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Fractional isolation, physico-chemical characterization and homogeneous esterification of hemicelluloses from fast-growing poplar wood

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Abstract

Extraction of the dewaxed and partially delignified fast-growing poplar wood with 1.5, 3.0, 5.0, 7.5 and 8.5% NaOH at 20°C for 16 h solubilized 65.6, 71.6, 73.8, 85.6 and 89.3% of the original hemicelluloses, respectively. Xylans were found to be the predominant hemicellulosic components in the cell walls of fast-growing poplar wood. The isolated five hemicellulosic preparations were further characterized by Fourier transform infrared (FT-IR), and carbon-13 magnetic resonance spectroscopy (^{13}C NMR) as well as gel permeation chromatography (GPC). The results showed that the hemicelluloses contain a main chain formed by D-xylopyranose residues linked by β-1,4 linkages.

The hemicellulosic preparation, isolated with 8.5% NaOH at 20°C for 16 h, was esterified with propionyl, hexanoyl, lauroyl, and palmitoyl chlorides, respectively, in homogeneous *N*,*N*-dimethylformamide/lithium chloride system using 4-dimethylaminopyridine as a catalyst and triethylamine as a neutralizer. The effects of molar ratio of acyl chloride/anhydroxylose unit, triethylamine concentration, and reaction time and temperature on the yield and degree of substitution of the hemicellulose derivatives have been primarily examined. The degree of substitution was controlled between 0.34 and 1.44 under the reaction conditions given. Under an optimum condition (sample 8, molar ratio 3:1), over 70% of the total hydroxyl groups in native hemicelluloses were palmitoylated at 75°C for 40 min. The results obtained from the molecular weight measurements (48 000–127 200 g mol^{-1}) revealed only a minimal degradation of the macromolecular hemicelluloses during the rapid reactions at 60–75°C for 25–40 min. © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: Fast-growing poplar wood; Alkali extraction; Hemicelluloses; Esterification; Homogeneous system

1. Introduction

More than 1.5-billion m^3 of woody plants are consumed by pulp and paper production and timber each year in the world (Kim, Kurahashi & Meshitsuka, 1996). This consumption still is increasing continuously, and this rapidly growing demand for paper in the last few years has not been fully met by the substitute products introduced lately, which may eventually result in massive deforestation of the planet (Patel, Angadiyavar & Srinivasa, 1985). One possible solution to this problem is to develop more useful woody plants by breeding and manipulation of genes. The developments of fast-growing woody plants is one of the achievements of investigations (Kim et al., 1996). These fast-growing poplar trees, developed by hybridization of normal poplar trees then bred successively by grafting, have been noticed widely from the viewpoint of an important renewable resource. The knowledge about lignin components of these woods has recently been investigated by Kim, Iiyama, Kurahashi and Meshitsuka (1995) and Kim et al. (1996). However, there is no information of their polysaccharide components, particularly hemicelluloses, nor of chemical modification of the hemicelluloses as novel materials for industrial use.

During the last 10 years, our research group has been involved in isolation, characterization, and modification of polysaccharides including starch from cereal crops and hemicelluloses from cereal straws (Lawther, Sun & Banks, 1995; Sun, Fang, Goodwin, Lawther & Bolton, 1998). Based on the uniform reactions of cellulose with control over the degree of substitution in homogeneous *N*,*N*-dimethylacetamide (DMAc)/lithium chloride (LiCl) system (McCormick & Callais, 1987; Williamson, Armentrout, Porter & McCormick, 1998), researchers in our laboratories investigated to find suitable reaction media to perform derivation reactions in homogeneous *N*,*N*-dimethylformamide (DMF)/LiCl system, in which the substitutions along

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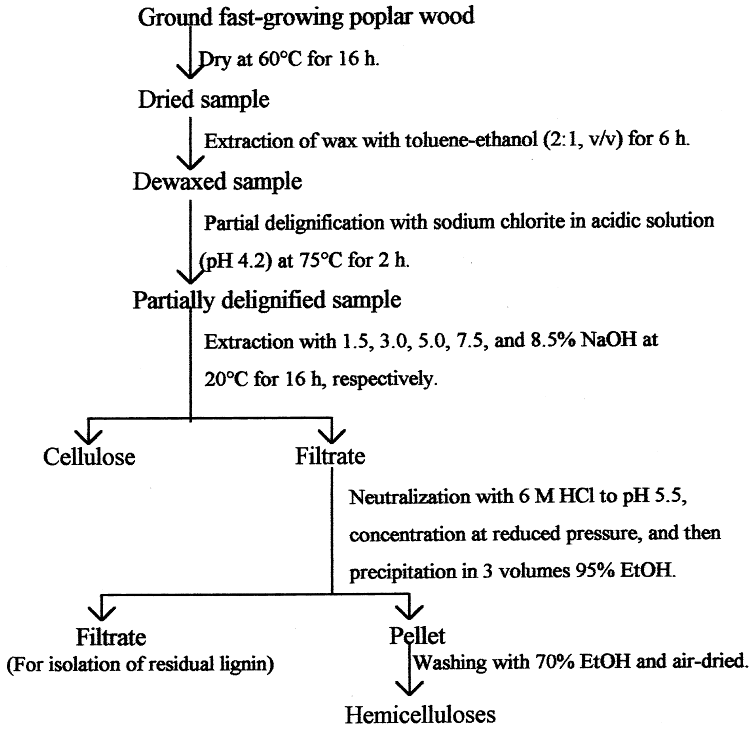

Fig. 1. Scheme for isolation of hemicellulose and cellulose from partially delignified fast-growing poplar wood.

the hemicellulosic backbone can be achieved with satisfactory yields and with little depolymerization of the hemicellulose chains. Up to that time, studies on chemical modification of hemicelluloses have been carried in heterogeneous and gel-like phase obtaining low degrees of substitution.

In this research, five preparations of hemicelluloses are obtained by extraction of the dewaxed and partially delignified fast-growing poplar wood with 1.5, 3.0, 5.0, 7.5 and 8.5% NaOH at 20°C for 16 h, respectively, and their physico-chemical properties are examined. Special attention is paid to their lignin association and composition. Furthermore, the hemicellulosic preparation, isolated with 8.5% NaOH, is esterified in homogeneous solution of DMF/LiCl. The products are characterized by yield of esterification, degree of substitution (DS), FT-IR spectroscopy, molecular size, and solubility.

2. Material and methods

2.1. Materials

Fast-growing poplar tree, 12 years old, was harvested in December of 1997, at the University Forest of the North-Western University of Agricultural and Forest Science and Technology (Yangling, P.R. China). After being peeled off the outer and inner barks and then chipped and dried, the chips were ground to pass through a 0.6–0.8 mm screen. The ground sample was further dried at 60°C for 16 h. Then the material was dewaxed by refluxing with toluene–EtOH (2:1, v/v) for 6 h in a Soxhlet apparatus. The dewaxed sample was then partially delignified with sodium chlorite at 75°C for 2 h in acidic solution (pH 4.2), adjusted by 10% acetic acid. After being filtered and extensively washed with distilled water and EtOH, the residue was dried in a cabinet oven with air circulation at 50–60°C for 16 h. The dried crude holocellulose was then kept at 5°C before alkali extraction. The composition (%, w/w) of the fast-growing poplar wood is 43.8% cellulose, 27.1% hemicelluloses, 23.3% lignin, 1.6% ash, and 2.0% wax on a dry weight basis. 4-Dimethylaminopyridine (DMAP) and triethylamine (TEA) were reagent grade. Anhydrous LiCl was used after drying at 130°C for 2 h. Other reagent grade chemicals including propionyl chloride (PC), hexanoyl chloride (HC), lauroyl chloride (LC) and palmitoyl chloride (PAC) were purchased from Sigma Chemical Company (England) and used as delivered.

2.2. Isolation and characterization of hemicelluloses

The scheme for isolation of hemicelluloses from the prepared crude holocellulose is shown in Fig. 1. The hemicelluloses were extracted with 1.5, 3.0, 5.0, 7.5 and 8.5% NaOH at 20°C for 16 h, respectively, from the above crude holocellulose with a liquor ratio of 1:18. The solubilized hemicelluloses were isolated by precipitation of the neutralized hydrolysate (pH 5.5) in 3 volumes of 95% EtOH. After filtration, the pellets of the hemicelluloses were washed with 70% EtOH and air-dried.

Table 1
Yield of hemicelluloses extracted with various concentrations of sodium hydroxide at 20°C for 16 h from the dewaxed and partially delignified fast-growing poplar wood

	NaOH concentration (%)				
Yield (%)	1.5	3.0	5.0	7.5	8.5%
Hemicelluloses	17.8	19.4	20.0	23.2	24.2
Residue	56.0	52.2	50.5	46.4	46.2

Hemicelluloses-bound saccharidic components in the fractions were firstly subjected to hydrolysis in 2 M trifluoroacetic acid for 2 h at 120°C. Trifluoroacetic acid was removed by vacuum evaporation at 40°C. The dried monosaccharides were then reduced with sodium borohydride in dimethyl sulphoxide and the resulting alditols acetylated using 1-methylimidazole as the catalyst. The neutral sugar composition of the isolated hemicellulosic preparations was determined by gas chromatography (GC) analysis of their alditol acetates (Blakeney, Harris, Henry & Stone, 1983). Alkaline nitrobenzene oxidation of the lignin associated in the isolated hemicelluloses was performed at 170°C for 3 h. The lignin content in hemicelluloses was calculated multiplying the yield of phenolics, obtained by nitrobenzene oxidation, by 1.96 (Sun et al., 1998). Methods of uronic acid analysis, determination of phenolic acids and aldehydes in nitrobenzene oxidation mixtures with high-performance liquid chromatography (HPLC), and measurement of the molecular weights of native hemicelluloses have been described in previous papers (Sun, Lawther & Banks, 1995, 1996).

FT-IR spectra were obtained on an FT-IR spectrophotometer using a KBr disc containing 1% finely ground samples. The solution-state ^{13}C NMR spectrum was obtained on a Bruker 400 AC spectrometer operating in the FT mode at 62.4 MHz under total proton-decoupled conditions. Spectra were recorded at 25°C from 180 mg of sample dissolved in 1.0 ml D_2O after 20 000 scans. A 60° pulse-flipping angle, a 3.9 μs pulse width and a 0.85 s acquisition time were used.

2.3. Esterification of hemicelluloses

To 30 ml of distilled water was added 0.6 g hemicelluloses (0.009 mol of hydroxyl functionality), extracted with 8.5% NaOH at 20°C for 16 h from the dewaxed and partially delignified fast-growing poplar wood. This mixture was heated to 60–80°C, with stirring, until the hemicelluloses completely dissolved (~5 min). Then a 30-ml volume of DMF was added and the reaction was stirred for another 5 min. The water was removed from the swollen gel by evaporation at diminished pressure at about 50°C. Then a solution of 0.15 g LiCl, 0.10 g DMAP, and corresponding moles of PC, HC, LC, and PAC together with required amounts of TEA (% of the native hemicelluloses, w/w), previously dissolved in 15 ml DMF, was added dropwise, and the homogeneous reaction mixture was stirred for a total period of 25, 30, 35 and 40 min at the temperatures given. The reaction was stopped at required times by cooling the reaction mixture with cold water. After being cooled to room temperature, the homogeneous reaction mixture was slowly poured into 120 ml of 95% EtOH with stirring. Organic solvents were separated from the precipitated products by dissolving in EtOH. The white product that separated from the solution was filtered off and collected. The filtrate was washed thoroughly with EtOH and acetone. The resulting product was purified by Soxhlet extraction with ethanol for 6 h followed by air drying for 24 h and then further dried in an oven at 55°C for another 16 h.

2.4. Characterization of the esterified hemicelluloses

The yield percentages were calculated based on the assumption that all of the hemicelluloses were converted to di-esterified hemicelluloses. In such a case, the yield percentage and the degree of substitution (DS) would be 100% and 2.0, respectively. The unreacted acyl chloride in the mixture of reactions was separated from the product by dissolving in 95% EtOH and acetone. If no reaction occurred and all of the hemicelluloses were recovered unreacted, the yield percentage would be 54.1% for propionylation, 40.2% for hexanoylation, 26.6% for lauroylation and 21.7% for palmitoylation, respectively.

The molecular-average weights of esterified hemicelluloses were determined by gel permeation chromatography (GPC) on a PLgel 5 μm Mixed-D column. The samples were dissolved in pyridine/LiCl (0.1%, w/w) at a concentration of 0.1%, and a 200 μl sample in solution was injected. The column was operated at 80°C and eluted with pyridine/LiCl at a flow rate of 1 ml min^{-1}. The column was calibrated using PL pullulan polysaccharide standards. The solubility was measured at 4.0% concentration in various organic solvents.

3. Results and discussion

3.1. Yield and sugar composition of the isolated hemicelluloses

During the alkaline treatment of the dewaxed and partially delignified fast-growing poplar wood, some alkali-labile linkages between lignin molecules, or between lignin and polysaccharides, might be broken by alkali. Acidic moieties such as carboxylic or phenolic groups, ionized in alkaline solution, might also promote the solubilization of the hemicelluloses and residual lignin, either by increasing the solubility of individual fragments or by inducing the swelling of the cell wall (Scalbert, Monties, Guittet & Lallemand, 1986). The effect of alkali concentration on the yield of released hemicelluloses during the treatment at 20°C for 16 h is given in Table 1. As expected, increase of

Table 2
The content of neutral sugars (relative % dry matter) and uronic acids (% dry matter) in hemicellulosic preparations

Sugars/uronic acids	NaOH concentration (%) 1.5	3.0	5.0	7.5	8.5%
Rhamnose	1.64	1.62	1.58	1.58	2.15
Arabinose	1.03	1.25	0.91	0.90	1.03
Xylose	88.79	88.15	86.98	79.11	78.65
Mannose	2.23	3.15	3.63	9.29	9.03
Glucose	3.52	3.68	4.70	7.21	7.21
Galactose	2.79	2.15	2.20	1.91	1.93
Uronic acids	12.25	10.00	9.70	9.12	8.38

alkali concentration resulted in an increment of the solubilized hemicelluloses. As shown in Table 1, treatment with 1.5, 3.0, 5.0, 7.5 and 8.5% NaOH at 20°C for 16 h removed 65.6, 71.6, 73.8, 85.6 and 89.3% of the original hemicelluloses from the crude holocellulose, respectively. As a result, the yield of residues decreased from 56.0 to 46.2% as the alkali concentration increased from 1.5 to 8.5% during the treatment under the conditions given. This phenomenon indicated that increase of alkali concentration favoured the release of hemicelluloses. One can, therefore, predict that if the alkali concentration continues to be increased during the treatment, there will be more hemicelluloses released.

The composition of neutral sugars and content of uronic acid in the solubilized hemicellulosic preparations are shown in Table 2. Obviously, xylose was the predominant sugar component in all of the hemicellulosic preparations, comprising 78.7–88.8% of the total sugars. Mannose and glucose were present in small amounts, and rhamnose, arabinose, and galactose were observed as minor sugar constituents. This indicated that xylans are the predominant hemicelluloses in the cell walls of poplar wood. Similar results have been reported from aspen wood (Puls & Schuseil, 1993). The authors stated that xylans represented more than 90% of the hemicelluloses in aspen wood. Further studies found that these polymers are heterosaccharide-branched hemicelluloses, which consist of about 200 β-xylopyranose residues, linked together by 1,4-glycosidic bonds. An increase in alkali concentration from 1.5 to 8.5% resulted in a decrease of xylose from 88.8 to 78.7%, but an increase of mannose from 2.2 to 9.0% and glucose from 3.5 to 7.2% in the hemicellulosic preparations. These increases in mannose and glucose with the growth of alkali concentration implied that the poplar wood may also contain small amounts of glucomannan, which is favourably extracted at a relatively high concentration of alkali. The same result was observed for hardwoods, which consist of 3–5% glucomannan, linked by β-1,4-glycosidic bonds (Puls & Schuseil, 1993). In addition, as can be seen in Table 2, the content of uronic acids, mainly glucuronic acid or 4-*O*-methyl-glucuronic acid (MeGlcA) ranged between 8.4 and 12.3%. This observation implied that MeGlcA substituents were more labile with increasing alkali concentration.

Table 3
The composition of neutral sugars (relative % dry matter) in the alkali extracted residues

Sugars	NaOH concentration (%) 1.5	3.0	5.0	7.5	8.5%
Rhamnose	0.50	Tr[a]	Tr[a]	Tr[a]	Tr[a]
Xylose	13.22	9.79	7.83	5.64	4.68
Mannose	1.18	0.93	0.81	0.62	0.20
Glucose	84.50	87.78	90.82	93.53	95.12
Galactose	0.60	0.50	0.44	0.20	Tr[a]

[a] Tr = trace.

Table 4
The yield (% hemicelluloses, w/w) of phenolic acids and aldehydes from alkaline nitrobenzene oxidation of associated lignin in the hemicellulosic preparations

Phenolic acids and aldehydes	NaOH concentration (%) 1.5	3.0	5.0	7.5	8.5
p-Hydroxybenzoic acid	0.11	0.11	0.087	0.069	0.068
p-Hydroxybenzaldehyde	0.30	0.28	0.17	0.15	0.16
Vanillic acid	0.39	0.36	0.21	0.18	0.18
Syringic acid	0.12	0.12	0.094	0.075	0.073
Vanillin	0.92	0.89	0.67	0.55	0.53
Syringaldehyde	0.62	0.60	0.39	0.33	0.33
p-Coumaric acid	0.022	0.023	0.021	0.018	0.016
Ferulic acid	0.025	0.024	0.023	0.023	0.022
Total	2.51	2.36	1.49	1.35	1.34
Content of lignin	4.92	4.63	2.92	2.65	2.63

The residues after alkali treatment were mainly formed of cellulose, as evidenced by sugar analysis. The data in Table 3 indicated that glucose was the extremely predominant sugar component, comprising 84.5–95.1% of the total sugar constituents. An increase of alkali concentration from 1.5 to 8.5% led to a growth of cellulose rich in the residues as shown by an increment of glucose from 84.5 to 95.1% and a decrease of xylose from 13.2 to 4.7%, which corresponded to the increasing yield of hemicelluloses solubilized. The residual fraction, extracted with 8.5% NaOH, still contained a small amount of xylose (4.7%) and minor quantities of mannose (0.2%). These resistances to extraction with a relatively high concentration of NaOH for 16 h implied that the hemicelluloses in the cell walls of fast-growing poplar wood are very strongly associated to the surface of cellulose.

3.2. Content of associated lignin in isolated native hemicelluloses

To verify whether lignin fragments contaminate the isolated hemicellulosic preparations, all the hemicellulosic fractions were treated to determine the associated lignin by alkaline nitrobenzene oxidation at 170°C for 3 h. This

Table 5
The yield (% residues, w/w) of phenolic acids and aldehydes from alkaline nitrobenzene oxidation of associated lignin in the alkali extracted residues

Phenolic acids and aldehydes	NaOH concentration (%) 1.5	 3.0	 5.0	 7.5	 8.5
p-Hydroxybenzoic acid	0.22	0.20	0.20	0.16	0.14
p-Hydroxybenzaldehyde	0.14	0.14	0.12	0.082	0.080
Vanillic acid	0.15	0.14	0.10	0.088	0.092
Syringic acid	0.022	0.020	0.016	0.016	0.012
Vanillin	0.28	0.26	0.24	0.22	0.22
Syringaldehyde	0.14	0.14	0.12	0.11	0.080
p-Coumaric acid	0.068	0.062	0.056	0.040	0.042
Ferulic acid	0.066	0.068	0.060	0.044	0.046
Total	1.09	1.03	0.91	0.76	0.71
Content of lignin	2.14	2.02	1.78	1.49	1.39

method provided an estimate of the amount of associated lignin and an indication of its composition. Results concerning the characterization of lignin bound to hemicelluloses are summarized in Table 4. The data showed that all the hemicellulosic preparations contained relatively low amounts of associated lignins, ranging between 2.6 and 4.9%. This low content of bound lignin in hemicelluloses indicated that the α-benzyl ether linkages between lignin and hemicelluloses were significantly cleaved during the treatment by alkali under the conditions given. This cleavage is particularly true at the relatively high concentration of alkali, since an increase in concentration of alkali from 1.5 to 3.0, 5.0, 7.5 and to 8.5% resulted in a decrease in lignin content from 4.9 to 4.6, 2.9, 2.7 and to 2.6%, respectively. The major products, obtained from alkaline nitrobenzene oxidation, were identified to be vanillin and syringaldehyde, which together represented for 61.4–71.1% of the total phenolic monomers. A measurable higher yield of vanillin than syringaldehyde implied that the hemicelluloses in the cell walls of fast-growing poplar wood are tightly associated with lignin by guaiacyl units. A noticeable amount of *p*-hydroxybenzaldehyde and vanillic acid, and traces of syringic acid, *p*-hydroxybenzoic acid, *p*-coumaric acid, and ferulic acid were also found to be present in the nitrobenzene oxidation mixtures. In addition to *p*-hydroxybenzoic acid esterified to lignin, the occurrence of a significant amount of ether linked *p*-hydroxybenzoic acid, trace amounts of both *p*-coumaric and ferulic acids with ester- and ether-forms were also detected by Kim et al. (1995) from the cell walls of normal and fast-growing poplar woods.

Table 6
Weight-average ($\overline{M_{\mathrm{w}}}$) and number-average ($\overline{M_{\mathrm{n}}}$) molecular weights and polydispersity ($\overline{M_{\mathrm{w}}}/\overline{M_{\mathrm{n}}}$) of the native hemicellulosic preparation

	NaOH concentration (%) 1.5	 3.0	 5.07.5	 8.5%	
$\overline{M_{\mathrm{w}}}$	38 830	38 360	39 840	42 230	39 250
$\overline{M_{\mathrm{n}}}$	7330	7580	7040	5560	4910
$\overline{M_{\mathrm{w}}}/\overline{M_{\mathrm{n}}}$	5.3	5.1	5.7	7.6	8.0

Table 5 summarizes the content of residual lignin and its phenolic composition obtained by alkaline nitrobenzene oxidation from the alkali extracted residues. Clearly, a much lower lignin content (1.4–2.1%) was observed in the alkali extracted residues, indicating that sodium hydroxide under the concentration used is an effective agent for removing the residual lignins from partially delignified fast-growing poplar wood. As can be seen from Table 5, an increase of alkali concentration from 1.5 to 8.5% resulted in a decrease of residual lignin from 2.1 to 1.4% in the residues. Vanillin, *p*-hydroxybenzoic acid, *p*-hydroxybenaldehyde, vanillic acid, and syringaldehyde and vanillin were found to remain the major constituents of phenolics in the nitrobenzene oxidation of bound lignin from the residues. Based on the evidence of ferulic acid ester–ether bridges in cell walls of cereal straws and temperate grasses, the presence of noticeable amounts of *p*-hydroxybenzoic acid in the cell walls of fast-growing poplar wood may contribute to the regulation of cell wall elongation by the formation of crosslinking between lignins and/or between lignin and polysaccharides (Kim et al., 1995).

3.3. Molecular weight distribution of native hemicelluloses

The weight-average ($\overline{M_{\mathrm{w}}}$) and number-average ($\overline{M_{\mathrm{n}}}$) molecular weights and the polydispersity ($\overline{M_{\mathrm{w}}}/\overline{M_{\mathrm{n}}}$) of the isolated hemicellulosic preparations are listed in Table 6. The data showed that the extraction with an increase in NaOH concentration from 1.5 to 7.5% under the conditions given led to, in general, slight growing of $\overline{M_{\mathrm{w}}}$ from 38 830 to 42 230 g mol^{-1}, suggesting that increasing alkali concentration from 1.5 to 7.5% at least in part, increased dissolution of large molecular size hemicelluloses from the poplar wood. Conversely, as the alkali concentration was further increased to 8.5%, the $\overline{M_{\mathrm{w}}}$ decreased to 39 250 g mol^{-1}, indicating a slight degradation of the solubilized hemicelluloses during the treatment with 8.5% NaOH at 20°C for 16 h.

The molecular weight distribution of the hemicellulosic fraction, extracted with 5.0% NaOH at 20°C for 16 h from the dewaxed and partially delignified fast-growing poplar wood, is shown in Fig. 2. As can be seen from the diagram, the molecular weight distribution ranged between over two million and 840 g mol^{-1} with three peaks having the molecular weight of 281 190, 16 850 and 1320 g mol^{-1}, respectively. The distribution showed a wide polymolecularity. The third small peak had a relatively lower molecular weight value of around 1320 g mol^{-1}, which was probably due to the degradation of the released hemicelluloses during the extraction with a relatively higher concentration of alkali.

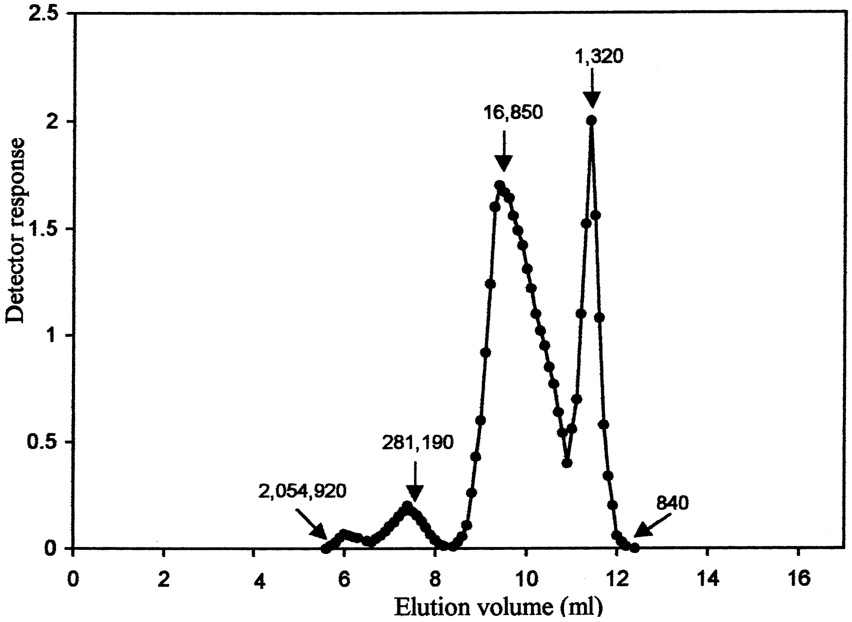

Fig. 2. GPC molecular weight distribution of the hemicellulosic preparation extracted with 5.0% NaOH at 20°C for 16 h from the dewaxed and partially delignified fast-growing poplar wood.

3.4. FT-IR spectra of native hemicelluloses

The FT-IR spectra of the four hemicellulosic preparations, extracted with 1.5% NaOH (spectrum a), 5.0% NaOH (spectrum b), 7.5% NaOH (spectrum c) and 8.5% NaOH (spectrum d) at 20°C for 16 h from dewaxed and partially delignified fast-growing poplar wood, appear in Fig. 3. The spectra were examined in the frequency range of 800–4000 cm^{-1}. The spectral profiles and relative intensities of the bands among the four spectra were rather similar, indicating similar structures of the hemicelluloses. The band at 1625 cm^{-1} is principally associated with absorbed water (Kacurakova, Belton, Wilson & Hirsch, 1998). The prominent absorption at 1045 cm^{-1} is attributed to the C–O, C–C stretching or C–OH bending in hemicelluloses (Kacurakova, Ebringerova, Hirsch & Hromadkova, 1994). The small sharp band at 897 cm^{-1} is characteristic of β-glycosidic linkage between the sugar units (Gupta, Madan & Bansal, 1987).

Fig. 4 shows the FT-IR spectra of four cellulosic preparations obtained by treatment the dewaxed and partially delignified fast-growing poplar wood with 1.5% NaOH

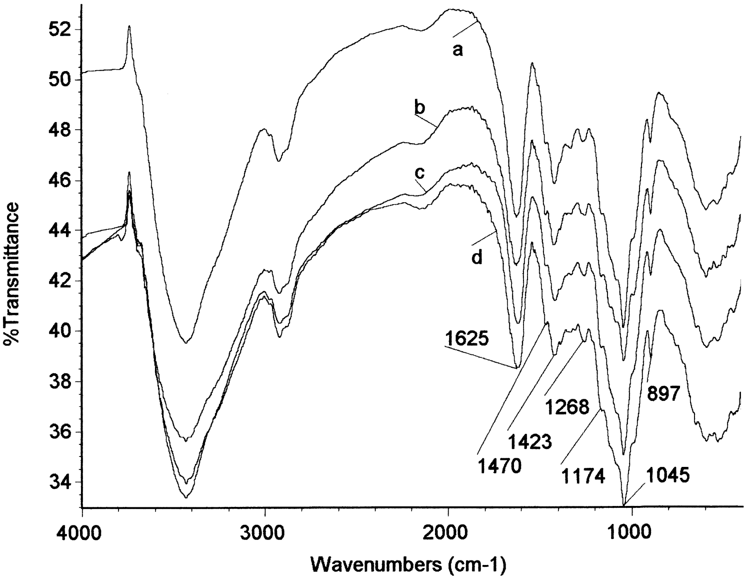

Fig. 3. The FT-IR spectra of hemicellulosic preparations extracted with 1.5% NaOH (spectrum a), 5.0% NaOH (spectrum, b), 7.5% NaOH (spectrum c), and 8.5% NaOH at 20°C for 16 h (spectrum d) from the dewaxed and partially delignified fast-growing poplar wood.

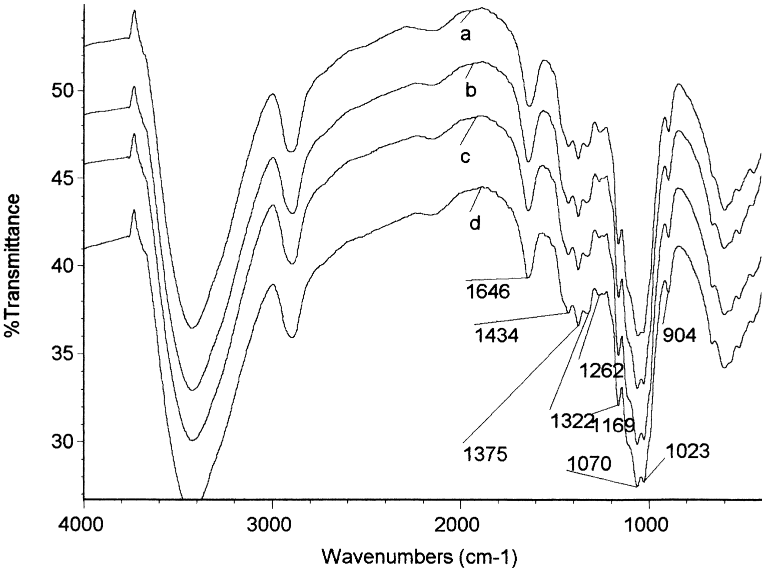

Fig. 4. The FT-IR spectra of cellulosic fractions obtained by treatment with 1.5% NaOH (spectrum a), 5.0% NaOH (spectrum b), 7.5% NaOH (spectrum c), and 8.5% NaOH at 20°C for 16 h (spectrum d) from the dewaxed and partially delignified fast-growing poplar wood.

(spectrum a), 5.0% NaOH (spectrum b), 7.5% NaOH (spectrum c) and 8.5% NaOH at 20°C for 16 h (spectrum d). Similar to the four spectra of hemicelluloses, no essential differences were encountered between the cellulosic spectra. All the spectra have an intense absorbed water-related absorbance at 1646 cm^{-1}. The absorbances at 1434, 1375, 1322, 1169, 1070, 1023 and 904 cm^{-1} in the four spectra are associated to the typical of cellulose.

3.5. ^{13}C NMR spectrum of native hemicelluloses

To get further information about the configuration of the glycosidic linkages, the ^{13}C NMR spectroscopic

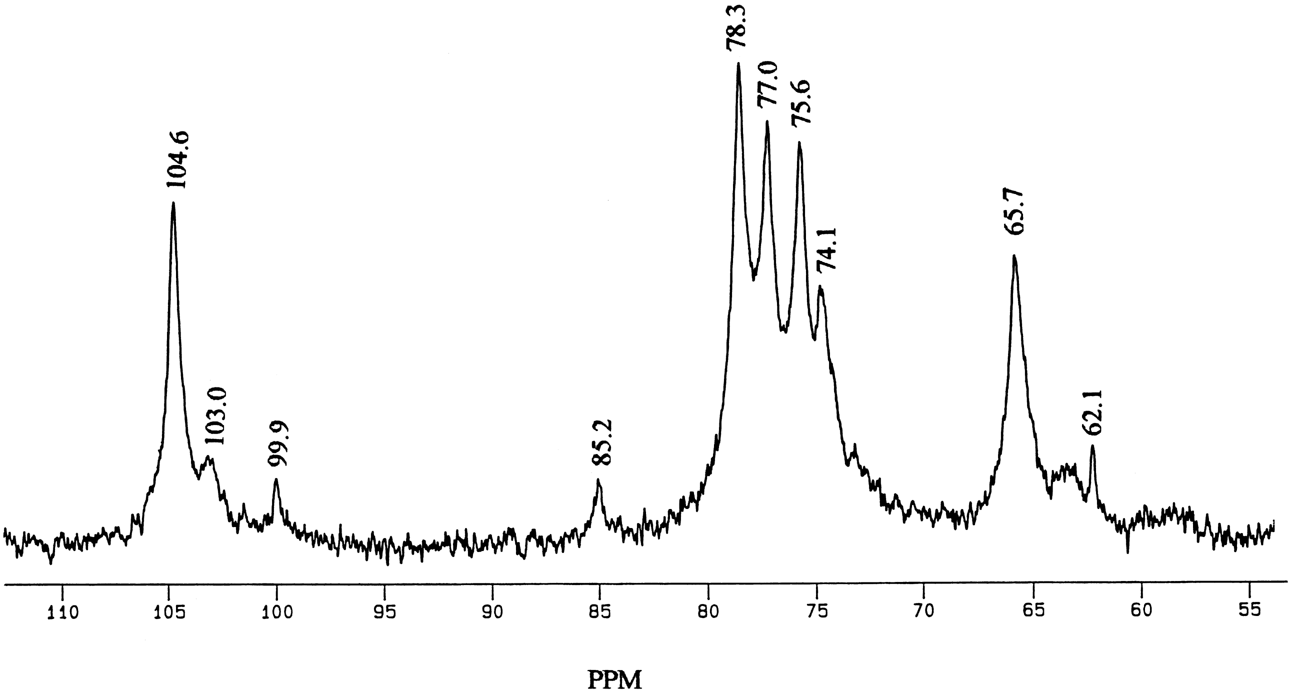

Fig. 5. ^{13}C NMR spectrum of hemicellulosic preparation extracted with 8.5% NaOH at 20°C for 16 h from the dewaxed and partially delignified fast-growing poplar wood.

Table 7
The yield of esterified hemicelluloses and the degree of substitution (DS) (based on assumption that all of the hemicelluloses are converted to di-esterified hemicelluloses (yield, 100%; DS, 2.0). If no reaction occurred and all of the hemicelluloses were recovered unreacted, the yield percentage would be 54.1% for propionylation, 40.2% for hexanoylation, 26.6% for lauroylation, and 21.7% for palmitoylation, respectively)

Molar ratio[a]	Esterification conditions			Esterified hemicelluloses		
	Temperature (°C)	Time (min)	TEA(%)[b]	Sample no.	Yield (%)DS	
3: 1 (PC:X)	60	25	250	1	71.2	0.74
3: 1 (PC:X)	75	35	280	2	84.3	1.31
3: 1 (HC:X)	65	25	260	3	75.6	1.18
3: 1 (HC:X)	75	35	280	4	82.8	1.42
2: 1 (LC:X)	70	30	120	5	39.0	0.34
3: 1 (LC:X)	75	40	250	6	75.2	1.32
2: 1 (PAC:X)	70	30	120	7	39.0	0.44
3: 1 (PAC:X)	75	40	270	8	78.1	1.44

[a] Moar ratio represents the mole of acyl chloride/mole of anhydroxylose (*X*) in hemicelluloses. PC, HC, LC, and PAC represent propionyl chloride, hexanoyl chloride, lauroyl chloride, and palmitoyl chloride, respectively.

[b] TEA (%) represents the percentage of native hemicelluloses (w/w).

analyses was performed. The ^{13}C NMR spectroscopy (in D$_2$O) of the hemicelluloses, extracted with 8.5% NaOH at 20°C for 16 h, is illustrated in Fig. 5. The spectrum was interpreted on the basis of reported data for structurally defined arabinoxylan-type, glucoronoxylan-type, and L-arabino-(4-*O*-methyl-D-glucurono)-D-xylan (Ebringerova, Hromadkova, Alfoldi & Berth, 1992; Imamura, Watanabe & Kuwahara, 1994; Kato, Azuma & Koshijima, 1987; Sun et al., 1996). The main 1,4-linked β-D-Xylp units are obviously characterized by five strong signals at 104.6, 78.3, 77.0, 75.6, and 65.7 ppm, which, respectively, are assigned to C-1, C-4, C-3, C-2 and C-5 of the β-D-Xylp units. Signals at 103.0, 74.1 and 62.1 ppm correspond to C-1, C-3 and C-6 of D-Manp residues, respectively. The signal at 62.1 ppm overlapped with C-6 in D-Glcp units. Signals attributed C-1 and C-4 in MeGlA residue in the hemicelluloses appear at 99.9 and 85.2 ppm, respectively. These values stated that the anomeric configuration of the glycosidic linkage of the D-xylopyranose residues in the main chain of the hemicelluloses is β. This structure is the same as that in the hemicelluloses, isolated from hardwood or various plants in the family of Gramineae such as cereal straws and grasses.

3.6. Yield of esterified hemicelluloses and degree of substitution

The yield of esterified hemicelluloses and the degree of substitution are listed in Table 7. The low, moderate, and high degrees of substitution were obtained although no attempts were made to optimize conditions (molar ratio of the reactant, concentration of TEA, and reaction temperature and duration) for maximum yields. There are no side products produced during the esterifying reactions and, therefore, only minor purification was required. In addition, due to the nearly lack of associated unreacted acyl chloride in isolated products, the DS values of the hemicellulosic derivatives were determined from the yield percentage of esterified hemicelluloses on the assumption of each xylose in xylan-containing two free hydroxyl groups per xylose unit. As shown in Table 7, the yield percentage and DS varied from 39.0 to 84.3% and DS from 0.34 to 1.44, respectively. The lowest yield (39.0%) and DS (0.34) were obtained from sample 5 when the lowest reactant molar ratio (mole of lauroyl chloride: mole of anhydroxylose, 2:1) and lowest concentration of TEA (120%) were used. This suggested that if the esterification reaction did not proceed to give a high degree of substitution mostly unreacted or lightly esterified hemicelluloses were obtained. Obviously, an increase of molar ratios of acyl chloride/anhydroxylose, TEA concentration, or reaction temperature and time resulted in a significant increment of the yield and DS. Increases of molar ratio from 2:1 (samples 5 and 7) to 3:1 (samples 6 and 8), reaction temperature from 70 to 75°C and time from 30 to 40 min, and TEA charge from 120 to 250 or 270% led to significant increases in yields from 39.0 to 75.2 or 78.1% with DS values from 0.34 to 1.32 or from 0.44 to 1.44, respectively. Similar observations were found between the samples 1 and 2 or between sample 3 and 4. The reason for this increasing yield with the concentration of acyl chloride could be interpreted in terms of greater availability of acyl chloride molecules in the proximity of the hemicellulosic molecules at higher concentration of the esterifying agent (Khalil, Hashem & Hebeish, 1995). It is probable that the hemicellulosic hydroxyls are immobile and their reactions will therefore rely on the availability of the acyl chloride molecules in the vicinity of hydroxyl groups. The enhancement of esterification by prolonging the reaction was a direct consequence of the favourable effect of time on diffusion and adsorption of the reactants onto the hemicellulose molecules. In sample 8, a highest DS value (1.44) implied that over 70% of the total hydroxyl groups in native hemicelluloses were palmitoylated under the reaction

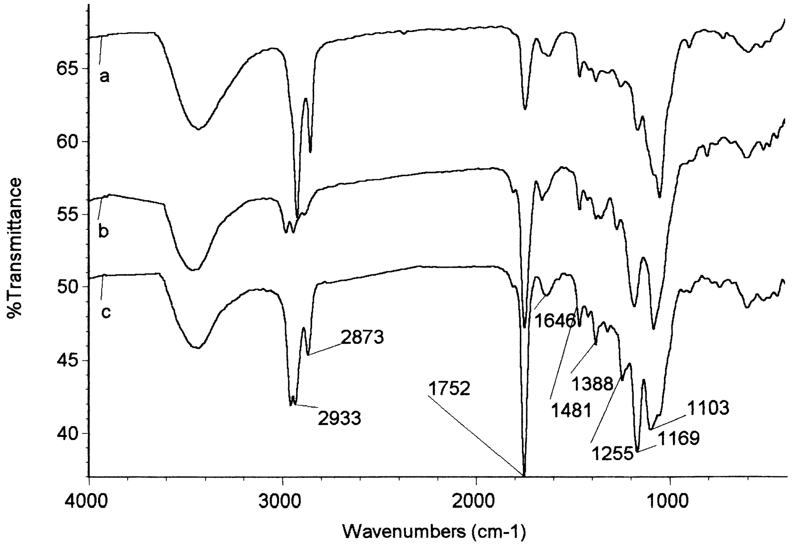

Fig. 6. FT-IR spectra of palmitoylated hemicelluloses (spectrum a, sample 7), propionylated hemicelluloses (spectrum b, sample 1), and hexanolyated hemicelluloses (spectrum c, sample 3).

condition given. In order to gain a complete substitution, addition of more moles of acyl chloride per mol of free OH groups or prolonging reaction time together with increase of TEA concentration is needed.

It was found that the reaction mechanism involves a attacking of acyl carbon centre by nucleophile such as DMAP and removal of hydrochloric acid. TEA, used as an acid acceptor, had a more significant efficiency than the pyridine. Hydrochloric acid, a by-product produced from the esterification of hemicelluloses, is a strong acid, which affects the reaction pH and causes a extensive degradation of the product. However, addition of TEA can counteract the strong acid by forming ammonium salt and stabilize the reaction system pH between 5.5 and 6.5. Treatment of the hemicelluloses suspended in a dipolar–aprotic solvent such as DMF/LiCl system can lead to a highly swollen gel suspension of the polymer and activate the polymers. Another obvious advantage of homogeneous system such as DMF/LiCl for the preparation of hemicellulose derivatives was to conduct a variety of organic reactions, producing high degrees of substitution under mild conditions. Additionally, DMF/LiCl acted as the solvent for the esterified hemicelluloses, which would ensure not only high substitution, but also more uniform substitution due to greater accessibility of the reagent (Lepeniotis & Feuer, 1997).

3.7. FT-IR spectra of esterified hemicelluloses

The FT-IR spectra of palmitoylated hemicelluloses (spectrum a, sample 7), propionylated hemicelluloses (spectrum b, sample 1), and hexanoylated hemicelluloses (spectrum c, sample 3) are illustrated in Fig. 6. The spectra provide evidence of esterification by showing the presence of three important ester bands at 1752 (C=O ester), 1255 (–C–O– stretching), and 1169 cm^{-1} (C–O–C vibration) (Saikia, Ali, Goswami & Ghosh, 1995). Other two prominent bands at 1481 and 1388 cm^{-1} in the spectra attribute to the CH_2 and C–H bending, respectively, in modified hemicelluloses. Two increasing peaks at 2873 and 2933 cm^{-1} in the spectra indicate the methyl and methylene C–H stretching in esterified hemicelluloses. Quantitative esterification is also evident from the decrease of the hydroxyl absorption at 3400 cm^{-1} in the spectra, which corresponds to the increase of degree of substitution from spectrum a to b and to c. The lack of peak at 1800 cm^{-1} in spectrum a, almost lack of this peak in spectra b and c, and no absorbance of carboxylic group at 1700 cm^{-1} indicate that the products are relatively free of the unreacted acyl chloride and the by-product of acyl acid.

Table 8
Weight-average ($\overline{M_w}$) and number-average ($\overline{M_n}$) molecular weights and polydispersity ($\overline{M_w}/\overline{M_n}$) of the esterified hemicellulosic preparations

	Esterified hemicellulosic preparations[a]							
	1	2	3	4	5	6	7	8
$\overline{M_w}$	48 000	55 600	68 200	73 200	53 100	99 600	64 100	12 7200
($\overline{M_n}$)	5900	6700	8400	8900	6600	11700	7700	14500
($\overline{M_w}/\overline{M_n}$)	8.1	8.3	8.1	8.2	8.0	8.5	8.3	8.8

[a] Corresponding to sample no. in Table 7.

3.8. Molecular weight of esterified hemicelluloses

In order to illustrate whether the extent of degradation occurred during the reaction in DMF/LiCl system, all the molecular weights of esterified hemicelluloses were determined by GPC, and the weight-average ($\overline{M}_w$) and number-average ($\overline{M}_n$) molecular weights and polydispersity ($\overline{M}_w/\overline{M}_n$) of the esterified hemicelluloses are listed in Table 8. The theoretical molecular weights were based on the molecular weight of the starting hemicelluloses ($\overline{M}_w = 39\,250$ g mol^{-1}), extracted with 8.5% NaOH at 20°C for 16 h from the dewaxed and partially delignified fast-growing poplar wood. The data showed that the molecular weights of eight esterified hemicellulosic preparations were slightly lower than the calculated values by 10%. These results revealed that only a very little degradation of the polymers occurred during the reaction at 75°C for a short period of 40 min in the homogenous solution of DMF/LiCl.

3.9. Solubility of the esterified hemicelluloses

The natural hemicelluloses with one or two hydroxyl group per xylose unit are hydrophilic. Introduction of hydrophobic acyl groups in the molecular structure of hemicelluloses would be expected to alter their solubility properties. Such alteration would rely essentially on the degree of substitution (Rahn, Diamantoglou, Klemm, Berghmans & Heinze, 1996). The current results showed that all of the esterified hemicellulose preparations were soluble in pyridine at 80°C. They were also soluble in dimethylsulfoxide (DMSO) at 40°C and partially soluble in chloroform, dichloromethane, toluene, and tetrahydrofuran (THF) at room temperature. This increasing lipophilicity would lead to potential use of esterified hemicelluloses in the production of plastic, particularly biodegradable and/or environmentally degradable plastics, resins, and films. Further work on this area is currently under investigation.

In short, treatments of the dewaxed and partially delignified poplar wood with 1.5, 3.0, 5.0, 7.5 and 8.5% NaOH at 20°C for 16 h solubilized 65.6, 71.6, 73.8, 85.6 and 89.3% of the original hemicelluloses, respectively, which contained 2.6–4.9% associated lignin, and had a degree of polymerization giving weight-average molecular weights between 38 830 and 42 230 g mol^{-1}. Xylose was a predominant sugar component in all of the hemicellulosic preparations. Mannose and glucose were present in small amounts, and rhamnose, arabinose, and galacotose were observed as minor sugar constituents. This indicated that the hemicelluloses in the cell walls of fast-growing poplar wood mainly consist of xylans together with small amounts of glucomannans. Further studies by ^{13}C NMR analysis indicated that the hemicelluloses contain a main chain formed by D-xylopyranose residues linked by β-1,4 linkages.

Esterification of the hemicelluloses, isolated with 8.5% NaOH at 20°C for 16 h, in homogeneous DMF/LiCl system introduces a suitable, rapid, and effective method for the esterification of the hemicelluloses obtained from fast-growing poplar wood. The degree of substitution can be simply controlled by the stoichiometry of reagent such as the molar ratio of acyl chloride/anhydroxylose unit and amounts of TEA used, together with the controlling reaction time and temperature. For example, under a reaction condition (sample 8, molar ratio 3:1, 270% TEA, 75°C, 40 min), the product with a high DS value of 1.44 can be obtained without a significant degradation of the native hemicelluloses, in which over 70% of the total hydroxyl groups in native hemicelluloses were palmitoylated.

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