H.; Hamer, R. J.; Voragen, A. G. J. Water-unextractable cell wall material from wheat 2. Fraction of alkali-extracted polymers and comparison with water-exactable arabinoxylans. *J. Cereal Sci*. **1992**, *13*, 53–67.
(20) Sun, X. F.; Sun, R. C.; Fowler, P.; Baird, M. S. Extraction and characterization of original lignin and hemicelluloses from wheat straw. *J. Agric. Food Chem*. **2005**, *53*, 860–870.
(21) Eriksson, O.; Lindgren, B. O. About linkage between lignin and hemicelluloses in wood. *Sven., Papperstidn*. **1977**, *80*, 59–63.
(22) Sun, J. X.; Sun, X. F.; Sun, R. C.; Fowler, P.; Baird, M. S. Inhomogeneities in the chemical structure of sugarcane bagasse lignin. *J. Agric. Food Chem*. **2003**, *51*, 6719–6725.
(23) Kacuráková, M.; Wilson, R. H. Developments in mid-infrared FT-IR spectroscopy of selected carbohydrates. *Carbohydr. Polym*. **2001**, *44*, 291–303.
(24) Kacurakova, M.; Belton, P. S.; Wilson, R. H.; Hirsch, J.; Ebringerova, A. Hydration properties of xylan-type structures: an FTIR study of xylooligosaccharides. *J. Sci. Food Agric*. **1998**, *77*, 38–44.
(25) Kacurakova, M.; Ebringerova, A.; Hirsch, J.; Horomadkova, Z. Infrared study of arabinoxylans. *J. Sci. Food Agric*. **1994**, *66*, 423–427.
(26) Gupta, S.; Madan, R. N.; Bansal, M. C. Chemical-composition of pinus-caribaea hemicellulose. *Tappi J*. **1987**, *70*, 113–114.
(27) Bock, K.; Duus, J. O.; Norman, B.; Pedersen, S. Assignment of structures to oligosaccharides produced by enzymic degradation of a β-D-glucan from barley by ^{1}H- and ^{13}C-n.m.r spectroscopy. *Carbohydr. Res*. **1991**, *211*, 219–233.
(28) Bengtsson, S.; Aman, P. Isolation and chemical characterization of water-soluble arabinoxylans in rye grain. *Carbohydr. Polym*. **1990**, *12*, 267–277.
(29) Hoffmann, R. A.; Geijtenbeek, T.; Kamerling, J. P.; Vliegenthart, J. F. G. ^{1}H-n.m.r. study of enzymatically generated wheat-endosperm arabinoxylan oligosaccharides: structures of hepta- to tetradecasaccharides containing two or three branched xylose residues. *Carbohydr. Res*. **1992**, *223*, 19–44.
(30) Kawagishi, H.; Kanao, T.; Inagaki, R.; Mizuno, T.; Shimura, K.; Ito, H.; Hagiwara, T.; Nakamura, T. Formolysis of a potent antitumor (1−6)-beta-d-glucan protein complex from agaricus-blazei fruiting bodies and antitumor-activity of the resulting products. *Carbohydr. Polym*. **1990**, *12*, 393–403.
(31) Joseph, P. V.; Joseph, K.; Thomas, S.; Pillai, C. K. S.; Prasad, V. S.; Groeninckx, G.; Sarkissova, M. The thermal and crystallisation studies of short sisal fibre reinforced polypropylene composites. *Composites, Part A* **2003**, *34*, 253–266.
(32) Zafeiropoulos, N. E.; Baillie, C. A.; Matthews, F. L. A study of transcrystallinity and its effect on the interface in flax fibre reinforced composite materials. *Composites, Part A* **2001**, *32*, 525–543.
(33) Choi, M. J.; Soottitantawat, A.; Nuchuchua, O.; Min, S. G.; Ruktanonchai, U. Physical and light oxidative properties of eugenol encapsulated by molecular inclusion and emulsion-diffusion method. *Food Res. Int*. **2009**, *42*, 148–156.

Received for review September 21, 2009. Revised manuscript received November 18, 2009. Accepted November 18, 2009. This work was supported by the grants from the Natural Science Foundation of China (No. 30930073, 30871997 and 30710103906), China Ministry of Education (No. 111, 2007B55 and NCET-07-0082), Ministry of Science and Technology (973 project, 2010CB732204), and State Forestry Administration (200804015).