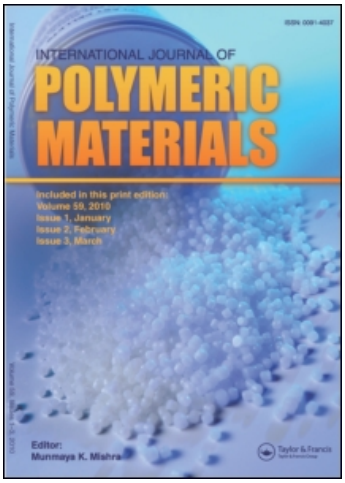


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# Fractionation and Characterization of Water-soluble Hemicelluloses and Lignin from Steam-exploded Birchwood

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# Fractionation and Characterization of Water-soluble Hemicelluloses and Lignin from Steam-exploded Birchwood

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Two crude hemicellulosic fractions, obtained by extraction of steam-exploded birchwood with hot water, were treated with 8% NaOH at 20°C for 1 and 4 h, and subsequently sub-fractionated into four lignin and four hemicellulosic fractions. Acid hydrolysis, alkaline nitrobenzene oxidation, ultraviolet (UV), gel-permeation chromatography (GPC), Fourier transform infrared (FT-IR), and carbon-13 nuclear magnetic resonance (NMR) spectroscopies were used to investigate the chemical compositions and structural features of the fractionated hemicelluloses and lignins. The sugar analyses indicated that xylose was the predominant sugar component in the four hemicellulosic fractions. Due to the autohydrolysis at elevated temperature and lower in acidity during the steam treatment processes, all the four hemicellulosic fractions showed a low degree of polymerization (DP, 38–41), with molecular-average weights between 5620 and 6160. Assignments of all the signals in the NMR spectrum led to the conclusion that the four lignin fractions, which differ from the organosolv lignins obtained from steam-exploded aspen wood meals, are still mainly composed of $\beta$—O—4 ether bonds, together with small amounts of less common $\beta$—$\beta$ and $\beta$—1 carbon–carbon linkages between the lignin structural units. The weight-average molecular weights were found to be 2250–2620 with the polydispersity of 1.5.

*Keywords:* Birchwood; hemicelluloses; lignin; sugars; phenolic acids and aldehydes; alkaline nitrobenzene oxidation; molecular weight

*Corresponding author.

## INTRODUCTION

Steaming is a rather effective pretreatment for certain hardwoods and for materials like straw [1]. It is generally agreed that the steaming process is basically one of autohydrolysis because acetic acid is liberated early in the process by cleavage of acetyl groups mainly emanating from hemicelluloses [2]. It provides the acidic medium (pH 3–4) important for the reactions during steaming at elevated temperature. Portion of the starting material, mainly hemicelluloses, are converted into water soluble products by hydrolysis. The cellulose is not significantly solubilized but undergoes a change in its crystallinity or partially depolymerized [3].

Steam-aqueous pretreatments are also the basis for the so-called fractionation processes, which enable the lignocellulosic materials to be separated into hemicelluloses, lignin, and cellulose with reasonable yields and purity [4]. During the treatment, lignin is profoundly changed. In particular, the $\beta$-aryl ether linkages, which constitute the most abundant linkages connecting the phenylpropane units, are to a large extent cleaved. This type of reaction results in a depolymerisation of the lignin with a concomitant formation of new free phenolic hydroxyl groups. Furthermore, an elimination of terminal hydromethyl groups has been observed and a certain demethylation of aromatic methoxyl groups takes place. On the other hand, depending on the treatment conditions, condensation reactions, *e.g.*, between benzylic carbon atoms and adjacent C-6 positions in aromatic rings, may also occur. These reactions results in a certain extent counterbalance the degradation of lignin caused by the $\beta$-aryl ether cleavage reaction [5].

The present paper deals with the chemical composition and structural characterization of the water-soluble hemicelluloses and lignin from steam-exploded birchwood. In addition, it is hope that such characterization would be further the utilization of the autohydrolysis hemicelluloses and lignin which represents a potential source of xylitol, a sugar substitute of the same sweetness and caloric value as saccharose, and phenolic compounds, as well as medically interesting compounds.

## EXPERIMENTAL

### Fractionation of Water-soluble Hemicelluloses

Water-saturated birchwood meals was steamed for 10 min at temperatures of 190°C and 210°C, respectively. After the steam explosion, the fibrous material was then washed with hot water to remove the degraded hemicellulosic components together with the associated lignins. The extracts were concentrated on a rotary evaporator under reduced pressure, and then mixed with 4 vols ethanol. The precipitated crude hemicelluloses and the fragments were filtered, washed with 70% ethanol, and air-dried. The lignin, associated in the two crude hemicellulosic fractions, was liberated by treatment of the crude hemicelluloses with 8% NaOH at 20°C for 1 and 4 h, respectively. After neutralization to pH 6.5, the solution was mixed with 4 vols ethanol. The precipitated hemicelluloses were filtered, washed with 70% ethanol, and air-dried. The alkali soluble lignins were recovered by reprecipitation at pH 1.5 from the supernatant solution (Fig. 1).



### Characterization of Hemicelluloses and Lignin

Neutral sugar composition of the isolated hemicellulosic fractions and the associated hemicelluloses in lignin preparations was determined by GC analysis of their alditol acetates [6]. Alkaline nitrobenzene oxidation of 8% NaOH soluble lignin and residual lignin in 8% NaOH treated hemicelluloses was performed at 170°C for 3 h with 3.5% O-phenanthroline as a catalyst. Methods of uronic acid analysis and determination of phenolic acids and aldehydes in nitrobenzene oxidation mixtures with HPLC have been described in previous papers [7–10]. All the nitrobenzene oxidation results and neutral sugar analyses represent the mean of at least triplicate determinations, and each mixture was chromatographed twice. The standard errors or deviations were observed to be lower than 6.6% except for the variations among the triplicate nitrobenzene oxidations (8–16%).

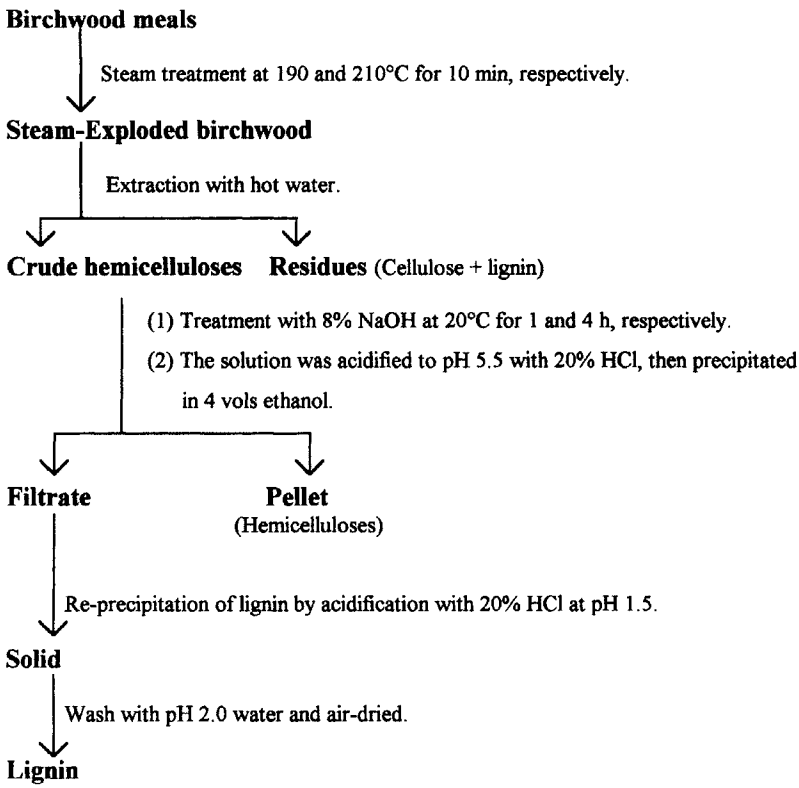


FIGURE 1 Scheme for isolation of hemicelluloses and lignins from crude hemicelluloses obtained from steam-exploded birchwood.

UV spectra were recorded on a Hewlett-Packard 8452A Diode Array spectrophotometer. Lignin sample (5 mg) was dissolved in 95% (v/v) dioxane-water (10 ml). A 1 ml aliquot was diluted to 10 ml with 50% (v/v) dioxane-water, and the absorbances between 200 and 350 nm were measured. FT-IR spectra were obtained on an FT-IR spectrophotometer (Nicolet, 750) using a KBr disc containing 1% finely ground samples. The solution-state of $^{13}$C-NMR spectrum was obtained on a Brucker 250 AC spectrometer operating in the FT mode at 62.4 MHz under total proton decoupled conditions. It was recorded at 25°C from 250 mg sample dissolved in 1.0 ml DMSO-$d_6$ after 30000 scans. A 40° pulse flipping angle, a 3.0 μs pulse width and a 0.85 s acquisition time were used.

The molecular-average weights of the four hemicellulosic fractions were determined by gel permeation chromatography on a PL aquagel-OH 50 column. The samples were dissolved with 0.02 M NaCl in 0.005 M sodium phosphate butter, pH 7.5, at a sample concentration of 0.1%, and 200 µl of this solution was injected. The columns were operated at 40°C, and eluted with 0.02 M NaCl in 0.005 M sodium phosphate buffer, pH 7.5, at a flow rate of 0.3 ml $min^{-1}$. The column was calibrated with PL pullulan polysaccharide. The average molecular weight of the 8% NaOH soluble lignin was determined by gel permeation chromatography on a PLgel 5 µ Mixed-D column (Polymer Laboratories Inc, USA). The sample (200 µl) was injected following dissolution in tetrahydrofuran at a concentration of 0.2%. The column was operated at 40°C and eluted with tetrahydrofuran at a flow rate of 1 ml $min^{-1}$. The column was calibrated using polystyrene standards.



## RESULTS AND DISCUSSION

After steam treatment, the birchwood meals were exploded by rapid decomposition into a fibrous material. A subsequent washing with hot water resulted in the removal of most of the solubilized and degraded hemicelluloses, together with some associated water-soluble lignin. Although the lignin structure might have changed to a certain extent, no free lignin was precipitated from the supernatant of the crude hemicelluloses, indicating that the ether bonds between polysaccharides and lignin may not significantly cleaved during the steam treatments. Similar results have been reported by Hua *et al.* [11]. To liberate and verify the associated lignin in the crude hemicellulosic fractions, the two crude hemicellulosic preparations, obtained by hot water extraction from the steam-exploded birchwood, were treated with 8% NaOH at ambient temperature for 1 and 4 h, respectively. The yield of the 'purified' hemicelluloses and free lignins is given Table I. Obviously, treatment of the crude hemicelluloses I, obtained by steam-explosion at 190°C for 10 min from birchwood and subsequently by hot water extraction, with 8% NaOH for 1 and 4 h yielded 52.8% and 41.9% hemicelluloses, and 5.7% and 4.9% lignin, respectively. While treatment of the crude hemicelluloses II, obtained

TABLE I The yield (% dry weight of sample) of isolated hemicelluloses and lignin fractions obtained from the crude hemicelluloses of steam-exploded birchwood

| *Hemicelluloses/lignin fractions* | *Yield*(%) | |
|---|---|---|
| | *Crude hemicelluloses* I[a] | *Crude hemicelluloses* II[b] |
| Hemicelluloses 1[c] | 52.80 | 18.33 |
| Lignin 1[c] | 5.70 | 6.63 |
| Hemicelluloses 2[d] | 41.90 | 19.20 |
| lignin 2[d] | 4.91 | 7.20 |

[a] Obtained by hot water extraction of steam-exploded birchwood (190°C, 10 min).
[b] Obtained by hot water extraction of steam-exploded birchwood (210°C, 10 min).
[c] Obtained by treatment of the crude hemicelluloses with 8% NaOH at 20°C for 1 h.
[d] Obtained by treatment of the crude hemicelluloses with 8% NaOH at 20°C for 4 h.

by steam-explosion at 210°C for 10 min from birchwood and subsequently by hot water extraction, with same concentration of NaOH for a same period produced a much lower yield of hemicelluloses (18.3% and 19.2%), but a slightly higher yield of lignin (6.6% and 7.2%). These results implied that the hemicelluloses I mainly contained the solubilized hemicelluloses, whereas the hemicelluloses II, contained significantly degraded hemicellulosic fragments, which were not recovered by precipitation in 4 vols ethanol. This suggested that increasing temperature of steam treatment caused a significant degradation of hemicelluloses and a slightly condensation or repolymerization of the lignin. The results obtained were consistent with the findings by Overend and Chornet [12] from a thoroughly study of lignocellulosics by steam-aqueous pretreatments. The authors reported that with increasing severity, the total mass of hemicellulose-derived material diminished as the soluble material was progressively converted to a furfural and/or incorporated into what is known as pseudolignin by condensation reactions. At very high severity, almost all of the hemicelluloses were destroyed.

Table II shows the neutral sugar composition and uronic acid content in the four hemicellulosic preparations and four lignin fractions. It is apparent that xylose was the predominant sugar component in all the hemicellulosic fractions. Galactose, glucose, mannose, rhamnose, arabinose, and uronic acids presented in relatively minor quantities. These data implied that the hemicelluloses comprised a xylan as a major constituent. Increasing alkaline treatment time from 1 to 4 h resulted in growth of xylose content from 81.8% to 86.3% in hemicelluloses I and 83.4% and 91.5% in

TABLE II The content of neutral sugars (relative % for hemicellulosic fractions and % dry weight of the fractions for lignins) and uronic acids (% dry weight of the fractions) of the isolated hemicelluloses and lignin fractions

| *Hemicelluloses/lignin fractions* | *Neutral sugars* | | | | | | *Uronic* |
|---|---|---|---|---|---|---|---|
| | *Rha* | *Ara* | *Xyl* | *Man* | *Glc* | *Gal* | *acids* |
| Cryde hemicelluloses I | | | | | | | |
| Hemicelluloses 1[a] | 1.86 | 0.52 | 81.77 | 1.81 | 3.14 | 10.91 | 5.58 |
| Lignin 1[a] | N | 1.56 | 0.46 | 0.61 | 1.02 | 0.50 | 1.50 |
| Hemicelluloses 2[b] | 1.78 | 0.89 | 86.32 | 1.24 | 3.93 | 5.84 | 4.13 |
| Lignin 2[b] | N | 2.05 | 0.30 | N | 1.08 | 0.59 | 1.76 |
| Crude hemicelluloses II | | | | | | | |
| Hemicelluloses 1[a] | 1.76 | 0.58 | 83.84 | 4.42 | 4.70 | 4.70 | 5.63 |
| Lignin[a] | N | 1.80 | 0.42 | 0.30 | 0.98 | 0.48 | 1.66 |
| Hemicelluloses 2[b] | 1.68 | 0.49 | 91.46 | 1.12 | 3.19 | 2.06 | 5.50 |
| Lignin 2[b] | N | 2.44 | 0.11 | N | 0.86 | 0.45 | 1.85 |

[a] Obtained by treatment of the crude hemicelluloses with 8% NaOH at 20°C for 1 h.
[b] Obtained by treatment of the crude hemicelluloses with 8% NaOH at 20°C for 4 h.



hemicelluloses II, indicating that extension of alkaline treatment time mainly cleaved the side chains of xylan. These data were in general agreement with the results obtained from native birchwood hemicelluloses. It has been reported that the predominant native birchwood hemicelluloses consist of about 200 $\beta$-xylopyranose residues, linked together by 1,4-glycosidic bonds. Approximately every tenth xylose unit carries a single, terminal side chain, consisting of 4-O-methylglucuronic acid attached directly to the 2-position of xylose. Seven out of 10 xylose residues contain an O-acetyl group at C-2, C-3, or at both positions [13]. Apart from these units, the hardwood xylans contain minor amounts of rhamnose and galacturonic acid, which were shown to be integral parts of the xylan main chain [14]. Furthermore, hardwoods also consist of 3–5% glucomannan [15]. The content of uronic acid in the four hemicellulosic fractions ranged between 4.1% and 5.6%, which was slightly higher than in the hemicelluloses extracted by aqueous sodium hydroxide. Similar result has been observed by Roy and Timell [16]. They showed that $\alpha$-1,2-glycosidic bond between the xylan backbone and the 4-O-methylglucuronic acid substituent to be by far more stable than the xylosidic bonds within the chain.

The four lignin fractions contained rather low levels of associated neutral sugars (3.9–4.2%), implying that most of the ether bonds

between lignin and hemicelluloses were cleaved during the 8% NaOH treatments. A relatively higher proportion of arabinose in the lignin fractions indicated that the arabinose residues linked to the lignin components were not significantly cleaved by the steam and alkaline treatments. Chemical studies on linkages between lignin and hemicelluloses, especially arabinoxylans, have emphasized the important role of arabinose residues in forming the linkages. Chesson *et al.* [17] suggested the presence of a covalent association between arabinose side-chains of xylan and phenolic substances including lignin in forage species. The presence of lignin–arabinose linkages was also suggested for spruce wood [18]. A relatively higher concentration of uronic acids in the four lignin fractions was presumed due to the abundance of ester bonds between lignin and glucuronic or 4-O-methylglucuronic acids in associated hemicelluloses, which was confirmed by a signal at 174.6 ppm in the $^{13}$C-NMR spectrum (Fig. 6) [19, 20].

The UV spectra of the two lignin fractions, isolated by 8% NaOH at 20°C for 1 h from the crude hemicelluloses II (a) and from the crude hemicelluloses I (b), are shown in Figure 2. The two fractions exhibited the basic UV spectrum typical of lignins with a maximum at 220 nm. The second maximum at 275 nm originated from non-conjugated phenolic groups in the lignin [21]. A slightly lower absorption coefficient of the lignin fraction (b), obtained from the crude

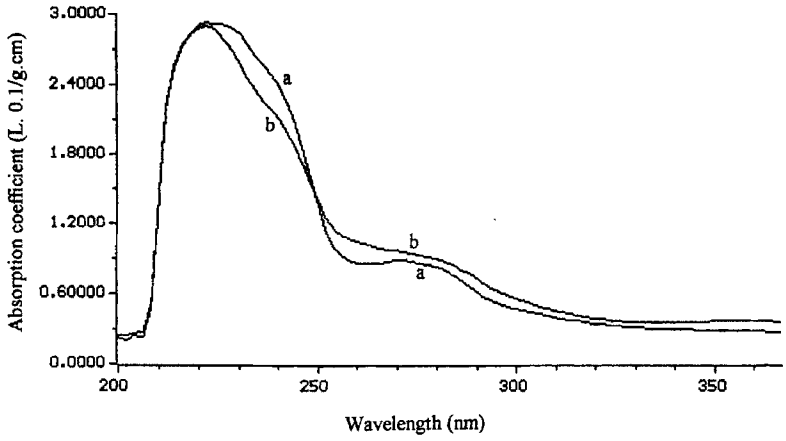


FIGURE 2 UV spectra of lignin fractions extracted with 8% NaOH at 20°C for 1 h from crude hemicelluloses II (a) and crude hemicelluloses I (b).

hemicelluloses I, was presumed due to the co-precipitation of more non-lignin materials such as ash or salts. The lignin fractions, isolated by methanol extraction of aspen exploded wood lignin, were observed to have the absorption maximum at a higher wavelength (238 nm) than was reported for aspen ball milled lignin. Further studies showed that the absorption coefficient of steam-exploded aspen lignin was considerably higher than that of the corresponding ball milled lignin throughout the region of 260 to 350 nm and higher or comparable to softwood ball milled lignin [22]. Furthermore, the lignin fractions, obtained by extraction of steamed aspen wood meal with 90% dioxane at 70°C, appeared to have a absorption maximum at 366 nm, indicating that the lignin fractions contained phenolic hydroxyls conjugated to $\alpha$-carbonyl groups, carbon–carbon double bonds, or biphenyl groups [23]. These different phenomena indicated that the lignin fractions, isolated by organic solvents from steamed aspen wood meal, was a 'modified' lignin and characteristically had a low methoxyl content [22].

Steam treatment at elevated temperature and lower in acidity is more drastic in terms of cleavage of lignin–lignin and lignin–carbohydrate bonds than mild acidolysis due to the higher temperature involved [23]. The previous studies clearly showed that a comprehensive cleavage of $\beta$-aryl ether bonds takes places during the steam treatment [5]. After subsequent washing with hot water to remove most of the solubilized or degraded hemicelluloses together with small amounts of associated lignins, the steam-exploded residues consisting mainly of cellulose and lignin, could be extracted with organic solvents such as acetone, ethanol, or dioxane to recover a substantial portion of the lignin. On the other hand, during the steam treatments, the depolymerized lignin fragments remain in the proximity of condensation sites in the wood matrix, and are more liable to recondense than in acidolysis, where degradation products go into solution [23]. In our experiments, no free lignin was recovered in water from the steam-exploded birchwood, although in the case of the steam treatments, almost 10–15% of the original lignin has been reported to be extracted in water, and in aqueous systems, 30–40% of the original lignin is extracted in water [12]. This result implied that lignin condensation takes an important role during the steam treatments in our experiment. Similar result has been observed by Klemola [24]. He obtained only the more Hibbert ketones in the

degradation products on steam hydrolysis of birchwood at 180°C for 2 h.

After alkaline treatment of the water solubilized crude hemicelluloses I and II, the liberated four lignin fractions and the alkaline treated four hemicelluloses were undergone the alkaline nitrobenzene oxidation, and the results are given in Table III. Syringaldehyde was found to be predominant oxidation product in all the lignin and hemicellulosic fractions, which comprised 75.4–83.3% of the total nitrobenzene oxidation products in the four lignin fractions, and resulted from the degradation of non-condensed syringyl units. Vanillin appeared as the second major product, and came from the oxidation of non-condensed guaiacyl units. Other six phenolic monomers such as *p*-hydroxybenzoic acid, *p*-hydroxybenzaldehyde, vanillic acid, syringic acid, acetovanillone, and acetosyringone were also found in small or trace amounts in the degradation products. The current findings correspond to the normal hardwood lignins, which are comprised of sinapyl alcohol- and coniferyl alcohol-derived units in varying ratios.



Gel permeation chromatography has proved to be a fast and effective method of fractionating polymers according to molecular size. However, it is important to mention here that the molecular weights discussed throughout this work were obtained from a calibration performed with pullula polysaccharide (for hemicelluloses) and polystyrene (for lignin) standards. These values, therefore, should only be considered as relative molecular weights, not absolute. The weight-average ($M_w$) and number-average ($M_n$) molecular weights, and the polydispersity ($M_w/M_n$) of the four lignin and four hemicellulosic fractions are given in Table IV. Obviously, due to the mild acid hydrolysis during the steam treatments and the alkaline hydrolysis during the extractions of the lignins from the crude hemicelluloses, the four hemicellulosic fractions had a much lower degree of polymerization (DP, 38–41), with molecular-average weights ranging from 5620 to 6160. This reduction in chain length of the hemicelluloses resulted partially also from the action of heat and pressure produced during the steam treatment processes. Depending on the steaming conditions the chain lengths of the hemicelluloses ranged from DP 2 to 100 [15]. An increase of steam temperature from 190°C to 210°C or alkaline treatment time from 1 to 4 h resulted in a

TABLE III The content (% sample, w/w) of phenolic acids and aldehydes from nitrobenzene oxidation of lignin in isolated hemicelluloses and lignin fractions

| *Hemicelluloses/lignin fractions* | *Phenolic acids and aldehydes* | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | *PHBA* | *PHBAL* | *VA* | *SYA* | *VAN* | *SYAL* | *AV* | *AS* | *Total* |
| Crude hemicelluloses I | | | | | | | | | |
| Hemicelluloses I[a] | N | 0.063 | 0.012 | 0.038 | 0.11 | 0.38 | 0.022 | 0.015 | 0.64 |
| Lignin 1[a] | 0.26 | 0.24 | 0.32 | 4.90 | 10.14 | 57.84 | 3.06 | 0.0046 | 76.76 |
| Hemicelluloses 2[b] | N | 0.095 | N | 0.10 | 0.054 | 0.20 | 0.017 | N | 0.47 |
| Lignin 2[b] | 0.38 | 0.27 | 0.30 | 4.72 | 8.91 | 64.82 | 3.00 | 0.0045 | 82.40 |
| Crude hemicelluloses II | | | | | | | | | |
| Hemicelluloses 1[a] | N | 0.081 | N | 0.11 | 0.081 | 0.30 | 0.010 | N | 0.58 |
| Lignin 1[a] | 0.32 | 0.31 | 0.49 | 3.71 | 6.82 | 63.38 | 2.30 | 0.15 | 77.48 |
| Hemicelluloses 2[b] | N | 0.0078 | N | 0.090 | 0.043 | 0.24 | 0.015 | 0.0053 | 0.40 |
| Lignin 2[b] | 0.18 | 0.16 | 0.24 | 3.18 | 5.49 | 56.87 | 2.03 | 0.14 | 68.29 |

[a] Obtained by treatment of the crude hemicelluloses with 8% NaOH at 20°C for 1 h.
[b] Obtained by treatment of the crude hemicelluloses with 8% NaOH at 20°C for 4 h.
(PHBA, *p*-Hydroxybenzoic acid; PHBAL, *p*-Hydroxybenzaldehyde; VA, Vanillic acid; SYA Syringic acid; VAN, Vanillin; SYAL, Syringaldehyde; AV, Acetovanillone; AS, Acetosyringone; N = Not detectable).

TABLE IV The weight-average ($M_w$) and number-average ($M_n$) molecular weights, and the polydispersity ($M_w/M_n$) of the isolated hermicelluloses and lignin fractions

| *Hemicelluloses/lignin fractions* | $\overline{M_w}$ | $\overline{M_n}$ | $\overline{M_w/M_n}$ |
|---|---|---|---|
| Crude hemicelluloses I | | | |
| Hemicelluloses 1[a] | 6160 | 4670 | 1.32 |
| Lignin 1[a] | 2300 | 1570 | 1.46 |
| Hemicelluloses 2[b] | 6000 | 5150 | 1.16 |
| Lignin 2[b] | 2250 | 1520 | 1.49 |
| Crude hemicelluloses II | | | |
| Hemicelluloses 1[a] | 5800 | 3560 | 1.63 |
| Lignin 1[a] | 2600 | 1710 | 1.52 |
| Hemicelluloses 2[b] | 5620 | 43100 | 1.31 |
| Lignin 2[b] | 2620 | 1730 | 1.52 |

[a] Obtained by treatment of the crude hemicelluloses with 8% NaOH at 20°C for 1 h.
[b] Obtained by treatment of the crude hemicelluloses with 8% NaOH at 20°C for 4 h.



slight decrease of hemicellulose molecular weights by 6% and 3%, respectively.

The data in Table IV showed that the four lignin fractions appeared to have low molecular-average weights, ranging between 2250 and 2620. Occurrence of these low molecular weights of lignins resulted mainly from the hydrolysis of the easily broken ether bonds, both inter and intra to the basic phenylpropane ($C_6$-$C_3$) moieties of the lignin structure [12]. Chum *et al.* [26] and Glasser *et al.* [27] have independently reached similar results by alkaline extraction of the steamed materials. Chum *et al.* [26] found that the mass-average molecular weight of alkali-extracted steam-treated aspen was 2100 with a polydispersity of 2.7. Glasser *et al.* [27] reported that the lignin fractions, obtained by alkali extraction of steamed aspen and poplar, had mass-average values of 2300 and 3000 g $mol^{-1}$, respectively. In our experiments, the lignin fractions, obtained by alkali extraction of the crude hemicelluloses II, showed slightly higher molecular-average weights (2600–2620) than those of the lignin fractions (2250–2300), obtained by alkali extraction of the crude hemicelluloses I. This indicated that steam treatment at higher temperature (210°C) favoured lignin condensation.

The molecular weight distribution of the hemicellulosic fraction, obtained by treatment of crude hemicelluloses II with 8% NaOH at 20°C for 4 h, is shown in Figure 3. The main peak had a molecular weight equal to 5080. The second small peak corresponded to the

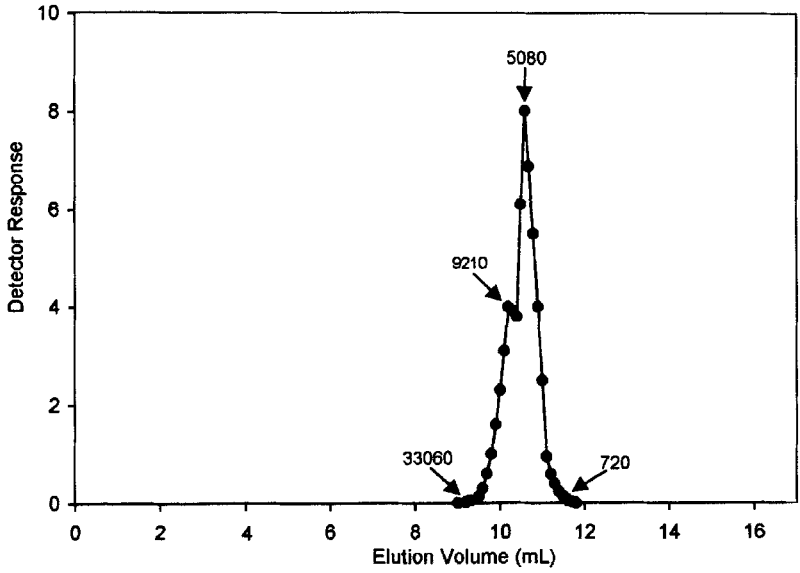


FIGURE 3 GPC molecular weight distribution of hemicelluloses obtained by treatment of crude hemicelluloses II with 8% NaOH at 20°C for 4 h.



relatively higher molecular weight of 9210. The elution profiles showed a relatively wide polymolecularity, ranging from oligosaccharides up to polysaccharides with high molecular weight of 33060.

Figure 4 shows the FT-IR spectra of two hemicellulosic fractions, extracted with 8% NaOH at 20°C for 1 h from the crude hemicelluloses I (spectrum a) and with 8% NaOH at 20°C for 4 h from the crude hemicelluloses II (spectrum b). The band at 1628 $cm^{-1}$ represents the carbonyl groups and is presumed due to the uronic acid in salt form. A band at 1255 $cm^{-1}$ can be assigned to the corresponding ester C—O stretch for esterified uronic acids [28]. The prominent band at 1050 $cm^{-1}$ is attributed to the C—OH bending [29]. The small sharp band at 897 $cm^{-1}$ is characteristic of $\beta$-glycosidic linkages between the sugar units [30]. The additional hemicelluloses-related absorbances appeared at 1420, 1388, 1361, 1328, 1215, 1169, 1122, 1096, and 990 $cm^{-1}$.

Typical FT-IR spectra of the four lignin fractions are shown in Figure 5. The spectral profiles and relative intensities of the bands among the four spectra were rather similar, indicating similar

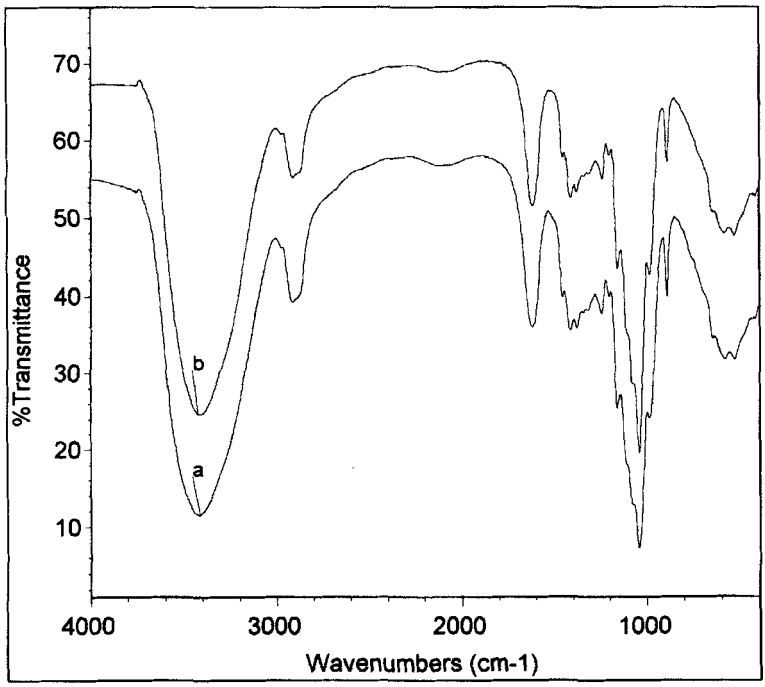


FIGURE 4 FT-IR spectra of birchwood hemicellulosic fractions: (a) extracted with 8% NaOH at 20°C for 1 h from crude hemicelluloses I; (b) extracted with 8% NaOH at 20°C for 4 h from crude hemicelluloses II.



structures of the lignins. The presence of an intensive band at 1713 $cm^{-1}$ after saponification is assigned to unconjugated carbonyl groups such as unconjugated ketone and/or aldehyde groups as well as carboxyl ester groups. In the spectra of lignin preparation including ball milled lignin and solvolysis lignins, the bands observed at 1710 – 1750 $cm^{-1}$ are attributed to unconjugated carboxyl ester groups or unconjugated aliphatic or $\beta$-ketone groups [22]. Aromatic skeleton vibrations in lignin appear at 1596, 1510, and 1420 $cm^{-1}$. There is also observed a shift from a relatively lower to a slightly higher frequency of absorptions between 1596 and 1510 $cm^{-1}$ from spectra *a* and *c* to spectra *b* and *d*. The lignin fractions (spectra *a* and *c*), obtained from the crude hemicelluloses I, show a relatively higher frequency of absorption at 1510 $cm^{-1}$ than at 1596 $cm^{-1}$. While the reverse trend is

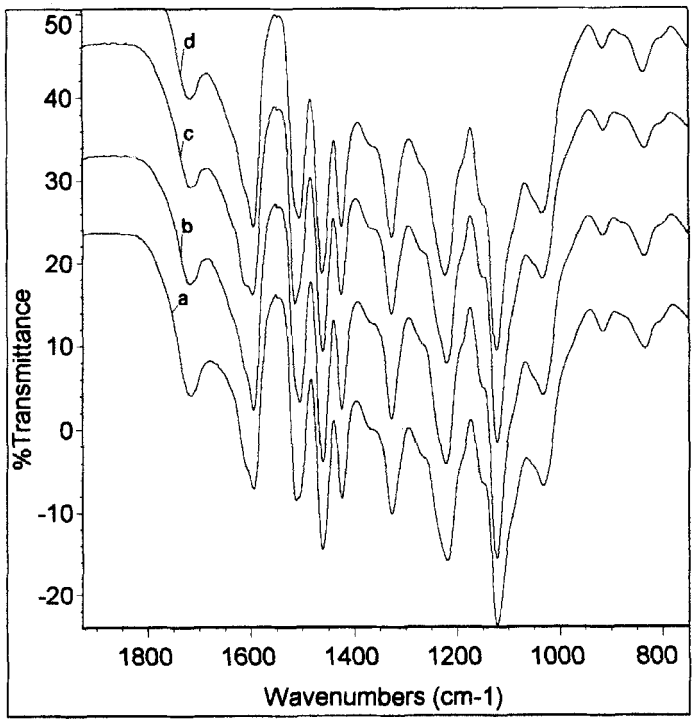


FIGURE 5 FT-IR spectra of birchwood lignin fractions: (a) extracted with 8% NaOH at 20°C for 1 h from crude hemicelluloses I; (b) extracted with 8% NaOH at 20°C for 1 h from crude hemicelluloses II; (c) extracted with 8% NaOH at 20°C for 4 h from crude hemicelluloses I; (d) extracted with 8% NaOH at 20°C for 4 h from crude hemicelluloses II.

observed in the lignin fractions (Spectra *b* and *d*), obtained from the crude hemicelluloses II. Thus the results suggested that the steam treating temperature has a strong effect on the lignin structures. The C=O ester groups in lignin molecules can be verified with two small bands at 1272 and 1180 $cm^{-1}$, indicating the esterified uronic acids. The bands at 1328 and 1225 $cm^{-1}$ correspond with syringyl and/or guaiacyl ring breathing with CO stretching. The aromatic CH in-plane deformations appear at 1120 $cm^{-1}$ (syringyl type) and 1030 $cm^{-1}$ (guaiacyl type), respectively.

The $^{13}$C-NMR spectrum of the lignin fraction, extracted with 8% NaOH at 20°C for 1 h from the crude hemicelluloses I, is shown in Figure 6. The assignment of the peaks in the spectrum was made based

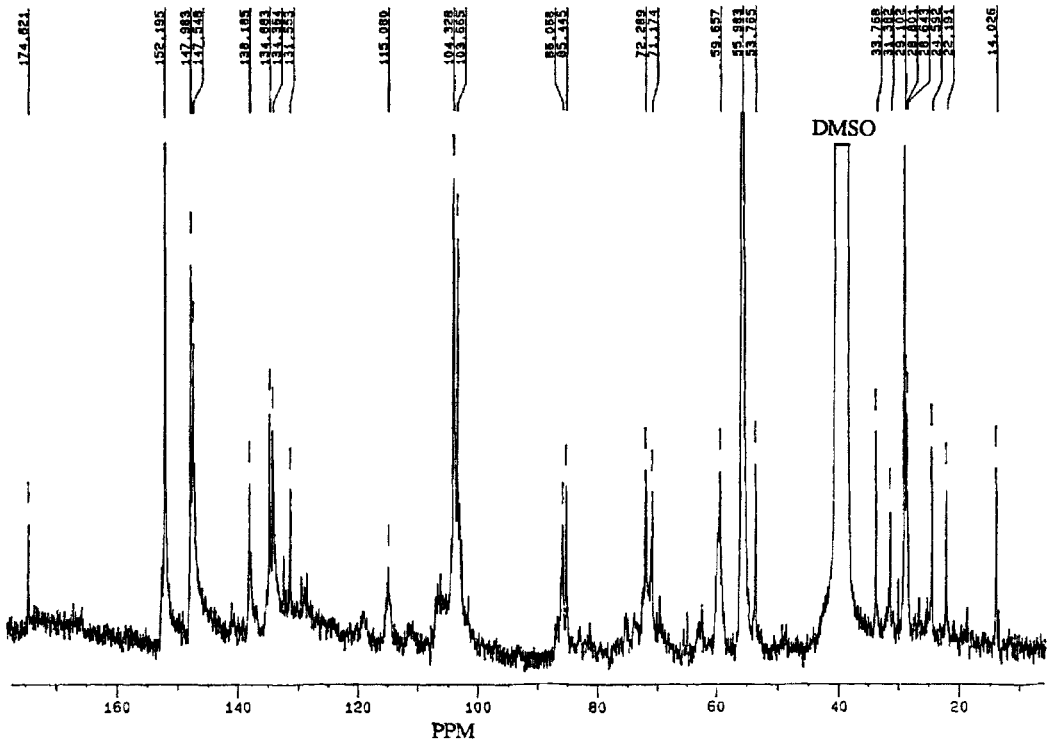


FIGURE 6 $^{13}$C-NMR spectrum of lignin fraction extracted with 8% NaOH at 20°C for 1 h from crude hemicelluloses I.

on comparison with the results of lignin model compounds and native lignin reported by Robert *et al.* [5], Harchessault *et al.* [22], Chua and Wayman [23], Nimz *et al.* [31], and Pan *et al.* [32].

There are no carbonyl signals towards 200 ppm which would be assigned to unconjugated ketone groups although the FT-IR spectra indicates the presence of such groups. A signal at 174.6 ppm is ascribable to C-6 in methyl uronates (C=O in aliphatic acids or esters), and it is particularly important in the spectrum of steam-exploded birchwood lignin but it is absent in the aspen exploded wood lignin [22]. In the aromatic carbon region between 104 and 160 ppm, the syringyl (S) residues were indicated by signals at 152.2 (C-3/C-5 in S, etherified), 148.0 and 147.5 (C-3/C-5 in S, non-etherified), 138.2 (C-1/C-4 in S, non-etherified), 134.9 and 134.4 (C-4 in S, non-etherified), 131.6 (C-1 in S, non-etherified), and 104.3 and 103.7 ppm (C-2/C-6 in S, etherified). Guaiacyl (G) residues gave signals at 148.0 and 147.5 (C-3 in G, etherified), 134.9 and 134.4 (C-1 in G, etherified), 131.6 (C-1 in G, non-etherified), 119.6 (C-6 in G, etherified), 115.1 (C-5 in G, non-etherified), and 111.5 ppm (C-2 in G, etherified), respectively. The *p*-hydroxyphenyl (H) residues appeared as a small signal at 128.7 ppm (C-2/C-6 in H). These observations indicated that the lignin, obtained from the steam-exploded birchwood hemicelluloses, is still characteristically etherified as compared to ball milled birchwood lignin [22], which was not consistent with the results obtained from the lignin fraction, extracted with methanol from the steam-exploded aspen wood meals by Marchessault *et al.* [22]. They reported that there was an increase of non-etherified units and an increase of *p*-hydroxyphenyl units in the isolated lignin fractions. This phenomenon suggested that the lignin fractions, obtained from exploded birchwood and aspen wood may have different structures.

Signals at 72.3 and 71.2, 86.1 and 85.4, and 59.7 ppm are assignable to C-$\alpha$, C-$\beta$, and C-$\gamma$ carbons of the propane side chains when $\beta$—O—4 linkages are present. This indicated that $\beta$—O—4 ether band is still the major linkages between the phenylpropane units after explosion of birchwood. This result was not thoroughly agree with the findings by Domburg and coworker's study [33] on organosolv lignins from exploded aspen wood. They reported that the thermal stability of alkyl–aryl linkages was less in hardwoods than in softwoods which was in keeping with the lack of success of the explosion process with

gymnosperms. In a personal opinion, The weakness of such β—O—4 linkages in organosolv lignins, obtained from exploded aspen wood meals, was probably that the de-etherification occurred at the β—O—4 linkages during the organosolv extraction process. In addition, the less common carbon–carbon linkages such as β—β (C-β in β—β, 53.8 ppm) and β—1 (C-γ and C-β in β-1, 62.8 ppm) were also present. A distinct signal at 56.0 ppm is assigned to $-OCH_3$ in syringyl and guaiacyl groups. Finally, the peaks between 14.0 and 33.8 ppm represent the aliphatic carbons in *n*-propyl side chains of lignin molecules.

The above studies showed that the four lignin fractions, isolated by 8% NaOH at 20°C for 1 and 4 h from crude hemicelluloses of steam-exploded birchwood, contained very small amounts of associated polysaccharides. All the lignin preparations had a high proportion of non-condensed syringyl units and small amounts of non-condensed guaiacyl units. They seem less condensed than straw lignins and the lignins from un-exploded hardwoods. In addition, the extracted lignins were also observed to contain unconjugated ketone and/or aldehyde groups, which resulted from the depolymerization reactions of the lignin macromolecule under acidic conditions during the steam treatment processes. Further studies showed that the lignin fractions are still mainly composed of β—O—4 ether bonds, together with small amounts of less common β—β and β—1 carbon–carbon linkages between the lignin structural units. This differs from the results obtained from the organosolv lignins from steam-exploded aspen wood, in which a substantial cleavage of β-aryl ether bonds and an increase of *p*-hydroxyphenyl units were observed.



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