Physicochemical and Structural Characterization of Hemicelluloses Isolated by Different Alcohols from Rice Straw

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Six alcohol-soluble hemicellulosic preparations from rice straw were comparatively studied, and their physicochemical characterizations were examined. The treatments of the dewaxed rice straw with 60% methanol, 60% ethanol, 60% n-propanol, 60% n-butanol, 60% ethanol containing 0.01 M HCl, and 60% ethanol containing 0.25 M NaOH at 75 ºC for 3 h were able to solubilize 10.4, 10.4, 6.2, 6.5, 7.4, and 55.0% of the original hemicelluloses, respectively. The results showed that methanol and ethanol had similar solubilization capacity of hemicelluloses and gave slightly higher solubility compared to n-propanol and n-butanol. The major monosaccharide of the four neutral alcohol-soluble hemicellulosic fractions was xylose (31.78-36.80%) followed by glucose (26.35-39.39%), galactose (15.05-17.17%), and arabinose (14.93-15.58%), whereas the alkaline ethanol-soluble hemicellulosic fraction contained the highest amount of xylose (59.62%). By combining 1H, 13C, and 2D-HSQC NMR with FT-IR spectroscopy, the alkaline ethanol-soluble hemicellulosic fraction can be structurally defined as 4-*O*-methyl-α-D-glucurono-L-arabino-D-xylans.

Keywords: Rice straw; Physicochemical characterization; Alcohol-soluble hemicelluloses; NMR spectroscopy

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INTRODUCTION

Polymers from renewable resources are receiving increased attention in the scientific community because of the projected shortage of petroleum-based raw materials in the near future. Tremendous effort is being focused on the development of new materials that can replace synthetic polymers by bio-based polymers as viable alternatives. Polysaccharides have been used in a wide range of applications such as food, packaging, agricultural chemicals, and biomedical devices, for which non-toxicity, biocompatibility, and biofunctionality are required. Hemicelluloses are the second most abundant natural polymer in the vegetal world after cellulose, representing about 20 to 35% of lignocellulosic biomass (McMillian 1994). Hemicelluloses are branched polymers with a degree of polymerization in the range of 80 to 200. The most common sugars found in hemicelluloses are D-glucose, D-mannose, D-xylose, L-arabinose, and D-galactose (Gabrielii and Gatenholm 1998). Despite their abundance in nature, they have been under-utilized commercially. In the last decade, there has been renewed interest in exploring potential applications for this available resource, but the main difficulty is

extracting hemicelluloses without deteriorating the polymer chain. Therefore, advances have been done in this area, offering new perspectives of utilization (Goksu *et al.* 2007). For example, researchers have been working on the conversion of hemicellulose into value-added products such as xylose, xylitol, ethanol, xylooligosaccharides, and xylan-based biodegradable films (Akpinar *et al*. 2007; Jeoh and Agblevor 2001).

Rice straw is one of the most abundant lignocellulosic waste materials in the world. In terms of total production, rice is the third most important grain crop in the world behind wheat and corn. As per FAO statistics, the world annual rice production in 2012 was about 729 million tons. Every kilogram of grain harvested is accompanied by the production of 1 to 1.5 kg of straw (Ravoof *et al.* 2012). These values make it possible to estimate that about 650 to 975 million tons of rice straw are produced per year globally. The options for the disposition of rice straw are limited by the low bulk density, slow degradation in the soil, harboring of rice stem diseases, and high mineral content. Rice straw has traditionally been used as animal feed for cattle and horse breeding, feedstock for paper industry, or organic fertilizer by burning it on the open field or burying it into the soil (Caviglioli *et al.* 2002; Vlasenko *et al.* 1997). However, recent points of animal breeding practices and a growing attention to environmental problems have tended to limit these uses. Especially, as climate change is extensively recognized as a threat to development, there is growing interest in alternative uses of agro-industrial residues for energy applications.

Efficient separation of biomass components is one of the major obstacles to the efficient utilization of renewable resources. In order to achieve a complete and portable utilization of lignocellulosic biomass, many efforts have been made all over the world (Johansson *et al.* 1987). Among the various pretreatment methods currently studied for the production of pulp and/or ethanol, the organosolv process is very promising (Hage *et al.* 2009). Organosolv pulping processes have been presented as proven alternatives to the kraft process due to their economy, environmental amity, and ability to produce substantial quantities of useful by-products (lignin, sugars, furfural, and acetic acid) in addition to pulp (Dahlmann and Schroeter 1990; Ni *et al.* 1997; Botello *et al.* 1999; Vila *et al.* 2003; Pan *et al.* 2006;). The most widely used solvents for organosolv process have been reported to be primary alcohols with low boiling point such as ethanol and methanol, although other solvents, namely acetic and formic acids have also been used (Aziz and Sarkanen 1989; Oliet *et al.* 2000, 2002). Alcohols have been used to split lignocelluloses into their components for many years (Foulon *et al*. 1934). Methanol improves penetration of the pulping liquor into the lignocellulosic material and therefore increases the delignification rate. It also prevents lignin from condensing during the process, which results in low pulp kappa numbers (Muurinen 2000). As with most of the organosolv pulping processes, ethanol is one of the most promising organic pulping chemicals. Aqueous ethanol penetrates easily into the structure of wood, resulting in uniform delignification. Alkaline ethanol pulping (Marton and Granzow 1982) seemed to be the most economical of the processes studied (Muurinen 2000). Kleinert (1974) has studied the mechanism of ethanol pulping and confirmed that it is a clean delignification method. Pisarnitskii *et al.* (2006) analyzed the hemicelluloses in oak wood ethanol extracts by aqueous-alcoholic media (40 to 90%), and found that the hemicelluloses with different compositions were extracted from wood at the different concentrations of ethanol. Recently, this pretreatment technology has been successfully developed for hybrid poplar and lodgepole pine killed by mountain pine beetle, producing substrates with very good enzymatic digestibility (Pan *et al.* 2007; 2008).

Methanol and ethanol are the most popular alcohols used in pulping because of their low boiling characteristics, and therefore they can be relatively easily recovered by distillation. However, several other alcohols, such as propanol, butanol, and glycols have also been proposed to be used as pulping chemicals (Muurinen 2000). In a word, alcohol organosolv pulping represents an alternative new chemical pulping process for the future. It promises higher pulp yields than the kraft process, good bleachability, small economies of scale, lower processing costs, simplicity of cooking chemical recovery, and the possibility of substantial co-product (fermentable sugars, lignin, and extractives) recoveries, as well as simplicity of pulping and recovery hardware in production of a wide range of chemical papermaking pulps (Yawalata and Paszner 2004).

In order to develop its new uses and increase its potential value, the isolation and composition of rice straw have been extensively studied in recent years (Assavanig *et al.* 1992; Jin and Chen 2007; Sun and Cheng 2002; Sun *et al.* 2002; Xu *et al*. 2006). But to the best of our knowledge, there has been no research about the separation and structural characterization of hemicelluloses isolated by different alcohols from rice straw. Therefore, the aim of this study was to investigate the physicochemical and structural characterization of hemicelluloses isolated by different alcohols from rice straw. In this study, six hemicellulosic fractions of rice straw were isolated by the treatments with 60% methanol, 60% ethanol, 60% *n*-propanol, 60% *n*-butanol, 60% ethanol containing 0.01M HCl, and 60% ethanol containing 0.25 M NaOH at 75 ºC for 3 h, respectively. The chemical composition and the structural features of the hemicellulosic fractions were determined and characterized by high performance anion exchange chromatography (HPAEC), Fourier transform infrared (FT-IR), gel permeation chromatography (GPC), and ^{13}C nuclear magnetic resonance spectroscopy (^{13}C NMR) along with 2D ^{1}H–^{13}C Hetero-nuclear Single Quantum Correlation (HSQC) spectra to pave the way for the use of rice straw hemicelluloses in further industrial applications.

EXPERIMENTAL

Materials

Rice straw was obtained from the experimental farm of the Northwest Agricultural and Forestry University (Yangling, China). It was dried in sunlight and cut into small pieces. Samples were dried at 60 ºC for 16 h in an oven until the weights were constant, and then ground using a Christie Laboratory mill to pass a 0.8 mm size screen. The powder was further dried in a cabinet oven with air circulation at 60 ºC for 16 h and stored in a desiccator. The main composition (w/w) of the rice straw used was cellulose 36.5%, hemicelluloses 33.8%, chlorite lignin 12.3%, and wax 3.8%, on a dry weight basis, obtained according to the method of Lawther *et al.* (1995). The contents of mineral compounds (13.3%) and silica (70.8%) were determined according to the methods described by Pan *et al.* (1999). The sample was transferred to a crucible and carbonised gently in a muffle furnace at 550±10 ºC for 6 h. The mineral compounds obtained were treated with concentrated HCl. The acid-insoluble residue was filtered, washed with hot water until no chlorides were detectable, ignited, and finally weighed as silicon dioxide. Prior to treatment, rice straw was first dewaxed with toluene-ethanol (2:1, v/v) in a Soxhlet extractor for 6 h.

Extraction of Hemicelluloses

The different alcohol treatments of rice straw were carried out according to the scheme in Fig. 1. The dewaxed rice straw was extracted with 60% methanol, 60% ethanol, 60% *n*-propanol, 60% *n*-butanol, 60% ethanol containing 0.01 M HCl, and 60% ethanol containing 0.25 M NaOH as a catalyst at 75 ºC for 3 h at a solid to liquor ratio of 1:25 (g mL^{-1}) under stirring, respectively. The solubilized filtrations were recovered, neutralized in the pH range 5.5 to 6.0 with 6 M HCl, concentrated to about 50 to 60 mL under reduced pressure, and then precipitated by pouring the concentrated supernatant fluid into 3 volumes of 95% ethanol. After filtration, the six pellets of the isolated hemicellulosic fractions were thoroughly washed with acidified 70% ethanol and air-dried, and were labeled as hemicellulosic fraction H_1, H_2, H_3, H_4, H_5, and H_6, respectively. The yields of the residual hemicelluloses and lignins are given on a dry weight basis related to the initial dewaxed rice straw.

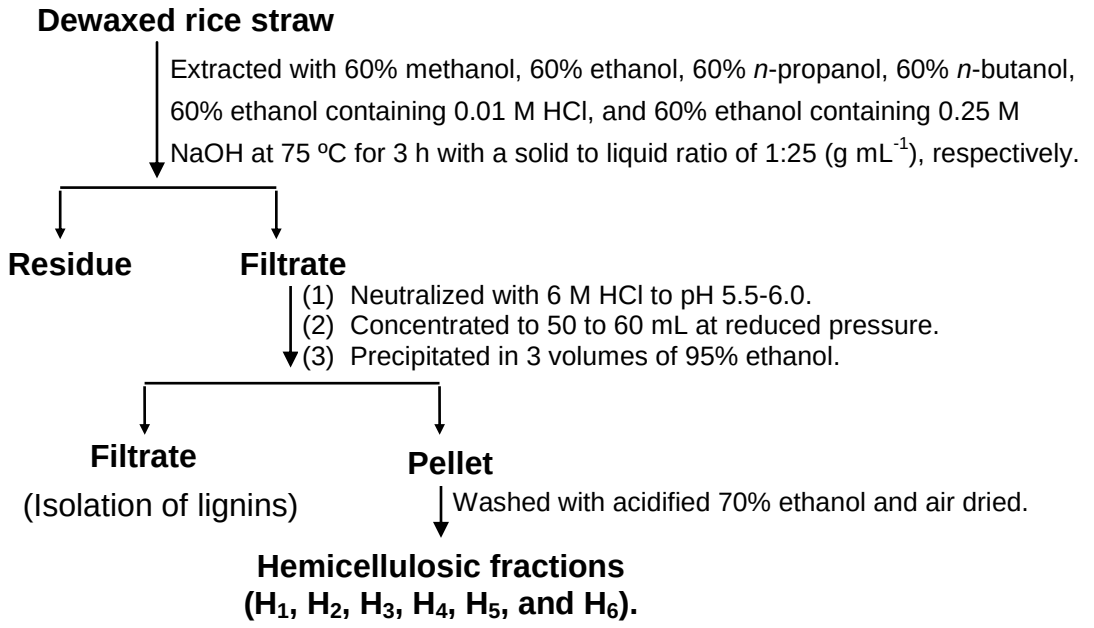


Fig. 1. Scheme for isolation of hemicelluloses from rice straw

Characterization of Hemicellulosic Fractions

The composition of monosaccharides in the hemicellulosic fractions was determined by high performance anion exchange chromatography (HPAEC). The neutral sugars in the hemicellulosic fractions were liberated by hydrolysis with 10% H_2SO_4 for 2.5 h at 105 ºC. After hydrolysis, the sample was diluted to 30-fold, filtered, and injected into a HPAEC system (Dionex ICS–3000) with an amperometric detector, an AS50 autosampler, and a Carbopac 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^{-1}. Run time was 45 min, followed by a 10 min elution with 18 mM NaOH re-equilibrate the column. The uronic acid was eluted with 0.4 M NaOH for 20 min at a rate of 1 mL min^{-1} with postcolumn addition of 0.3 M NaOH at a rate of 0.5 mL min^{-1}. Calibration was performed with standard solutions of L-

arabinose, D-glucose, D-xylose, D-rhamnose, D-mannose, D-galactose, glucuronic acid, and galacturonic acid.

The chemical composition of phenolic acids/aldehydes from the lignin fractions was measured by alkaline nitrobenzene oxidation and determined by high-performance liquid chromatography (HPLC) on a ZORBAX Eclipse XDB-C_{18} HPLC column of dimensions 250×4.6 mm (1200 series, Agilent Technologies, USA). In addition, GPC, FTIR, NMR, and TGA/DTG are used to determine molecular weight, functional groups, structural characteristics, and thermal stability of hemicelluloses extracted from rice straw, respectively.

The average molecular weights ($\bar{M}$) of hemicelluloses were determined by gel permeation chromatography (GPC, Agilent 1200, USA) on a PL aquagel-OH 50 column (300×7.7mm, polymer laboratories Ltd.), calibrated with PL pullulan polysaccharide standards (peak average molecular weights 738, 12 200, 100 000, and 1 600 000, polymer laboratories Ltd). The column oven was maintained at 30 ºC. Detection was achieved with a differential refractive index detector (RID), which was eluted with 5 mM sodium phosphate buffer (pH 7.5) containing 0.02 M NaCl and 0.1% hemicelluloses at a flow rate of 0.5 mL min^{-1}.

The FT-IR spectra were measured on a Nicolet 750 spectrophotometer using KBr discs containing 1% finely ground hemicellulosic fractions. Thirty-two scans at a resolution of 4 cm^{-1} were averaged. Background spectra were taken in an empty chamber. The samples were dried in an oven for 10 h at 50 ºC before the spectra were recorded. The solution-state ^{1}H NMR spectra were recorded on a Bruker AVIII NMR spectrometer at 400 MHz using 20 mg hemicelluloses in 1.0 mL D_2O. The chemical shifts were calibrated relative to the signals from D_2O, used as an internal standard, at 4.7 ppm for the ^{1}H NMR spectra. The acquisition time was 4 s, and the relaxation time was 1 s. ^{13}C NMR spectra were obtained on the same NMR spectrometer at 100 MHz. The sample (80 mg) was dissolved in 1 mL of D_2O (99.8%D) overnight at room temperature. The ^{13}C NMR spectra were recorded at 25 ºC after 30 000 scans. A 30^{o} pulse flipping angle, a 9.2 μs pulse width, and a 4 s delay time between scans were used. The proton-detected HSQC spectra were acquired by HSQCETGP experiment mode, over a t_1 spectral width of 10 000 Hz and a t_2 width of 1800 Hz, and the AQ was 0.13 s. The scanning time was 64. The delay between transients was 1.5 s, and the delay for polarization transfer was correspond to an estimated average ^{1}H-^{13}C coupling constant of 145 Hz. Data processing was performed using a standard Bruker Topspin-NMR software.

Thermal analysis of the samples was performed using thermogravimetric analysis (TGA) on a simultaneous thermal analyzer (Pyris Diamond TG/DTA, PE instrument). The apparatus was continually flushed with nitrogen at a flow rate of 25 mL min^{-1}. The sample weighed between 8 and 15 mg and was heated from room temperature to 700 °C at a heating rate of 10 °C min^{-1}.

RESULTS AND DISCUSSION

Yield of Hemicelluloses

The hemicellulosic polymer is a mixture of a number of different polysaccharides, and the yield and composition of the polymer can vary depending on the method of isolation (Peng *et al*. 2009). To isolate the pure hemicelluloses, a prior treatment by extraction with organic solvents is required to remove the non-cell wall components such

as wax and chlorophyll. It was found that pre-treatment with toluene-ethanol (2:1, v/v) could remove most of the chlorophyll, wax, and other extractives. In addition, organosolv degradation processes have attracted increasing attention as alternatives to conventional degradation processes, because they entail lower environmental impact and lower energy consumption. By-products such as the degraded lignin and hemicelluloses can be utilized for many purposes (Bian *et al.* 2010). Therefore, the rice straw was isolated by different alcohols after it was dewaxed with toluene-ethanol (2:1, v/v) in this study.

The yield (% initial dewaxed rice straw, w/w) of the alcohol-soluble hemicellulosic fractions in this experiment is shown in Table 1. As can be seen, the extraction of the dewaxed rice straw with 60% methanol, 60% ethanol, 60% *n*-propanol, 60% *n*-butanol, 60% ethanol containing 0.01 M HCl, and 60% ethanol containing 0.25 M NaOH at 75 ºC for 3 h yielded 3.5, 3.5, 2.1, 2.2, 2.5, and 18.6% hemicelluloses, corresponding to 10.4, 10.4, 6.2, 6.5, 7.4, and 55.0% of the original hemicelluloses, respectively. It should be noted that the yield of alkaline ethanol-soluble hemicelluloses obtained from the 60% ethanol containing 0.25 M NaOH treatment was much higher than the other treatments. This indicated that much more hemicellulosic macromolecules were solubilized during the treatment with alkaline ethanol than the other ones. Interestingly, the hemicelluloses yields of 60% methanol and 60% ethanol treatments were the same value (3.5% of initial dewaxed rice straw); meanwhile the yields of 60% *n*-propanol and *n*-butanol treatments were very similar (2.1 and 2.2% of initial dewaxed rice straw), indicating that methanol and ethanol had similar soluble capacity of hemicelluloses from rice straw at 60% concentrate, and had slightly higher solubility than *n*-propanol and *n*-butanol.

Furthermore, in comparison with the yield of H_2 (3.5%) obtained by extraction with 60% ethanol, the yields of H_5 obtained by 60% ethanol containing 0.01 M HCl and H_6 obtained by 60% ethanol containing 0.25 M NaOH were 2.5 and 18.6%, respectively, corresponding to 7.4, and 55.0% of the original hemicelluloses. Obviously, the yield is in the order of $H_6 > H_2 > H_5$. This result clearly showed that the alkali plays an important role in cleaving the linkages between lignin and hemicelluloses. In contrast, acid gave a much lower level for extraction of hemicelluloses from rice straw.

Table 1. Yield (% initial dry rice straw, w/w) of Solubilized Hemicelluloses, Lignin, and Cellulose-rich Residue

	Yield					
	F_1[a]	F_2[a]	F_3[a]	F_4[a]	F_5[a]	F_6[a]
Hemicelluloses	3.5	3.5	2.1	2.2	2.5	18.6
Lignin	1.8	1.6	2.0	1.5	1.6	9.3
Residue	89.0	89.4	91.2	86.9	86.3	56.6

[a] F_1, F_2, F_3, F_4, F_5, and F_6 represent the solubilized polymeric preparations of hemicelluloses, lignin, and residue obtained by treatment of dewaxed rice straw with 60% methanol, 60% ethanol, 60% propanol, 60% butanol, 60% ethanol containing 0.01 M HCl, and 60% ethanol containing 0.25 M NaOH at 75 ºC for 3 h, respectively

Monosaccharide Composition

The neutral monosaccharide composition and content of uronic acids of the six alcohol-soluble hemicellulosic subfractions were identified and quantified as shown in Table 2. As can be seen, the major monosaccharide in hemicellulosic fractions H_1, H_2, H_3, and H_5 was xylose (31.78-36.80%) followed by glucose (26.39-29.82%), galactose

(15.05-17.17%) and arabinose (14.93-15.94%). This result showed that the alcohol soluble hemicelluloses from rice straw had a relatively lower amount of xylose and a higher amount of glucose, galactose, and arabinose than those of wheat straw and sweet sorghum stem in comparison with our previous results (Xu *et al.* 2006; Nie *et al.* 2011). Meanwhile, the major monosaccharide of hemicellulosic fraction H_4, which was isolated by 60% *n*-butanol was glucose (39.39%) followed by xylose (26.96%), indicating that the treatment with 60% *n*-butanol mainly broke the xylan-backbone, while the treatments of 60% methanol, 60% ethanol, and 60% *n*-propanol solubilized the side chain of hemicelluloses. In comparison, the contents of xylose among hemicellulosic fractions H_1, H_2, and H_3 displayed little difference, revealing the hemicelluloses extracted by alcohols from rice straw shared similar structures of xylans.

Additionally, it was also found that the dominating monomeric sugar components of H_5 and H_6 were 36.21 and 59.62% xylose, respectively. In comparison with the fraction H_2, the contents of xylose in H_5 and H_6 were much higher than that in H_2 (32.69%). It indicated that the treatment with acid or alkaline had effective dissolving capability to linear hemicelluloses while the treatment with alcohol mainly solubilized the branched ones.

Table 2. Contents of Neutral Sugars and Uronic Acids (relative % of hemicellulosic sample, w/w) in the Hemicellulosic Fractions

Neutral Sugars	Hemicellulose Fractions[a]					
	H_1	H_2	H_3	H_4	H_5	H_6
Rhamnose	1.62	1.73	2.11	1.68	1.51	1.32
Arabinose	15.29	15.58	14.93	12.27	15.94	18.10
Galactose	17.17	16.21	15.05	15.42	15.61	7.48
Glucose	29.82	28.19	26.51	39.39	26.39	8.17
Xylose	31.78	32.69	36.80	26.96	36.21	59.62
Mannose	1.83	2.12	1.62	1.74	1.98	0.80
Glucuronic acid	2.08	3.09	2.61	2.11	2.00	4.40
Galacturonic acid	0.41	0.39	0.37	0.43	0.37	0.11

[a] H_1, H_2, H_3, H_4, H_5, and H_6 represent the solubilized hemicellulosic preparations obtained by treatment of dewaxed rice straw with 60% methanol, 60% ethanol, 60% propanol, 60% butanol, 60% ethanol containing 0.01 M HCl, 60% ethanol containing 0.25 M NaOH at 75 ºC for 3 h, respectively

The associate lignin polymers in the isolated hemicelluloses are not amenable to direct chemical analysis. However, lignin in such samples can be characterized by chemical degradation to release phenolic monomers including derivatives of cinnamic and benzoic acids, benzaldehyde, and acetophenone that can be quantified by a variety of chromatographic techniques (Hedges and Ertel 1982). To verify the lignin associated in the isolated hemicelluloses, all of the six solubilized hemicellulosic preparations were subjected to alkaline nitrobenzene oxidation at 170 ºC for 2.5 h, and the phenolic acids and aldehydes resulted from the degradation and oxidation of the bound lignin in the hemicellulosic preparations are given in Table 3. Obviously, all the hemicellulosic fractions contained relatively low amounts of associated lignins because the total yield of phenolic acids and aldehydes ranged only from 0.12 to 0.33%, which indicated that a substantial cleavage of ether linkages between lignin and hemicelluloses occurred during the different alcohols treatments. This is in accordance with previous research results (Pisarnitskii *et al.* 2006; Pan *et al.* 2006; Peng *et al.* 2012).

Table 3. Yield of Phenolic Acids and Aldehydes (% hemicellulosic sample, w/w) Obtained by Alkaline Nitrobenzene Oxidation of the Associated Lignin in the Solubilized Hemicellulosic Fractions

Phenolic Acids and Aldehydes	Hemicellulosic Fractions[a]					
	H_1	H_2	H_3	H_4	H_5	H_6
Syringaldehyde	0.079	0.063	0.068	0.068	0.069	0.061
Syringic acid	0.112	0.081	0.080	0.079	0.084	0.032
Vanillin	0.055	0.057	0.046	0.044	0.047	0.026
Ferulic acid	T[b]	0.049	0.055	T	0.126	T
Total	0.246	0.250	0.249	0.190	0.325	0.120

[a] Corresponding to the hemicellulosic fractions in Table 2. [b] T = trace.

Molecular Weight

To illustrate the extent of degradation or hydrolysis caused by different alcohols, the molecular weights of the six hemicellulosic fractions were determined by GPC, and their weight-average ($\bar{M}_w$) and number-average ($\bar{M}_n$) molecular weights and poly-dispersity ($\bar{M}_w/\bar{M}_n$) are given in Table 4. As can be seen, the weight-average ($\bar{M}_w$) molecular weights of the four different alcohol-soluble hemicellulosic preparations was in the following order, $H_4 > H_1 > H_2 > H_3$, suggesting that the aqueous solution of 60% *n*-butanol had a lower ability of hemicelluloses degradation than that of 60% methanol and 60% ethanol. Meanwhile, the treatment with 60% *n*-propanol aqueous solution had a relatively higher degradation ability of hemicelluloses.

Interestingly, the weight-average ($\bar{M}_w$) molecular weights increased from 21950 g mol^{-1} (H_5) of the acidic-ethanol treatment to 23690 g mol^{-1} (H_2) of the neutral-ethanol treatment, and to 48750 g mol^{-1} (H_6) of the alkali-ethanol treatment. This indicated that 60% ethanol treatment with acid catalyst favored the cleavage of some glycosidic linkages in hemicelluloses while the 60% ethanol treatment with alkali catalyst dissolved some large molecular-size hemicelluloses.

Table 4. Weight-average ($\bar{M}_w$) and Number-average ($\bar{M}_n$) Molecular Weights and Polydispersity ($\bar{M}_w/\bar{M}_n$) of the Hemicellulosic Fractions

	Hemicellulosic Fractions[a]					
	H_1	H_2	H_3	H_4	H_5	H_6
$\bar{M}_w$	25310	23690	18850	30380	21950	48750
$\bar{M}_n$	9690	9500	8220	10110	8750	15890
$\bar{M}_w/\bar{M}_n$	2.61	2.49	2.29	3.00	2.51	3.07

[a] Corresponding to the hemicellulosic fractions in Table 2

FT-IR Spectra

FT-IR spectroscopy has been shown to be a very valuable technique for the evaluation of cell wall monosaccharide composition and for monitoring their changes during the isolation process (Coimbra *et al.* 1999). In particular, it can possible solve the problems of identification of polysaccharides, check their purity, carry out semi-quantitative functional analyses, determine structure, and investigate complexing and intermolecular interactions (Filippov 1992). The FT-IR spectra of hemicellulosic fraction H_1 (spectrum 1), H_2 (spectrum 2), and H_3 (spectrum 3) are illustrated in Fig. 2. The

absorptions at 3421 and 2925 cm^{-1} are attributed to the stretching of –OH groups and C-H stretching, respectively. The absorption at 1639 cm^{-1} was principally associated with absorbed water, because the hemicelluloses usually have a strong affinity for water, and in the solid state these macromolecules may have disordered structures that can easily be hydrated (Kačuráková *et al.* 2000).

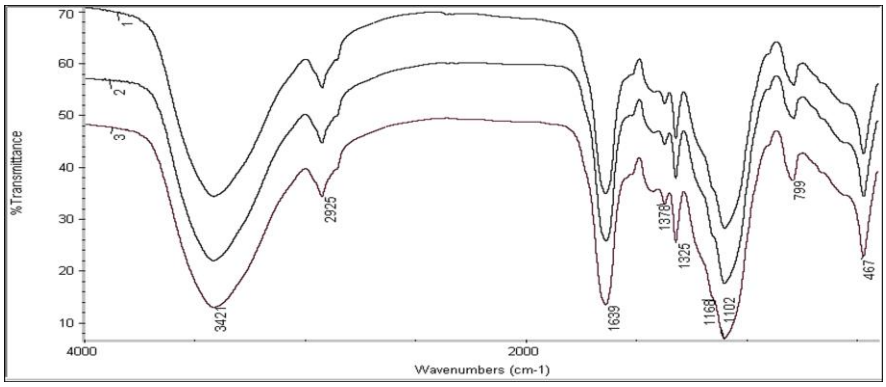


Fig. 2. FT-IR spectra of rice straw hemicellulosic fractions isolated with 60% methanol (spectrum 1), 60% ethanol (spectrum 2), and 60% n-propanol (spectrum 3) at 75 ºC for 3 h

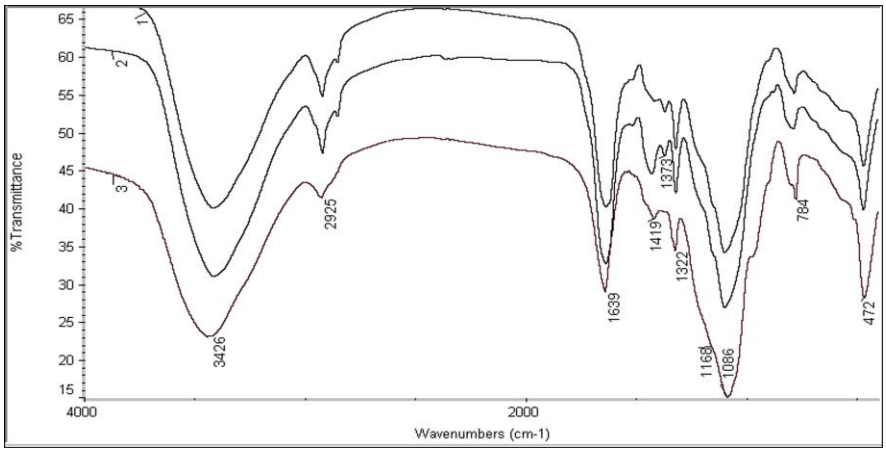


Fig. 3. FT-IR spectra of rice straw hemicellulosic fractions isolated with 60% n-butanol (spectrum 1), 60% ethanol containing 0.01 M HCl (spectrum 2), and 60% ethanol containing 0.25 M NaOH (spectrum 3) at 75 ºC for 3 h

The FT-IR spectra in the 1200 to 800 cm^{-1} region give more information about the polysaccharides. Previous FT-IR research showed that each particular polysaccharide has a specific band maximum in the 1200-1000 cm^{-1} region and this region is dominated by ring vibrations overlapped with stretching vibrations of (C-OH) side groups and the (C-O-C) glycosidic bond vibration. However, the xylan units may also affect the frequency of the IR band (Kačuráková *et al.* 2000). Because of the relatively high amount of glucose, galactose, and arabinose in rice straw compared to wheat and sorghum stem (Xu *et al.* 2006; Nie *et al.* 2011), the band shape of the hemicelluloses are influenced by the glucan, galactan, and arabinan from the side chain, whose original maximum absorptions at 1104 and 1076, 1118 and 1078, and 1097 and 1070 cm^{-1}, respectively, led to the maximum absorptions of the C-O, C-C stretching or C-OH bending in the hemicellulosic fractions H_1, H_2, H_3, H_4, and H_5 and shown at 1102 cm^{-1} in Fig. 2. However, this

maximum absorption of hemicellulosic fraction H_6 shifted to 1086 cm^{-1} (Fig. 3) because of its higher amount of xylose (59.62%), which has the original maximum absorptions at 1078 and 1041 cm^{-1}. The absorbance peak at 1373 cm^{-1} originates from C-H ester bands due to partial acetylation of hydroxyl groups in hemicelluloses. The peak at 1168 cm^{-1} relates to the C-O anti-symmetric stretching.

^{1}H, ^{13}C, and 2D-HSQC NMR Spectra

NMR spectroscopy is the most powerful and noninvasive physicochemical technique for determining polysaccharide structures from suitably isolated fractions. It can provide detailed structural information of carbohydrates, including identification of monosaccharide composition, elucidation of *α*- or *β*-anomeric configurations, establishment of linkage patterns, and sequences of the sugar units in oligosaccharides and/or polysaccharides (Cui 2005). To further investigate the configuration of the glycosidic linkages and the structural features of the solubilized hemicelluloses, hemicellulosic fraction H_6, which was extracted with 60% ethanol containing 0.25 M NaOH from rice straw at 75 ºC for 3 h, was determined by ^{1}H and ^{13}C NMR spectroscopy and 2D-HSQC spectrometry, and the spectra are shown in Figs. 4 to 6.

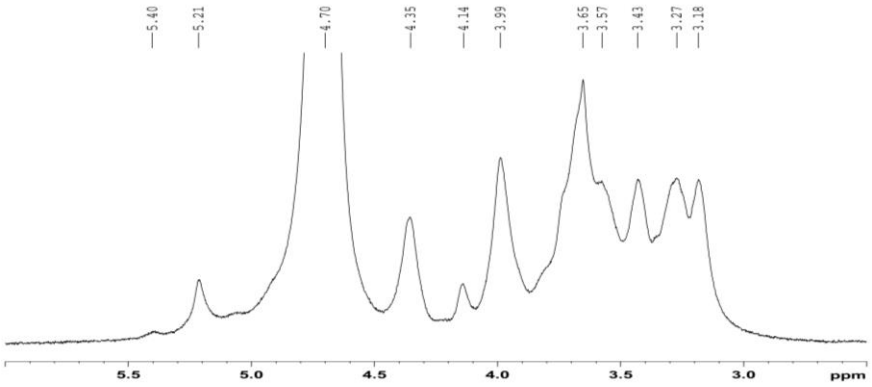


Fig. 4. ^{1}H-NMR spectrum of hemicellulosic fraction H6 isolated with 60% ethanol containing 0.25 M NaOH at 75 ºC for 3 h

Figure 4 shows the ^{1}H NMR spectrum of the hemicellulosic fraction H_6. As can be seen, the spectrum gives the typical signal pattern expected for a hemicellulosic moiety. The signals at δ 3.1 to 5.4 ppm are caused by the protons of the arabinose and xylose residues except for the strong signal at δ 4.7 ppm, which is HDO from the solvent (D_2O). The main signals at δ 4.35 (H-1), 3.99 (H-5eq), 3.65 (H-4), 3.43 (H-3), 3.27 (H-5ax), and 3.18 ppm (H-2) are assigned to *β*-D-xylopyranosyl residues since the region between 4.3 and 4.9 ppm corresponds to the *β*-configuration (Kawagishi *et al.* 1990), while the minor signals at δ 5.21 (H-1), 4.14 (H-5), 3.43 (H-2) ppm correspond to 4-*O*-methyl–D-glucuronic acid residues (Habibi and Michel 2005). Anomeric protons of terminal *α*-D-arabinofuranosyl residues give rise to the peak at δ 5.4 ppm (Teleman *et al.* 2000). The anomeric proton was distinguished at δ 4.35 ppm, which is assigned as (1→4)-*β*-D-Xyl*p* of the hemicellulosic fraction H_6. This confirmed that (1→4)-*β*-D-Xyl*p* is *β*-glycosidically linked, which is consistent with the presence of the very small peak at 902 cm^{-1} (data not shown) in the FT-IR spectrum.

Although ^{13}C-NMR has a much weaker signal, it has significant advantages over ^{1}H-NMR spectroscopy in the analysis of polysaccharides because the chemical shifts in

^{13}C-NMR spectrum are spread out over a broader range. This broad distribution of signals helps to overcome the severe overlapping problems associated with the proton spectrum. The hemicellulosic subfraction H_6 was investigated using ^{13}C NMR spectroscopy (Fig. 5) to elucidate the polymer backbone and the type of side-chain branching along the backbone. The five main signals at δ102.0, 76.0, 74.4, 73.0, and 63.2 ppm originate from C-1, C-4, C-3, C-2, and C-5 of *β*-D-xylans, respectively. The signals at δ 109.3, 86.0, 80.4, 78.3, and 61.5 ppm originate from C-1, C-4, C-2, C-3, and C-5 of *α*-L-arabinofuranosyl residues linked to *β*-D-xylans, respectively. These data revealed that the anomeric configuration of the D-xylopyranose residues is *β* type while the L-arabinofuranose residue is *α* type.

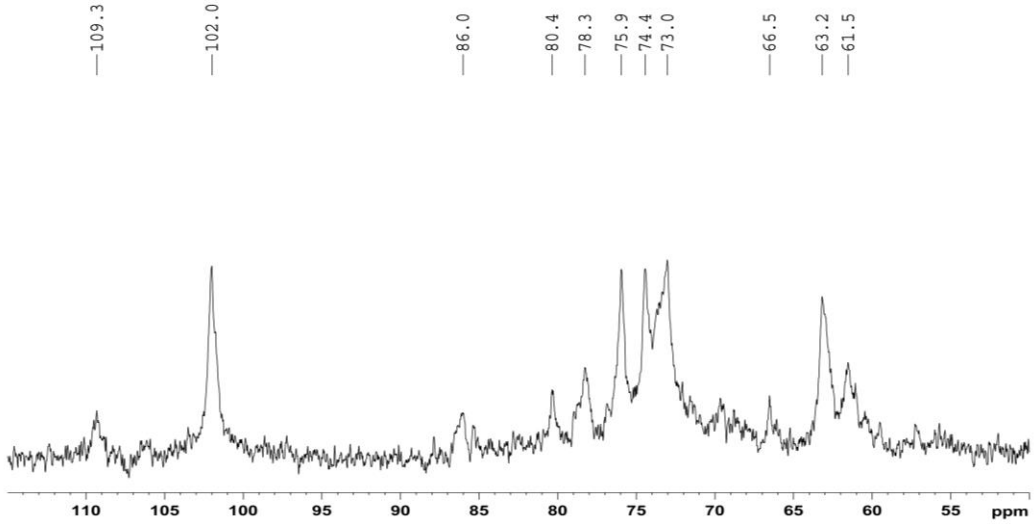
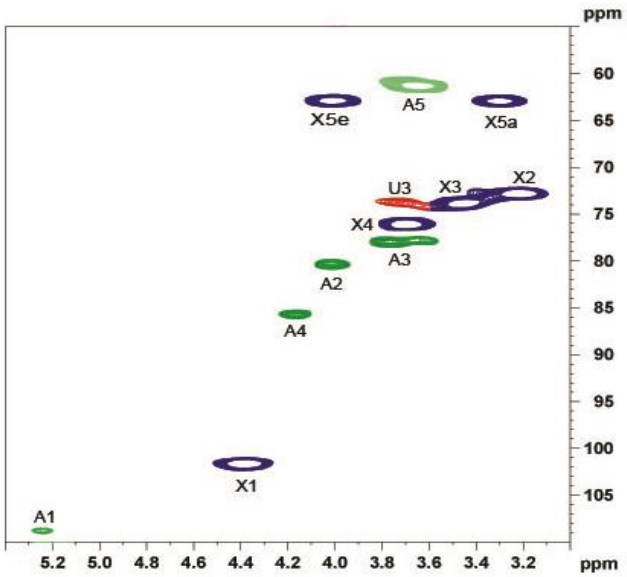


Fig. 5. ^{13}C-NMR spectrum of hemicellulosic fraction H_6 isolated with 60% ethanol containing 0.25 M NaOH at 75 ºC for 3 h

Even with the availability of high field spectrometers, severe proton spectral overlap can often complicate the assignment of structures of organic molecules in the assignment of ^{1}H NMR spectra. However, acquisition of a high-resolution coupled heteronuclear single quantum correlation (HSQC) spectrum overcame this problem (Powder *et al.* 2012). A 2D-HSQC spectrum provides a useful method for determining vicinal proton coupling constants between strongly coupled protons; therefore, it is useful for assigning all the proton and C-13 resonances. The 2D-HSQC spectra of the hemicelluloses fraction H_6 is shown in Fig. 6. As can be seen, the dominant five cross-peaks could be easily identified at δ 102.0/4.35, 78.3/3.65, 74.4/3.57, 73.0/3.27, and 63.2/3.99+3.27 ppm, which are assigned to C-H_1, C-H_4, C-H_3, C-H_2 and C-H_5 of the (1→4)-linked-*β*-D-Xyl*p* units, respectively. Furthermore, some weak cross-peaks at δ 109.3/5.21, 86.0/4.14, 80.4/4.04, 78.3/3.65, 61.5/3.57 ppm, which represented the C-H_1, C-H_4, C-H_2, C-H_3, and C-H_5 of L-arabinose, were also observed. In short, these results implied that the hemicellulosic fraction H_6, which was extracted with 60% ethanol containing 0.25 M NaOH from rice straw at 75 ºC for 3 h, can be structurally defined as 4-*O*-methyl-α-D-glucurono-L-arabino-D-xylans. Similar structural characterization of hemicelluloses was also reported in *Caragana korshinskii* (Bian *et al.* 2010), bamboo (Peng *et al.* 2011a, b), and sugarcane bagasse (Peng *et al.* 2009).



X: (1→4)-*β*-D-Xylp
U: 4-*O*-Me-*α*-D-GlcpA
A: *α*-L-Araf

Fig. 6. 2D-HSQC spectrum of hemicellulosic fraction H_6 isolated with 60% ethanol containing 0.25 M NaOH at 75 ºC for 3 h

Thermogravimetric Analysis

Thermogravimetric analysis (TGA) and derivative thermogravimetry (DTG) were used to investigate the thermal stability of hemicellulosic samples H_2 and H_6. As shown in Fig. 7, the TGA curves show an initial decrease in the weight of samples between 50 and 150 ºC due to the release of moisture remaining in the samples. Furthermore, it can be clearly seen that weight loss of hemicelluloses took place mainly in the temperature range of 200 to 400 ºC. Thus, the weight loss of H_2 and H_6 were 27.8 and 24.1% at 300 ºC, respectively. While the temperature further was increased to 400 ºC, the weight loss of H_2 and H_6 were 45.2 and 37.1%, respectively. Those results suggested that the thermal stability of H_2 was lower than that of H_6.

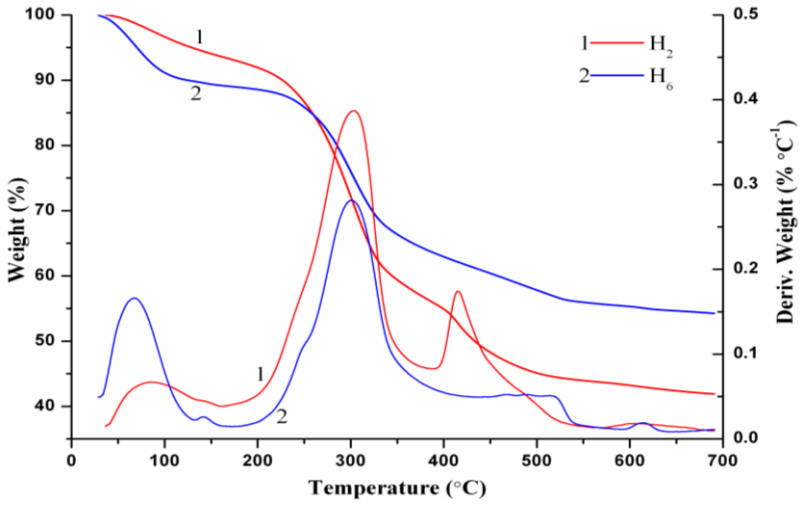


Fig. 7. TGA/DTG curves of H_2 and H_6

The TGA results indicated that linear hemicelluloses had greater thermal stability than that of branched hemicelluloses, which was in agreement with previous results reported by Peng *et al.* (2011a). Moreover, the DTG curve of H_2 exhibited the peak maximum at 303 ºC and a shoulder at 415 ºC were 0.38 and 0.17 % ºC^{-1}, respectively. Similar two decomposition steps in DTG curve were also found during the thermal degradation of hemicelluloses (Peng *et al.* 2011b). In comparison to H_2, the peak maximum of H_6 at 300 ºC was 0.28 % ºC^{-1}. This phenomenon is also supported by the result that the thermal stability of H_2 was lower than that of H_6.

CONCLUSIONS

1. The 60% aqueous solution of methanol and ethanol had a similar soluble capacity of hemicelluloses from rice straw and a slightly higher yield than those of *n*-propanol and *n*-butanol.
2. The major monosaccharide in neutral alcohol-soluble hemicellulosic fractions H_1, H_2, H_3, and H_4 is xylose (31.78-36.80%), followed by glucose (26.51-39.39%), galactose (15.05-17.17%), and arabinose (14.93-15.58%), while alkaline ethanol-soluble hemicellulosic fraction contained the highest amount of xylose (59.62%) compared to the other hemicellulosic fractions.
3. The weight-average ($\bar{M}_w$) molecular weights increased from 21950 g mol^{-1} of the hemicelluloses dissolved in the acidic-ethanol treatment, to 23690 g mol^{-1} of that isolated from the neutral-ethanol treatment, and to 48750 g mol^{-1} of polymer preparation released during the alkali-ethanol treatment. This indicated that 60% ethanol treatment with acid catalyst favored the cleavage of some glycosidic linkages in hemicelluloses, whereas the 60% ethanol treatment with alkali catalyst dissolved some large molecular-size hemicelluloses.
4. The combination of FTIR, ^{1}H, ^{13}C, and 2D-HSQC NMR revealed that the alkaline ethanol-soluble hemicellulosic fraction can be structurally defined as 4-*O*-methyl-α-D-glucurono-L-arabino-D-xylans.

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