# Characterization of Hemicelluloses Obtained from Partially Delignified *Eucalyptus* Using Ionic Liquid Pretreatment

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Lignocellulosic biomass is a relatively inexpensive and abundant feedstock for biofuel production. The key to unlocking the recalcitrance of lignocelluloses is an effective pretreatment process. A promising new pretreatment method for lignocellulosic biomass is the use of ionic liquids (ILs). In this study, wood flour was partially dissolved in the novel ionic liquid 1-butyl-3-methylimidazolium acesulfamate ([BMIM]Ace) mixed with different organic solvents (1,4-dioxane, acetone, methanol, DMSO, and DMF) followed by precipitation in water. Hemicelluloses were successfully extracted from the carbohydrate-enriched residues by an alkaline ethanol solvent. Sugar analysis of the hemicellulosic fractions indicated that xylose (63.25-74.85%) was the major sugar component, while small amounts of glucose (4.85-14.40%) and galactose (4.49-7.32%) were also observed. Molecular weights of these fractions varied between 49.330 and 60.760 g/mol as determined by GPC. NMR studies revealed that the hemicelluloses had a backbone of β-(1→4)-linked-D-xylopyranosyl units and were branched mainly through 4-*O*-methyl-α-D-glucuronic acid. The thermal degradation behavior of the hemicellulosic fractions showed that the most significant degradation occurred between 242 and 300 °C.

*Keywords: Pretreatment; Ionic liquid; Hemicelluloses; Characterization*

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## INTRODUCTION

Today, the world's fossil fuel-based economy is facing problems and challenges. The depletion of fossil resources and global warming concerns have led to an intensified search for alternative resources to supply modern society. A potential solution to the problem is the utilization of lignocellulosic biomass as an alternative, sustainable energy source. Therefore, extensive research on the conversion of lignocellulosic biomass is currently being undertaken all over the world. In addition to its renewability, it can be used to produce chemicals and biofuels that do not compete with food production (Huber *et al.* 2006; Lynd *et al.* 1999). The term "lignocellulosic biomass" is often used to describe the material that composes the plant cell wall and is made up of cellulose, hemicelluloses, and lignin. In recent years, hemicelluloses have received greater attention because of their practical applications for bioconversion into fuels and chemicals.

Hemicelluloses, the second most abundant constituent of lignocellulosic biomass, are not chemically well-defined compounds, but rather a family of polysaccharides, composed of different five- and six-carbon monosaccharide units (Rubin 2008). In general, hemicelluloses are mainly composed of pentoses (β-D-xylose, α-L-arabinose),

hexoses (β-D-mannose, β-D-glucose, α-D-galactose), and/or uronic acids (α-D-glucuronic, α-D-4-*O*-methylgalacturonic, α-D-galacturonic acids). Other sugars, such as α-L-rhamnose and α-L-fucose, may also be present in small amounts. The hydroxyl groups of sugars can be partially substituted with acetyl groups (Gírio *et al.* 2010). Moreover, the structure of hemicelluloses varies significantly, depending on the plant species (Timell 1967). Hemicelluloses are applicable as gels, films, coating, adhesives, additives, and a variety of hemicellulose-based biomaterials in food and pharmacy as well as other industries (Ebringerova *et al.* 2005).

The removal and recovery of hemicelluloses is an essential feature of a successful pretreatment process for biological conversion to ethanol or other products. However, separation of hemicelluloses from the cell wall is restricted by the presence of a lignin network, lignin-hemicelluloses linkages, and physical intermixing between hemicelluloses and cellulose (Freudenberg 1965; Hansen and Plackett 2008). The pretreatment stage is, therefore, critical to the overall process because it increases the accessibility and reactivity of polysaccharides by deconstructing the three-dimensional structure of lignocellulose. During successful pretreatment, delignification occurs and the crystalline cellulose and hemicelluloses are broken down without significant degradation of polysaccharides. Multiple pretreatment strategies have been investigated to remove the lignin and improve the yield of fermentable sugars for the production of biofuels. Conventional methods that convert lignocellulosic materials to sugars have been in the form of acid hydrolysis or through the use of high pressure and temperature. These methods are either energy-intensive or generate toxic byproducts that affect their economic viability.

In the last decades ionic liquids (ILs) have become popular for the pretreatment of biomass (Cetinkol *et al.* 2010; Fu *et al.* 2010; Li *et al.* 2010a; Samayam and Schall 2010; Simmons *et al.* 2010; Singh *et al.* 2009). Commonly defined as salts with a melting temperature <100 °C, ILs possess unique characteristics that distinguish them from molecular solvents. The unique ability of ILs to completely solubilize biomass and its biopolymeric constituents, such as cellulose or lignin, can potentially lead to improved pretreatment and fractionation of the biomass (Fort *et al.* 2007; Swatloski *et al.* 2002; Stark 2011; Wang *et al.* 2011). Until now, this process has been too expensive for industrial application, mainly due to the high cost of ILs. Therefore, advanced technology using ILs is required to make the process more efficient while minimizing the cost.

ILs can be an important tool for the pretreatment of lignocellulosic biomass. It was previously proposed that ILs could be utilized as cleaning chemicals for biomass pretreatment to reduce both crystallinity and lignin content, thereby improving the accessibility of cellulolytic enzymes to the biomass (Lee *et al.* 2009; Hendriks and Zeeman 2009; Yang *et al.* 2008). However, very few studies on the characterization of the structural and chemical features of hemicelluloses after IL pretreatment of biomass have been carried out to date. The object of this work was to establish a pretreatment method to fractionate hemicelluloses from regenerated carbohydrate-enriched residues, which have been pretreated by novel IL 1-butyl-3-methylimidazolium acesulfamate ([BMIM]Ace). A combination of ionic liquid and organic solvents was used in the pretreatment of *Eucalyptus* wood flour, and the physical and chemical changes of hemicelluloses extracted from partially delignified *Eucalyptus* wood were assessed. The hemicellulosic preparations obtained were characterized by high performance anion exchange chromatography (HPAEC), gel permeation chromatography (GPC), Fourier transform infrared (FT-IR), 1D ($^{13}$C) and 2D (HSQC) NMR spectra, and

thermogravimetric analysis (TGA). This work provides critical knowledge for the development of an ionic liquid fractionation approach to produce hemicellulosic fractions with a minimum of degradation products from lignocellulosic biomass.

## EXPERIMENTAL

### Materials

Three-year-old *Eucalyptus* was harvested in October 2010, at the arboretum of South China University of Technology (Guangzhou, China). The leaves and bark were removed so that only the stalks were used. The material was first dried in sunlight, ground, and screened to obtain a uniform particle size (125 to 300 µm). It was then dried again in a cabinet oven with air circulation at 50 °C for 24 h and stored at 5 °C before ball milling. The chemical composition of the raw material was 39.8% cellulose, 27.5% hemicellulose, 27.4% acid-insoluble lignin, 3.6% acid-soluble lignin, 0.6% extractives, and 1.1% ash, according to the method of NREL LAP (Sluiter *et al.* 2008). All weights and calculations were made on an oven-dry basis. All standard chemicals, such as monosaccharide used as an internal standard, were of chromatographic purity. Other chemical agents were analytically pure. The chemicals were purchased from Sigma Chemical Company (Beijing) and were used without further purification.

### Preparation of IL

The ionic liquid 1-butyl-3-methylimidazolium acesulfamate ([BMIM]Ace) (Fig. 1) was synthesized according to the literature (Tan *et al.* 2009).

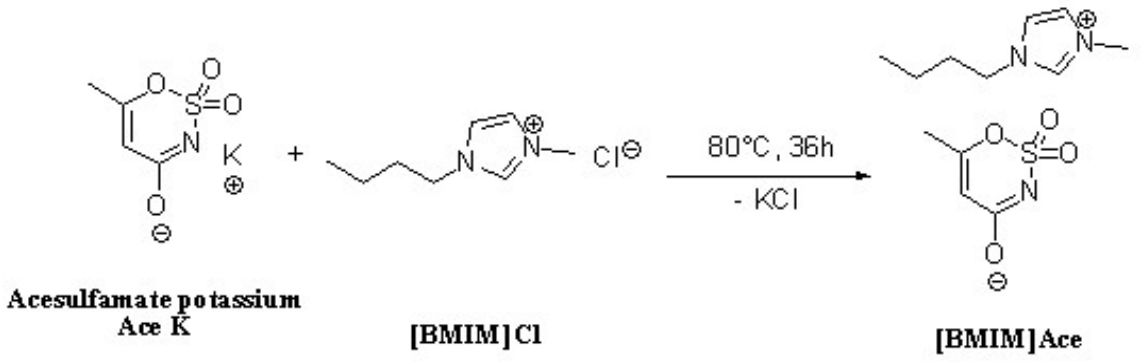


**Fig. 1.** The synthetic reaction and structure of ionic liquid 1-butyl-3-methylimidazolium acesulfamate ([BMIM]Ace)

[BMIM]Ace was prepared from a stoichiometric ratio between acesulfame potassium (Ace K) and 1-butyl-3-methylimidazolium chloride ([BMIM]Cl). Twenty-five g of Ace K was dissolved in 100 mL of hot water and added to 21.66 g of [BMIM]Cl. The mixture was stirred at 80 °C for at least 36 h. The water was removed under vacuum and 150 mL of dry acetone was added. Insoluble potassium chloride was removed by filtration. The IL was dried under vacuum at 80 °C for 12 h. It was then redissolved in 250 mL of dry acetone and refrigerated overnight. Residual precipitations of potassium chloride were filtered off, and acetone was removed under vacuum. The IL was dried at 80 °C and 10 Pa for 12 h.

## Methods

*Preparation of the hemicellulosic fractions*

All experiments were conducted in an open atmosphere. Figure 2 describes the scheme for the fractionation of hemicellulosic polymers from *Eucalyptus*. Planetary ball milling was performed in a 2 L porcelain jar, which occupied 20% of the active jar volume. The *Eucalyptus* was loaded into the jar, and the mild milling process was conducted at room temperature for 4 h with a rotation speed of 180 rpm.

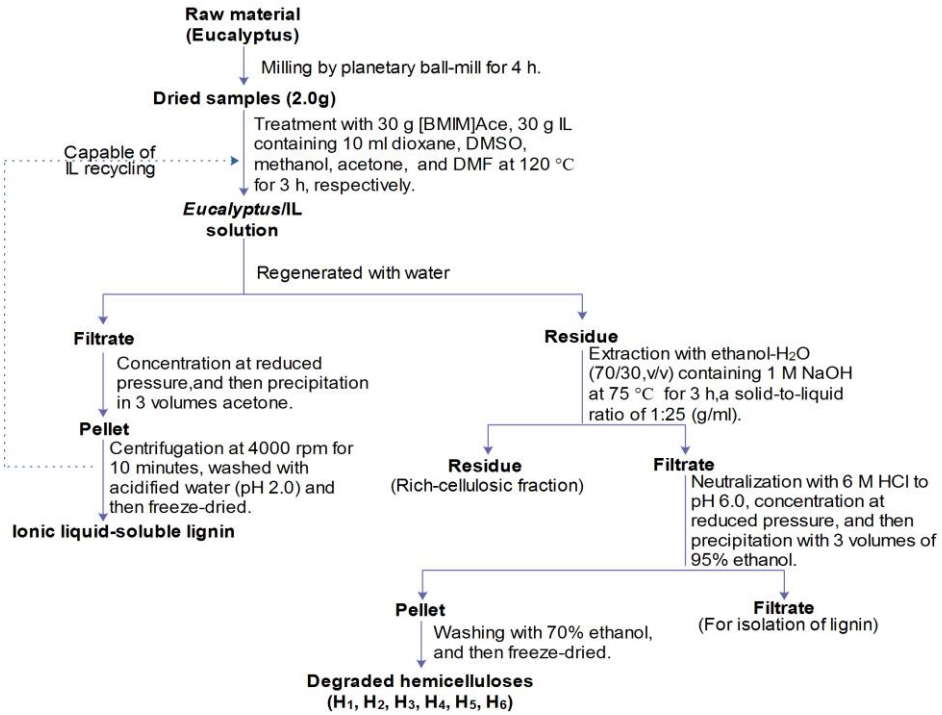


**Fig. 2.** Schematic process of *Eucalyptus* wood fractionation based on dissolution in [BMIM]Ace system followed by precipitation in water and extraction with 70% aqueous ethanol containing 1 M NaOH

The ball-milled sample (2 g) was submitted to treatment with 30 g of 1-butyl-3-methylimidazolium acesulfamate ([BMIM]Ace) and [BMIM]Ace containing different organic solvents (10 mL) at 120 °C for 3 h. Five different organic solvents (1,4-dioxane, acetone, methanol, dimethyl sulphoxide (DMSO), dimethyl formamide (DMF)) were used in combination with IL for the dissolving process. The pH values of fresh IL and IL/organic solvents mixtures were in the range of 6.0 to 6.4. After 3 h of incubation, carbohydrate-enriched materials were regenerated by the addition of water. The precipitated carbohydrate-rich materials were separated by filtration under gentle vacuum, while the lignin fraction was subsequently precipitated in acetone from the filtrate by evaporating water and organic solvents under reduced pressure. The residue was washed with distilled water to ensure that the IL had been washed out, and then dried at 105 °C for 2 h. Subsequently, the dried residue was extracted with 70% aqueous ethanol containing 1 M NaOH at 75 °C for 3 h with a solid-to-liquid ratio of 1:25 (g/mL). Each of the insoluble residues was filtered off and washed thoroughly with distilled water until the filtrate was neutral. The combined supernatant was neutralized to pH 6.0 with 4 M HCl, concentrated on a rotary evaporator under reduced pressure to about 50 mL, and then mixed with three volumes of 95% ethanol for isolating solubilized hemicelluloses,

respectively. The resulting pellet was filtered out, repeatedly rinsed with 70% ethanol, and freeze-dried. The insoluble residue was collected by filtration and washed with distilled water three times. The hemicellulosic fractions separated from carbohydrate-enriched residues, which were partially delignified by pretreatment with [BMIM]Ace/ 1,4-dioxane, [BMIM]Ace/DMSO, [BMIM]Ace/methanol, [BMIM]Ace/acetone, [BMIM] Ace/DMF, or fresh [BMIM]Ace, were labeled $H_1$, $H_2$, $H_3$, $H_4$, $H_5$, and $H_6$, respectively.

## Characterization

*Sugar composition and content of uronic acids*

The chemical compositions of the six hemicellulosic fractions were determined by hydrolysis with dilute sulphuric acid. A 4 to 6 mg sample of hemicelluloses was hydrolyzed with 1.475 mL of 10% $H_2SO_4$ for 2.5 h at 105 °C with occasional vibration. After hydrolysis, the mixture was filtered and diluted 60-fold and analyzed with a high performance anion exchange chromatography (HPAEC) system (Dionex ICS3000, USA) with a pulsed amperometric detector, AS50 autosampler, Carbopac$^{TM}$ PA-20 column (4 × 250 mm, Dionex), and a guard PA-20 column (3 × 30 mm, Dionex). Neutral sugars and uronic acids were separated in a 5 mM NaOH solution (carbonate-free and purged with nitrogen) for 20 min, followed by a 0-75 mM NaAc gradient in 5 mM NaOH for 15 min. The columns were then washed with 2 mM NaOH to remove carbonate for 10 min, followed by a 5 min elution with 5 mM NaOH to re-equilibrate the column before the next injection. The total analysis time was 50 min and the flow rate was 0.4 mL/min. Calibration was performed with standard solutions of L-arabinose, D-glucose, D-xylose, D-rhamnose, D-mannose, D-galactose, glucuronic acids, and galacturonic acids. All experiments were performed at least in duplicate, and the average values were calculated for all of the hemicellulosic fractions.

*Molecular weight determination*

The weight-average ($M_w$) and number-average ($M_n$) molecular weights of the hemicellulosic fractions were determined by gel permeation chromatography (GPC) with a refraction index detector (RID) on a PL aquagel-OH 50 column (300 × 7.7 mm, Polymer Laboratories Ltd.), calibrated with PL pullulan polysaccharide standards (peak average molecular weights 738, 12,200, 100,000, 1,600,000, Polymer Laboratories Ltd.). Two mg of sample were dissolved with 0.02 N NaCl in a 0.005 M sodium phosphate buffer (pH 7.5) at a concentration of 0.1% before measurement. The experiments were performed at least in triplicate. The standard errors or deviations were always observed to be lower than 10%.

*Fourier transform infrared spectroscopy*

FT-IR spectra of the hemicellulosic fractions were obtained using a Thermo Scientific Nicolet iN10 FT-IR Microscope (Thermo Nicolet Corporation, Madison, WI) equipped with a liquid nitrogen cooled MCT detector. Dried preparations were ground and their spectra were recorded in the range of 4000 to 700 $cm^{-1}$ at 4 $cm^{-1}$ resolution and 128 scans per sample. Before data collection, background scanning was performed for correction.

*1D and 2D NMR analysis*

The solution-state $^1H$ and $^{13}C$ NMR spectra were obtained on a Bruker AVIII 400 MHz spectrometer operating in the FT mode at 100.6 MHz. The hemicellulosic samples

(20 mg for $^{1}H$, 80 mg for $^{13}C$) were dissolved in 1 mL $D_2O$. The $^{1}H$ NMR spectrum was recorded at 25 °C after 128 scans. A 30° pulse flipping angle, a 13.6 µs pulse width, a 9.37 s acquisition time, and a 1 s relaxation delay time were used. The $^{13}C$ NMR spectrum was recorded at 25 °C after 30,000 scans. A 30° pulse flipping angle, a 9.2 µs pulse width, a 1.75 s acquisition time, and a 2 s relaxation delay time were used. A $^{1}H$-$^{13}C$ correlation 2D (HSQC) NMR spectrum was also recorded at 25 °C on the same spectrometer equipped with a z-gradient double resonance probe. An approximately 20 mg sample was dissolved in 1 mL $D_2O$ and the 2D NMR spectrum was recorded. The spectral widths were 3497 and 18,750 Hz for the $^{1}H$- and $^{13}C$-dimensions, respectively. The number of collected complex points was 1024 for $^{1}H$-dimension with a relaxation delay of 1.5 s. The number of scans was 128 and 256. Time increments were always recorded in the $^{13}C$-dimension. The $^{1}J_{CH}$ used was 145 Hz. Prior to Fourier transformation, the data matrixes were zero, filled up to 1024 points in the $^{13}C$-dimension.

*Thermogravimetric analysis*

The thermal degradation of the hemicellulosic fractions was investigated using thermogravimetric analysis (TGA) on a simultaneous thermal analyzer (SDT-60, Shimadzu). The apparatus was continually flushed with a nitrogen flow of 25 mL/min. The sample weighed between 8 and 12 mg and was heated in an aluminium crucible with a heating rate of 10 °C/min from room temperature to 600 °C.

## RESULTS AND DISCUSSION

### Yield of the Hemicelluloses

In order to separate the lignocellulosic material into its components, the extraction of hemicelluloses from the regenerated carbohydrate-enriched residues was carried out at 75 °C for 3 h. The yields of regenerated materials were 81.7, 82.2, 87.5, 85.7, 81.5, and 86.5%, corresponding to the raw materials pretreated with [BMIM]Ace/1,4-dioxane, [BMIM]Ace/DMSO, [BMIM]Ace/methanol, [BMIM]Ace/acetone, [BMIM]Ace/DMF, and fresh [BMIM]Ace, respectively. The yields of hemicelluloses (on a dry-weight basis of the *Eucalyptus* wood) are listed in Table 1. As shown, the yields of hemicellulosic fractions were 16.8, 17.5, 20.0, 19.8, 16.1, and 17.3% of the initial *Eucalyptus*, corresponding to the percentage of 61.1, 63.6, 72.7, 72.0, 58.5, and 62.9% of the total original hemicelluloses, respectively. The presence of some organic solvents affected the extraction efficiency, probably due to effects on the delignification efficiency of pretreatment and the yield of the recovered wood flour. It should be noted that $H_3$ presented the highest yield of hemicelluloses, suggesting that pretreatment with the [BMIM]Ace/methanol system was conducive to the swelling of the cell walls, and then enhanced the dissolution of hemicelluloses and lignin in the following alkaline ethanol extraction processes. Using the ionic liquid [BMIM]Ace combined with organic solvents as a pretreatment, the yields of the hemicellulosic fractions separated from the carbohydrate-enriched residues were relatively higher than that extracted from the untreated feedstock (Sun *et al.* 2011). This can be explained by the fact that the recovered carbohydrate-enriched residues from ionic liquid/organic solvents pretreatment of wood have a high surface area, resulting in more extensive delignification. This disrupted and removed the cross-linked matrix of lignin and hemicelluloses that was embedded in the cellulose fibers. Furthermore, extensive hydrogen bonding between the individual

polysaccharide cell wall components will hinder the extraction of hemicelluloses. However, the hydrogen bonding can be broken during the pretreatment process. Ionic liquid pretreatment removed more lignin and fewer hemicelluloses than dilute acid pretreatment, producing recovered material with lower levels of residual lignin and higher levels of residual xylan (Li *et al.* 2010b; Mora-Pale *et al.* 2011). Accordingly, extraction using an organic alkaline solution can liberate greater amounts of hemicelluloses from the regenerated carbohydrate-enriched residues because the alkali may break some linkages between lignin and polysaccharides, or between lignin and hydroxyl-cinnamic acid, such as *p*-coumaric and ferulic acids. Acidic moieties, such as carboxylic or phenolic groups ionized in the alkaline solution, might promote solubilisation of the hemicelluloses due to swelling of the cell walls (Scalbert *et al.* 1986).

**Table 1.** Yield of the Hemicellulosic Fractions (%, dry matter)

| | Hemicellulosic fraction* | | | | | |
|---|---|---|---|---|---|---|
| | $H_1$ | $H_2$ | $H_3$ | $H_4$ | $H_5$ | $H_6$ |
| Yield (%) | 16.80 | 17.50 | 20.05 | 19.84 | 16.10 | 17.35 |

* Represents the hemicellulosic fractions extracted from partially delignified *Eucalyptus* wood based on pretreatment using [BMIM]Ace/1,4-dioxane, [BMIM]Ace/DMSO, [BMIM]Ace/methanol, [BMIM]Ace/acetone, [BMIM]Ace/DMF, and fresh [BMIM]Ace

## Content of Neutral Sugars and Uronic Acids

The neutral sugars and uronic acids of the six hemicellulosic preparations, expressed as a relative percentage of total sugars and uronic acids, are listed in Table 2. The results indicated that the hemicelluloses isolated from *Eucalyptus* wood consist of arabinose, galactose, glucose, xylose, mannose, and glucuronic acid. Evidently, the neutral sugar analysis of the hydrolysates showed that xylose (63.25 to 74.85%) was the main sugar of the six hemicellulosic fractions. Uronic acid (10.38 to 15.63%), mainly glucuronic acid (Glc*p*A) or 4-*O*-methyl-glucuronic acid (4-*O*-Me-α-D-Glc*p*A), was present in a substantial amount. Arabinose (0.80 to 2.36%), galactose (4.49 to 7.32%), and mannose (0.89 to 3.75%) were present in small amounts. It should be noted that considerable amounts of glucose (4.85-14.40%) were also observed in the hemicellulosic fractions. These results were quite different from the sugar composition of hemicelluloses extracted with DMSO from hybrid poplar wood (Zhang *et al.* 2011), in which the contents of glucose and mannose were considerably higher. This apparent difference was probably caused by the dissolution and elimination of glucomannans during the pretreatment process, since glucomannans are easily dissolved in hot water due to their relatively lower molecular weight (Teleman *et al.* 2003).

As previously reported, the xylose-to-arabinose ratio is indicative of the degree of linearity or branching of the hemicellulosic fraction (Wedig *et al.* 1987). According to the results, a high xylose-to-arabinose ratio would indicate a high degree of polymerization with little bonding to other monosaccharide constituents. Moreover, the ratios of uronic acids to xylose (0.16-0.22) of the six hemicellulosic fractions revealed that the alkali-soluble hemicelluloses consisted of a relatively low content of substituted xylans, which was beneficial for enzymatic saccharification because many xylanases do not cleave glycosidic bonds between xylose units that are highly substituted (Lee and Forsberg 1987). In short, the predominance of xylose and the substantial amount of uronic acids

indicated that these hemicellulosic fractions mainly consisted of glucuronoxylans. The structure and the broad range of Xyl/GlcA ratios (0.16-0.22) are similar to the hemicelluloses in hardwoods (0.10 to 0.25) (Ebringerova *et al.* 2005). Furthermore, the presence of glucose and mannose indicated that small amounts of glucomannans also existed in the hemicellulosic fractions. Sugar analysis showed that the monosaccharide composition differed only slightly among the six samples. The phenomenon was regarded as a direct consequence of partial delignification during pretreatment in this experiment. Further evidence through additional experimentation is required to support this hypothesis.

**Table 2.** Neutral Sugars, Uronic Acids, and Uro/Xyl Ratios of the Hemicellulosic Fractions*

| Sugar (%) | Hemicellulosic fraction** | | | | | |
|---|---|---|---|---|---|---|
| | $H_1$ | $H_2$ | $H_3$ | $H_4$ | $H_5$ | $H_6$ |
| Arabinose | 1.21 | 0.80 | 2.36 | 0.98 | 1.59 | 0.89 |
| Galactose | 5.06 | 4.49 | 5.97 | 5.12 | 5.88 | 7.32 |
| Glucose | 4.85 | 13.97 | 7.14 | 14.33 | 5.95 | 14.40 |
| Xylose | 74.85 | 67.60 | 70.81 | 66.37 | 70.00 | 63.25 |
| Mannose | 0.89 | 0.91 | 0.97 | 1.21 | 0.95 | 3.75 |
| Uronic acids | 13.13 | 12.23 | 12.75 | 11.99 | 15.63 | 10.38 |
| Uro/Xyl *** | 0.18 | 0.18 | 0.18 | 0.18 | 0.22 | 0.16 |

*Relative % hemicellulosic sample, w/w
** Corresponding to the hemicellulosic fractions in Table 1
*** Uro/Xyl, uronic acids/xylose

## Average Molecular Weight Determination

Molecular weight is an important parameter used to evaluate the physicochemical properties of polysaccharides, such as solubility, rheological, and thermal properties. It is useful in the exploration of the full potential of hemicelluloses as hydrocolloids (Zaleha *et al.* 2008). Generally, polysaccharides with molecular weights in excess of DPn 50 and polydispersities below 3 are indicative of molecularly uniform polymers with potential commercial utility (Glasser *et al.* 2000). In order to investigate the extent of degradation that occurred during the process of extraction, all six hemicellulosic fractions were analyzed by their weight-average ($M_w$) and number-average ($M_n$) molecular weights, as well as polydispersity ($M_w/M_n$).

The results are summarized in Table 3. It was evident that the molecular weight (49.330-60.760 g/mol) of these hemicelluloses showed a higher degree of polymerization compared with the organosolv hemicelluloses (Zhang *et al.* 2011). This result was probably caused by alkali-enhanced dissolution of polysaccharides with large molecule sizes. The reason for this difference takes both the variety of materials and different treatments into consideration. Furthermore, the hemicellulosic fraction $H_3$ had a broader molecular weight distribution, corresponding to a polydispersity index of 1.81, compared to the other hemicellulosic fractions (1.67-1.76). To be more specific, the molecular weight distribution curves showed that $H_4$ exhibited a small peak in the low molecular weight region while $H_6$ had no peak in this region. This result was in agreement with the values of $M_w$: 49.330 g/mol for $H_1$ and 60.760 g/mol for $H_6$. The polydispersity values ranged from 1.67 to 1.81, which illuminated the structural heterogeneity of the isolated hemicelluloses. On the basis of molecular weight and polydispersity index, most hemicellulosic fractions with relatively uniform structures that were released by fractional extractions from *Eucalyptus* wood possess potential commercial utility.

**Table 3.** Weight-average ($M_w$) and Number-average ($M_n$) Molecular Weights and Polydispersity ($M_w/M_n$) of the Hemicellulosic Fractions

| | Hemicellulosic fraction* | | | | | |
|---|---|---|---|---|---|---|
| | $H_1$ | $H_2$ | $H_3$ | $H_4$ | $H_5$ | $H_6$ |
| $M_w$ | 51960 | 54480 | 59825 | 49330 | 56195 | 60760 |
| $M_n$ | 31035 | 30920 | 33055 | 28920 | 32180 | 35430 |
| $M_w/M_n$ | 1.67 | 1.76 | 1.81 | 1.71 | 1.75 | 1.72 |

* Corresponding to the hemicellulosic fractions in Table 1

## FT-IR Spectra

Infrared spectroscopy is a widely used method for the determination of molecular structure, identification of compounds in biological samples, and investigation of complex polymers. In addition, it can be applied to explore structural features when combined with chemical methods or other spectrum approaches, such as NMR spectroscopy. The most pronounced FT-IR spectral features differentiating the polysaccharides are found in the 1160-1150 and the 1000-960 $cm^{-1}$ regions, which can also be used to distinguish the non-reducing sugars from the reducing sugars (Kacurakova and Wilson 2001). Most of the absorption peaks were assigned according to data presented in the literature (Sun *et al.* 2011).

As shown in Fig. 3, the six alkali ethanol-soluble hemicelluloses from ionic liquid pretreatment displayed very similar spectra. The spectra showed the typical signal pattern for a hemicellulosic moiety. The similarities in the polysaccharide finger-print region (1200 to 950 $cm^{-1}$) suggested that the hemicellulose samples contained similar types of sugars. The 1500 to 1200 $cm^{-1}$ region is characteristic of many noncrystalline polysaccharides whose carbon, hydrogen, and O-H bending absorption bands are broad and overlap (Marchessault and Liang 1962). The prominent signal observed around 3356 $cm^{-1}$ was assigned to the –OH stretching vibrations and hydrogen bonding of the hemicelluloses. The band at 2918 $cm^{-1}$ relates to the C–H stretching of methyl and methylene groups. The presence of the uronic acid was also manifested mainly by the appearance of antisymmetric and symmetric stretching from the –COO group at 1602 and 1414 $cm^{-1}$, respectively.

In addition, the occurrence of a small peak at 1461 $cm^{-1}$ is complex. It is influenced by $CH_2$ bending in xylan as well as $CH_3$ deformation (asymmetric) in lignin, suggesting that minor amounts of bound lignin were present in the isolated hemicellulosic fractions. The main absorptions are characteristic of glycosidic structures at 1155 and 1041 $cm^{-1}$ for the antisymmetric bridge C–O–C and C–O stretching, respectively (Roy *et al.* 1991). Evidently, the presence of an arabinosyl side chain was documented by the shoulder peak at 980 $cm^{-1}$, which was reported to be attached only to the xylopyranosyl constituents (Kačuráková *et al.* 1994). Moreover, signals for O–H in-plane bending occurred at 1318 $cm^{-1}$ and 1248 $cm^{-1}$.

The spectral pattern in the 1200 to 800 $cm^{-1}$ region gave information about the types of polysaccharides present. The intensive absorbance at 1041 $cm^{-1}$ was assigned to the C–O–C stretching of glycosidic linkages, which is typical of xylans (Sun *et al.* 2002). A small but sharp band at 896 $cm^{-1}$, which was assigned to the C-1 group frequency or ring frequency, is typical of β-glycosidic linkages between sugar units in all hemicellulosic fractions.

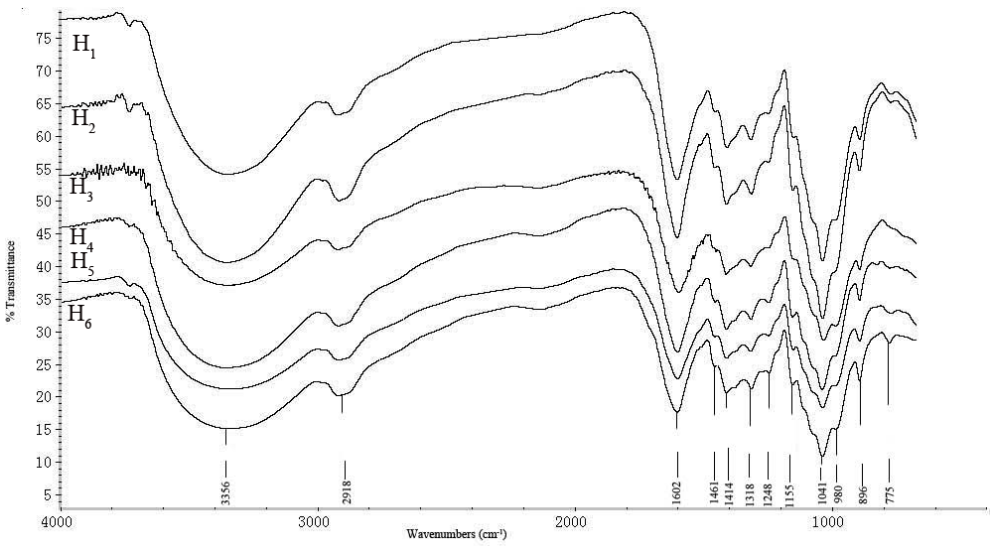


**Fig. 3.** FT-IR spectra of the hemicellulosic fractions $H_1$, $H_2$, $H_3$, $H_4$, $H_5$, and $H_6$

## 1D and 2D-HSQC NMR Spectra

NMR spectroscopy has a much higher resolution that enables a larger amount of information on high molecular weight polysaccharides to be obtained. To further elucidate the structural characterization of the extracted polymers, the hemicellulosic preparation $H_1$ with the highest xylan content was studied using 1D ($^{13}$C NMR) and 2D (HSQC) NMR spectroscopy. The 1D and 2D experiments caused chemical shifts of all of the carbons and protons in the major structural features of the polysaccharides. The $^{13}$C and HSQC spectra of the hemicellulosic fraction $H_1$ are shown in Fig. 4, and 5, respectively. The signals for $^{13}$C NMR were assigned on the basis of the HSQC spectra and previous literature.

*$^{13}$C NMR spectrum*

The $^{13}$C NMR spectrum allows elucidation of the polymer backbone and can also be employed to evaluate the types of side-chain branching along the backbone. It should be noted that there were five dominating signals at δ 102.39, 73.37, 74.99, 75.97, and 63.34 ppm, corresponding to C-1, C-2, C-3, C-4, and C-5 of β-D-Xyl*p* units, respectively (Gonzaga *et al.* 2005). The C-4 signal of the internal xylose units shifted to 75.97 ppm, thus confirming the involvement of this carbon in the (1→4)-linkage with β-D-xylose residues (Kardosova *et al.* 1998). The signal at 102.39 ppm is due to the anomeric carbons of β-configuration, as confirmed by the $^{1}$H NMR spectrum. The signal at 97.52 ppm corresponds to the anomeric carbons of 4-*O*-Me-α-D-Glc*p*A. Moreover, four interesting signals very close to the β-D-Xyl*p* units were observed. They appeared at 76.61, 71.92, and 74.66 ppm. It was deduced that these signals should be attributed to C-4, C-2, and C-3 of (1→4)-β-D-Xyl*p*-2-*O*-(4-OMe-α-D-Glc*p*A), respectively. The differences in chemical shifts of the NMR resonances between the (1→4)-linked β-xyl*p* units and (1→4)-β-D-Xyl*p*-2-*O*-(4-OMe-α-D-Glc*p*A) units can be explained by the formation of intra-molecular and inter-molecular hydrogen bonds in the β-D-Xyl*p* units. These hydrogen bonds reduce the probability density of electron cloud around the nucleus and engender a deshielding effect, which eventually makes for the differences in chemical shifts (Wen *et al.* 2011). In addition to the two signals of lower intensities occurring at 100.42 and 97.52 ppm characteristic for anomeric atoms, the signal in the

lowest field at 173.07 (C=O) ppm and the high-field resonance at 23.44 ($CH_3$) ppm indicate the presence of O-acetyl groups in the polysaccharide (Peter *et al.* 2002). The resonances of low intensity in the spectrum for arabinose residues occurred at 106.63 (C-1) ppm and glucose residues occurred at 103.73 (C-1) ppm. In addition to the aforementioned signals, signals at 177.07, 82.66, and 59.56 ppm were attributed to the C-6, C-4, and the methoxyl group of the 4-*O*-methyl-D-glucuronic acid residues (Vignon and Gey 1998).

*HSQC NMR spectrum*

The minor signals in the 1D NMR spectra were not able to be assigned due to overlapping. In order to confirm the detailed structural features of the polymers, direct correlations between hydrogen and carbon atoms of the glycosyl residues of $H_1$ were observed through the characterization cross-peaks in a HSQC spectrum by performing a 2D HSQC experiment. In the HSQC spectrum, the $^{13}C/^{1}H$ cross-peaks at 102.39/4.41, 73.37/3.23, 74.99/3.50, 75.97/3.70, 63.34/3.31 (H-$5_{ax}$), and 4.04 (H-$5_{eq}$) correspond unambiguously to C-1, C-2, C-3, C-4, and C-5 of the β-Xyl residues, in which chemical shifts of 3.31 and 4.04 ppm were assigned to the axial and equatorial protons linked at C-5, respectively. This confirmed that (1→4)-β-D-Xyl*p* is linked β-glycosidically, which is consistent with the presence of a small, sharp peak at 896 $cm^{-1}$ in the FT-IR spectrum of the $H_1$ fraction. Additionally, signals at 59.56/3.37 and 62.0/3.73, corresponding to $OCH_3$ and C-5 of the L-Ala*f* residues were also observed. All of the cross-peaks that can be clearly observed in the HSQC spectrum showed prominent signals corresponding to (1→4)-β-D-Xyl*p* linkages (blue color in Fig. 5), as well as signals of (1→4)-β-D-Xyl*p*-2-*O*-(4-OMe-α-D-Glc*p*A) linkages (red color). Signals of 4-OMe-α-D-Glc*p*A linkages can also be observed (green color). On the basis of the experimental data obtained from sugar analysis, FT-IR, and NMR spectra, it was demonstrated that $H_1$ is formed by a linear backbone of six (β-1→4)-xylopyranosyl residues and at least one of the xylose residues is monosubstituted at *O*-2 by a 4-*O*-methylglucuronic acid as a side chain, giving a typical ratio of 4-*O*-methylglucuronic acid to Xyl of 1 to 5. Moreover, because of the content of carboxyl functions, $H_1$ can be classified as an acidic xylan.

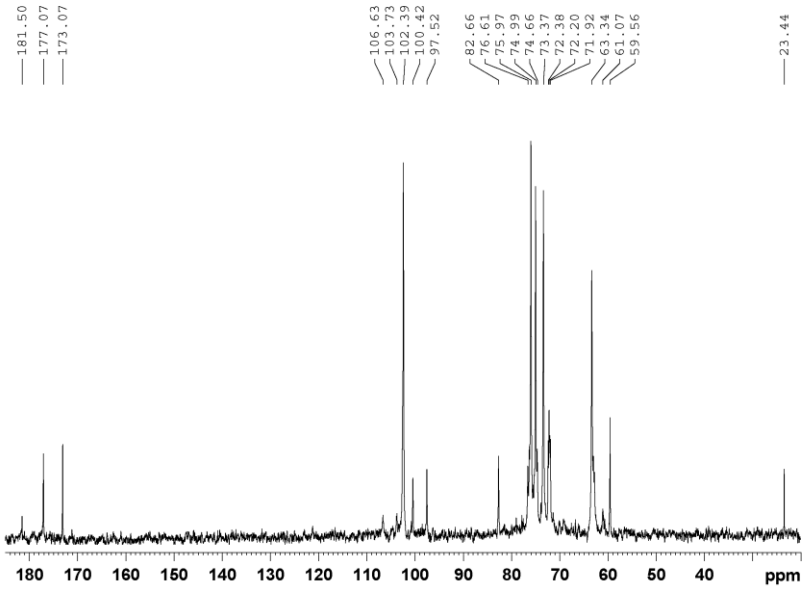


**Fig. 4.** $^{13}C$ NMR spectrum of the hemicellulosic fraction $H_1$

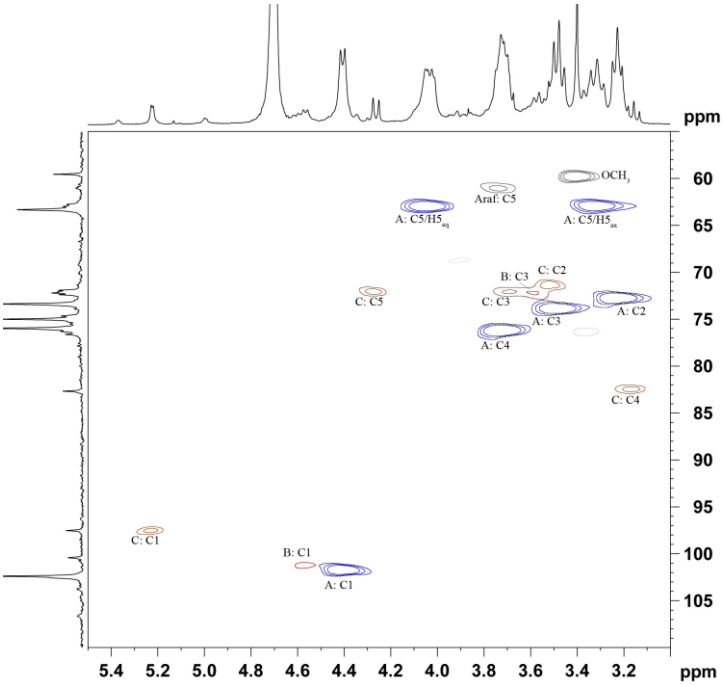


**Fig. 5.** HSQC spectrum of the hemicellulosic fraction $H_1$. A: (1→4)-β-D-Xyl*p* (shown in blue), B: (1→4)-β-D-Xyl*p*-2-*O*-(4-OMe-α-D-Glc*p*A) (shown in red), C: 4-OMe-α-D-Glc*p*A (shown in green)

## Thermal Analysis

Studying the thermal properties of hemicelluloses is very important in order to estimate their application for reinforced polymer composites because the processing temperature for many polymeric materials exceeds 100 °C. Thermal analysis is also valuable for the characterization of the physicochemical properties of macromolecules such as polysaccharides. In this work, the degraded hemicellulosic polymers were investigated using thermogravimetric analysis (TGA) and derivative thermogravimetry (DTG).

In comparison with other wood components, it has been reported that hemicelluloses are less thermally stable than cellulose and lignin (Ramiah 1970). However, little work has been published on this subject, particularly on the degradation stages and mechanisms of hemicelluloses. It is important to distinguish the behavior of *Eucalyptus* hemicelluloses for a better understanding of the pyrolysis process and to control the thermal decomposition process to make high value-added products.

In the present experiment, the two alkaline hemicellulosic fractions $H_3$ and $H_6$ were comparatively studied. The pyrolysis characteristics, from both TGA (%) and DTG (%/min) curves of the hemicellulosic fractions $H_3$ and $H_6$, are shown in Fig. 6. Both TGA and DTG curves of the pyrolysis process appeared to be divided into four weight loss stages. The first stage ranged from 30 °C to 149 °C. Both hemicellulosic fractions started decomposition similarly; the first weight loss for $H_3$ was 9.27% and it was 6.84% for $H_6$, mainly due to the removal of moisture when the preparations were heated. Further, the weight loss recorded for $H_3$ was 3.91% at 64.5 °C (1.64%/min) and 2.33% at 54.6 °C (1.07%/min) for $H_6$. The results confirmed the difference in moisture content of the two hemicelluloses. The second weight loss stage was between 149 °C and 243 °C for $H_3$, corresponding to around 5.29% of the total weight loss. For $H_6$, this stage ranged from 149 °C to 242 °C and corresponded to 4.78% of the total weight loss, which was

interpreted as being due to the pyrolysis of some of the lower molecular weight components (Yang *et al.* 2006). As seen from Fig. 6, the most significant degradation existed within a relatively narrow temperature range.

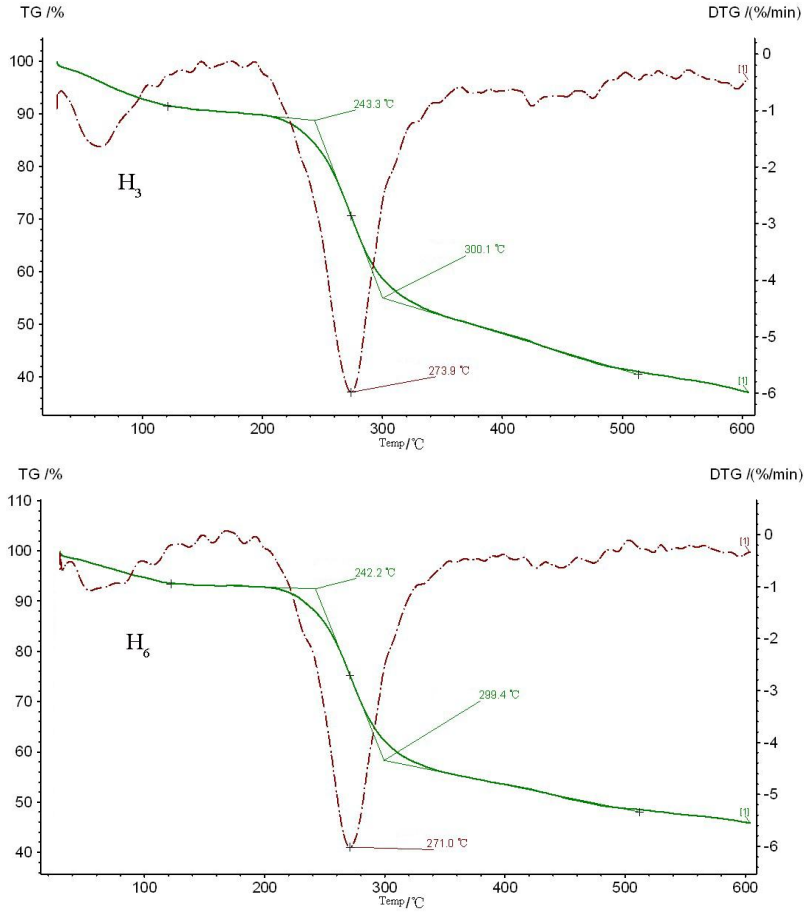


**Fig. 6.** TGA/DTG curves of the hemicellulosic fractions $H_3$ and $H_6$

The third weight loss stage occurred mainly within the range 243 to 300 °C, and the maximum mass loss rate (5.98%/min) was achieved at 273.9 °C for $H_3$. Similarly, for $H_6$ the third stage started at 242 °C and ended at 299 °C, and maximum mass loss rate (6.01%/min) occurred at 271 °C. In the third stage, 25.7% weight was pyrolyzed for $H_3$ and 26.1% for $H_6$, during which most of the hemicellulosic preparations were decomposed by a set of chemical reactions (depolymerization, hydrolysis, oxidation, *etc.*). The release of gaseous products was detected, such as $CO_2$, $CH_4$, CO, and some organics (a mixture of acids, aldehydes, alkanes, ethers, *etc.*) with $H_2O$. The release of $CO_2$ was mainly caused by the functional groups of carboxyl and COOH cracking and reforming (Ramiah 1970).

The last weight loss stage was found to decay smoothly from about 300 °C to the final temperature of 600 °C. There was still 37.16% solid residue remaining for $H_3$ and 45.85% for $H_6$, which could be explained by the combustion of volatile degraded components and the formation of a char residue that is oxidized in a subsequent stage (Shukry *et al.* 1991). The thermal properties of these two hemicellulosic fractions were very similar.

### Recovery and Recycling of IL

A major disadvantage in using IL as a pretreatment solvent for lignocellulosic biomass is its relatively high cost. Although many processes have been developed to decrease the production cost of ILs, typical ILs remain expensive. Therefore, the recovery and recycling of the solvent is very important for industrial utilization in light of environmental and economic concerns. In the present study, after the carbohydrate-enriched residue was regenerated from the IL solution, water and organic solvent was removed by evaporation under reduced pressure. The resulting liquor was dried at 75 °C under vacuum for 24 h to obtain recycled IL. The yield of the recycled [BMIM]Ace was around 95 to 99%. In addition, the colorless [BMIM]Ace became amber after recycling. The color was probably due to contaminants present in [BMIM]Ace, which could be degraded from [BMIM]Ace and lignocelluloses or even introduced with water, chemicals, or other solvents (Li *et al.* 2010b). Compared with that in the fresh [BMIM]Ace, the dissolution and regeneration of wood in the recycled [BMIM]Ace was still achieved within 3 h. However, the yield of regenerated wood dropped from 96.47% to 90.52% for the 4th recycle, indicating some loss in efficiency, which was probably due to the degradation of carbohydrate catalyzed with the enriched organic contaminants in the recycled IL. Similar decreased pretreatment efficiency of lignocelluloses has been reported in recycled [AMIM]Cl (Li *et al.* 2010b). These results indicated that more practical and effective methods for IL recycling should be further investigated in order to enhance the pretreatment efficiency of lignocelluloses.

## CONCLUSIONS

1. Based on partial delignification pretreatment using ionic liquid/organic solvents, successive extraction with an alkaline ethanol solution was found to be an effective technique for separating hemicelluloses from carbohydrate-enriched residue. However, the advantages were countered by the higher cost associated with the ionic liquid. This pretreatment technology should be explored and developed further.
2. It was found that xylose (63.25-74.85%) was the major sugar component. Small amounts of glucose (4.85-14.40%) and galactose (4.49-7.32%) were also observed in the isolated hemicelluloses.
3. It can be concluded that the alkali-soluble hemicellulosic fraction consisted of a linear backbone of (β-1→4)-xylopyranosyl residues and was relatively low substituted, which was beneficial for the enzymatic saccharification. From the results of FT-IR and NMR spectra, it was suggested that $H_1$ is formed by a linear backbone of six (β-1→4)-xylopyranosyl residues and at least one of the xylose residues is monosubstituted at *O*-2 by a 4-*O*-methylglucuronic acid as a side chain, giving a typical ratio of 4-*O*-methylglucuronic acid to xylose of 1 to 5.
4. Thermal analysis also showed that hemicelluloses were easily degraded, and the most significant degradation occurred from 242 to 300 °C.
5. Furthermore, the delignification efficiency in ionic liquid pretreatment suggests a promising option for recovering and converting lignin to a valuable commercial co-product.

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