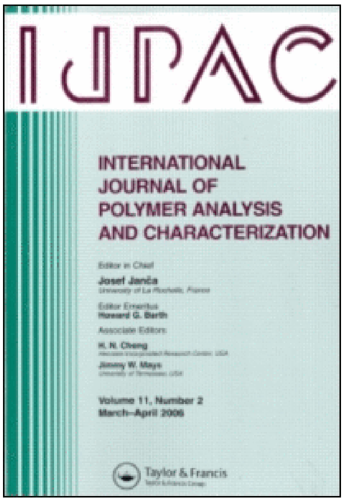

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Physicochemical Characterization of Hemicelluloses from Steamed Aspen and Birchwood

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The three hemicellulosic preparations, isolated from aspen by steam treatment (without explosion) at 180°C, 190°C, and 200°C for 10 min, and two hemicellulosic fractions, extracted from birchwood by steam-explosion treatment at 190°C and 210°C for 10 min, were comprised mainly of acetyl- and 4-*O*-methylglucuronosyl substituted xylans. When aspen was steam-treated without explosion, the hemicelluloses, extracted by water, contained relatively higher amounts of associated lignin (14.6–26.6%). Treatment of birchwood by steam-explosion yielded hemicelluloses which contained relatively lower amounts of associated lignin (6.2–9.5%). All five hemicellulosic fractions had a lower degree of polymerization (DP, 44–63), with molecular-average weights ranging between 6610 and 9390.

Keywords: Aspen; Birchwood; Hemicellulose; Lignin; Sugars

INTRODUCTION

The fractionation of lignocellulosic materials such as wood, straw, and grass into different polymeric constituents: cellulose, hemicelluloses, and lignin in varying degrees of modification is one of the main advantages of maximum utilization of these materials. The thermochemical or biochemical upgrading of each fraction leads to the production of separated chemicals, such as xylitol or furfural from hemicelluloses; sugars, ethanol or ruminant feed from cellulose; and phenols or fuels from lignin.[1] Such fractionation can be performed using different

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technologies such as pulping, solvent extractions, or steam-aqueous pretreatments.[2] The first step of the fractionation process is pretreatment which permits the solubilization of extractives and hemicelluloses. The residues thus obtained are more accessible to chemicals and enzymes.[3] Steam treatments with or without explosion have the advantage of using a widely available solvent without significant cost or environmental impact. During the steam processes, the lignocellulosic substrate is steam-treated at temperatures ranging from 140°C to 240°C for 1–10 min followed by a rapid pressure release (i.e., explosion) through a discharge valve. These steam-explosion processes have succeeded in separating lignocellulosic material into individual fibers. Moreover, the addition of acid or basic catalysts can profoundly change the product characteristics.[2,3] For example, the presence of SO_2, which leads to the *in situ* formation of acid, enhances the enzymatic digestibility of the carbohydrate fractions in coniferous materials,[2,4] whereas the presence of NaOH facilitates destructuring and defibration of hardwoods with very minor changes in cellulose, resulting in high-yield pulps having excellent paper-making characteristics.[2,5] It has also been demonstrated that the defibration by physicochemical steam-explosion treatment is due to softening and partial dissolution of the compound in the middle lamella, which is rich in lignin. During the treatment, the hemicelluloses are depolymerized and subsequently solubilized. A fraction of the lignin is partially depolymerized and carried into solution. Moreover, the cellulose undergoes a change in its crystallinity and can also partially depolymerize, depending on the treatment conditions.[3]

In the present work, aspen and birchwood were steamed without explosion at 180–200°C or with explosion at 190–210°C for 10 min, respectively. The solubilized hemicelluloses were extracted with water, and their physicochemical properties reported. Further attention is also paid to the composition of associated lignin.

MATERIAL AND METHODS

Aspen chips were steamed without explosion at 180°C, 190°C, and 200°C for 10 min, respectively. Birchwood chips were steamed with explosion at 190°C and 210°C, respectively, for 10 min. The steamed chips were then extracted with water. The extracts were concentrated on a rotary evaporator under reduced pressure, and then mixed with 4 vols ethanol. The precipitated hemicelluloses and the fragments were filtered, washed with 70% ethanol, and air-dried.

The neutral sugar composition of the isolated hemicelluloses was determined by GC analysis of their alditol acetates.[6] Hemicelluloses bound saccharidic components in the fractions were subjected firstly 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 sulfoxide and the resulting alditols acetylated using 1-methylimidazole as the catalyst. Alkaline nitrobenzene oxidation of the lignin associated in the isolated polysaccharides was performed at 170°C for 3 h. Methods of uronic acid analysis and determination of phenolic acids and aldehydes in nitrobenzene oxidation mixtures with HPLC have been described in previous papers.[7-9] FT-IR spectra were obtained on an FT-IR spectrophotometer (Nicolet, 750) using a KBr disk containing 1% finely ground samples.

The molecular-average weights of the hemicelluloses 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 using PL pullulan polysaccharide.

RESULTS AND DISCUSSION

Hardwood, such as aspen or birchwood, and straws contain 20–35% hemicelluloses. These polymers are a heterosaccharide branched polymer, which consists of about 200 β-xylopyranose residues, linked together by 1,4-glycosidic bonds. The content of the xylan component represented more than 90% of the hemicelluloses in aspen. In birchwood xylan, 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 position.[10] Apart from these units, hardwood xylans contain minor amounts of rhamnose and galacturonic acid, which were shown to be integral parts of the xylan main chain.[11] Furthermore, hardwoods usually consist of 3–5% glucomannan, linked by β-1,4-glycosidic bonds.[12] In the case of birchwood hemicelluloses, the main substituents of the β-*D*-xylopyranosidic backbone are *O*-acetyl and 4-*O*-methylglucurono residues.[13] During steam treatment, due to the hydrolytic attack achieved by the acetic acid formed during the steaming, the hemicelluloses were autohydrolyzed or solubilized.[14]

The chemical composition of the hemicellulosic fractions obtained after steaming aspen and birchwood is shown in Table I. There was no significant difference in the sugar composition among the three hemicellulosic fractions, obtained from steamed aspen, indicating that the three hemicellulosic preparations may have similar structures. Similar results were observed from the two hemicellulosic preparations, obtained from steam-exploded birchwood. Xylose was the major component sugar in the three aspen hemicellulosic fractions and two birchwood hemicellulosic fractions, comprising 58.5–81.3% of the total sugars. Glucose, mannose, galactose, arabinose, and rhamnose appeared as minor constituents. Increasing steam treatment temperature from 180°C to 200°C resulted in an increase of xylose from 58.5 to 68.7%, indicating that xylose in the main chain of xylan was more favorably extracted at a higher temperature from aspen.

TABLE I Composition of neutral sugars (relative% dry weight) and uronic acids (% dry weight) in aspen and birchwood hemicellulosic fractions

Steam treatment conditions	*HF*[a]	*Neutral Sugars*						*Uronic acids*
		Rha	*Ara*	*Xyl*	*Man*	*Glc*	*Gal*	
Steam treatment of aspen (no explosion)								
180°C, 10 min	1	3.14	3.33	58.46	12.03	16.70	6.34	5.25
190°C, 10 min	2	3.40	3.47	66.42	10.67	10.59	5.44	3.50
200°C, 10 min	3	2.54	2.55	68.68	10.73	11.26	4.25	3.00
Steam-explosion treatment of birchwood								
190°C, 10 min	4	2.84	2.26	72.90	1.82	6.48	13.69	5.60
210°C, 10 min	5	2.34	1.35	81.29	2.96	5.11	6.96	3.14

[a] Abbreviation for hemicellulosic fractions, obtained by water extraction of steam-treated aspen and steam-exploded birchwood, respectively.

A similar trend was observed for the two birchwood hemicelluloses. The content of xylose in the hemicelluloses increased from 190°C to 210°C and amounted to 81.3%, based on extracted material. This observation, however, did not agree with the results obtained from characterization and preparation of substituted xylo-oligosaccharides from steamed birchwood by Korte and co-workers.[15] The authors stated that an additional increase in temperature from 200°C to 210°C led to a decrease in xylan survival. The proportion of free xylose in the extract increased markedly at 210°C. At this temperature it comprised 13.0% of the extracted dry water, suggesting a shift from higher to lower oligomers as a function of steaming temperature. These different phenomena are probably due to the various methods used for isolation of solubilized hemicelluloses from steam-exploded birchwood. Further investigations are now needed to test this hypothesis.

The content of uronic acids, mainly as 4-*O*-methylglucuronic acid, decreased from 5.3% to 3.0% with an increase of steam treatment temperature from 180°C to 200°C from aspen, and from 5.6% to 3.1% with an increase of steam treatment temperature from 190°C to 210°C for birchwood. This observation implied that 4-*O*-methylglucuronic acid substituents were more labile with increasing steaming temperature. The results obtained was consistent with the finds by Korte *et al.*[15] The authors mentioned that in the steaming extract of 170°C, every 14th xylose unit remained substituted by a 4-*O*-methylglucuronic acid side group. At 190°C, every 38th xylose unit was substituted by a 4-*O*-methylglucuronic acid residue, whereas only every 90th xylose unit was substituted when birchwood was steamed at 210°C for 10 min. Such a conclusion has already been drawn for the hemicelluloses from steamed birchwood by Overend and Chornet.[16] They reported that high values of the reaction ordinate, the 4-*O*-methylglucuronic acid side groups on the xylan chain hydrolyzed rapidly.

In birchwood xylan, about 70% of the xylose units are substituted by an acetyl group.[13,17] In our experiments, the yield of acetyl group in birchwood hemicellulosic fractions 4 and 5 was found to be 7.4% and 6.4%, respectively. These results indicated that a noticeable amount of acetyl groups remained bound to hemicelluloses with little temperature dependence. As can be implied from the values given, the esterified acetyl side groups were more stable under the steam treatment conditions than were the 4-*O*-methylglucuronic acid substituents.

With a study of aqueous thermomechanical treatment of aspen in a batch reactor system, Carrasco[3] indicated that increasing steam treatment temperature or period significantly resulted in hydrolysis of the hemicelluloses. It was observed that there was no hemicelluloses removal at a temperature below 150°C. However, at a temperature about 240°C, almost all the hemicelluloses were removed. With increasing temperature or period of steam treatment, the total mass of hemicellulose-derived material diminished as the soluble material was progressively converted to furfural and/or incorporated into what is known as pseudolignin by condensation reactions.[6] Similarly, large amounts of the sugar degradation product 5-hydroxymethyl-2-furaldehyde were found in the water extracts of steamed aspen.[18] This phenomenon implied that in the later stages of the longer treatments, much of the hemicelluloses already solubilized in the earlier stages was further degraded to an insoluble material which has previously been termed pseudolignin.

During steam-explosion pulping, no lignin dissolution occurred, although the lignin structure might have changed to a certain extent.[19] when the hemicellulose content had been reduced to below 1%, the alkali-insoluble lignin content was still more than four times as great.[20] However, a fraction of the lignin in the middle lamella could be dissolved during water extraction of the steamed wood. Almost 10–15% of the original lignin was water soluble. In the aqueous systems, 30–40% of the original lignin is extracted in water.[16] In our experiments, the solubilized lignins were co-extracted with water in the hemicellulosic fractions. No significant amount of free lignin was isolated by precipitation from the aqueous solution by the addition of dilute acid to pH 1.5–2.0. This phenomenon suggested that the lignin in the cell walls of aspen and birchwood are strongly associated to the hemicelluloses, and the ether linkages between polysaccharides and lignin may not be cleaved during the given steam treatment conditions.

To further verify the presence of associated lignin, alkaline nitrobenzene oxidation of the isolated five hemicellulosic fractions was performed at 170°C for 3 h. The method provided an estimate of the amount of associated lignin and an indication of its composition.

TABLE II The content of associated lignin in hemicelluloses (% sample, w/w) and the yield (% sample, w/w) of phenolic acids and aldehydes from alkaline nitrobenzene oxidation of associated lignin in the hemicelloluse fractions obtained from steam-treated aspen and birchwood

Phenolic acids and aldehydes	*Hemicellulosic fractions*				
	1	2	3	4	5
p-hydroxybenzoic acid	0.08	0.06	0.07	0.022	0.014
p-hydroxybenzaldehyde	0.11	0.92	0.68	0.043	0.031
vanillic acid	0.10	0.28	0.23	0.023	0.015
syringic acid	0.43	1.04	0.92	0.25	0.16
vanillin	2.97	5.45	4.53	0.55	0.29
syringaldehyde	5.11	6.87	6.53	2.74	1.86
acetovanillone	0.17	1.41	0.79	0.038	0.023
acetosyringone	0.11	0.57	0.26	N[a]	N
Total	8.80	16.60	14.01	3.67	2.37
content of lignin	14.60	26.56	22.42	9.54	6.16

[a] N = not detected.

Table II gives the results of nitrobenzene oxidation of associated lignin in the isolated hemicellulosic fractions. As can be seen from the table, the three hemicellulosic preparations, obtained from the steamed aspen, produced syringaldehyde and vanillin as the major components, which amounted to 41.4–58.1% and 27.3–33.8% of the total phenolic monomers, respectively. Similarly, syringaldehyde was found to be a predominant component of phenolics in the mixtures of nitrobenzene oxidation of associated lignin from the steam-exploded birchwood hemicelluloses, which yielded 74.7–78.5% of the total phenolics. Small amounts of benzaldehydes and phenolic acids such as *p*-hydroxybenzaldehyde, acetovanillone, acetosyringone, syringic acid, vanillic acid, and *p*-hydroxybenzoic acid were also identified in the nitrobenzene oxidation products. This observation implied that the hemicelluloses, obtained from the steamed aspen, are linked to lignin via syringyl and guaiacyl units; while in the hemicelluloses, obtained from the steam-exploded birchwood, lignin is associated to hemicelluloses mainly via syringyl units.

Previous studies[18,21,22] have shown that steam hydrolysis resulted in an extensive liberation of phenolic groups, and undoubtedly this is primarily due to cleavage of the aryl ether bonds in *β*-*O*-4 structures. The liberated lignins are easily soluble in aqueous organic solvents such as ethanol–water and dioxane–water, and can be recovered by evaporating the solvent followed by precipitation from aqueous solution by addition of dilute acid. Carrasco[3] reported that 15% of the initial lignin has been solubilized at 237°C during the steam treatment of aspen in a batch reactor system although this value is below that of 20–25% reported by Sjöström.[23] Further studies found that these solubilized lignin fractions resulted from the middle lamella. Interestingly, the data in Table II exhibited that the content of associated lignin in the hemicellulosic fractions, obtained from steamed aspen, increased from 14.6% to 26.6% with a temperature increase from 180°C to 190°C. This increasing lignin content in the isolated hemicellulosic preparations was also probably due to the lignin recondensation reactions at a high temperature. However, the data in Table II showed that a slight decrease of associated lignin in the hemicellulosic fractions appeared at the higher temperature ranging between 190°C and 200°C for the steamed aspen and from 190°C to 210°C for the steam-exploded birchwood. This phenomenon implied that the repolymerization between soluble lignin and the degraded hemicellulosic products was favored at 190°C during the steamed treatment of aspen and birchwood.

Relative to alkaline hemicellulosic samples of the same species, the hemicelluloses were depolymerized significantly. A reduction in chain length of the hemicelluloses was brought about by the action of heat and pressure. Furthermore, due to the slightly acidic nature (pH 3–4) of the steam treatment process, most of the side chains did not survive in the isolated hemicellulosic fractions.[12] Depending on steaming conditions, the chain lengths of the hemicellulose fragments ranged from DP (degree of polymerization) 2 to 100.[24] Table III shows the weight-average M_w and number-average M_n molecular weights, the polydispersity M_w/M_n, and the degree of polymerization of the five

TABLE III The weight-average M_w and number-average M_n molecular weights, polydispersity (M_w/M_n), and the degree of polymerization (DP) of the hemicellulosic fractions

Hemicellulosic fraction	M_w	M_n	M_w/M_n	*DP*
1	9390	6380	1.47	63
2	7640	5850	1.31	51
3	7060	5460	1.29	47
4	7360	5580	1.32	49
5	6610	5360	1.23	44

hemicellulosic fractions. Obviously, the hemicelluloses, extracted from the steamed aspen or birchwood, had a much lower degree of polymerization (44–63), with molecular-average weights ranging between 6610 and 9390. An increase of steam temperature from 180°C to 200°C for aspen a reduce the molecular-average weight from 9390 to 7060. Similarly, an increase of steam temperature from 190°C to 210°C for birchwood brought about a molecular-average weight decrease from 7360 to 6610.

These results were in partial agreement with the studies of steamed birchwood carbohydrates by Puls *et al.*[24] The authors reported that 80% of the polysaccharides extracted from birchwood after steaming at 190–200°C for 10 min were acetyl- and 4-*O*-methylglucuronic substituted xylo-oligomers and xylose. Although most of the oligomers had a degree of polymerization of less than 10, monomeric xylose comprised only about 10% of the total xylo-oligomers. However, the current results were in a good agreement with the data reported by Puls and Schuseil[12] from organosolvent pulping of Norway spruce. They stated that a mixture of acetylgalactoglucomannan and arabino-4-*O*-methylglucuronoxylan were easily obtained from the pulping. The polymers within these mixtures can be precipitated easily with ethanol, to yield more or less intact hemicelluloses.

The gel permeation chromatogram of the hemicelluloses, extracted from the steamed aspen at 180°C for 10 min, is illustrated in Figure 1. The elution maximum corresponded to a polysaccharide molecular weight of 6350. Elution profile of the hemicelluloses showed a wide polymolecularity, ranging from oligosaccharides up to polysaccharides of molecular weight over 150 000.

The FT-IR spectra of the three aspen hemicellulosic fractions, extracted with water from steam treated for 10 min at 180°C (a), 190°C (b), and 200°C, are shown in Figure 2. The spectral profiles and relative intensities of the bands among the three spectra were rather similar, indicating similar structures of the hemicelluloses. The occurrence of acetyl and 4-*O*-methylglucuronic ester groups of hemicelluloses was verified at 1740 cm^{-1}. The band at 1613 cm^{-1} is principally associated with the C=O stretch of carboxylic anions (salt) for uronic acids in hemicelluloses.[25] The prominent absorption at 1056 cm^{-1} is attributed to C–OH bending. The small sharp band at 897 cm^{-1} is characteristic of the β-glycosidic linkage between sugar units.[26]

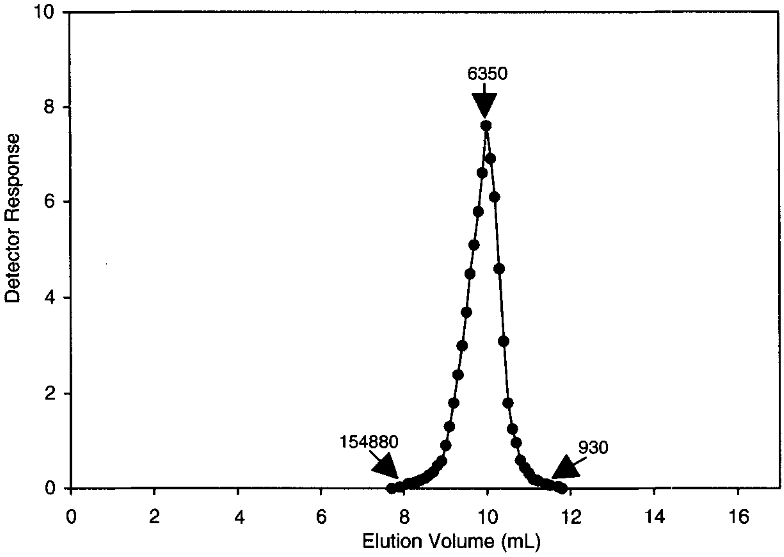

FIGURE 1 GPC molecular weight distribution of hemicelluloses obtained by steam treatment at 180°C for 10 min from aspen.

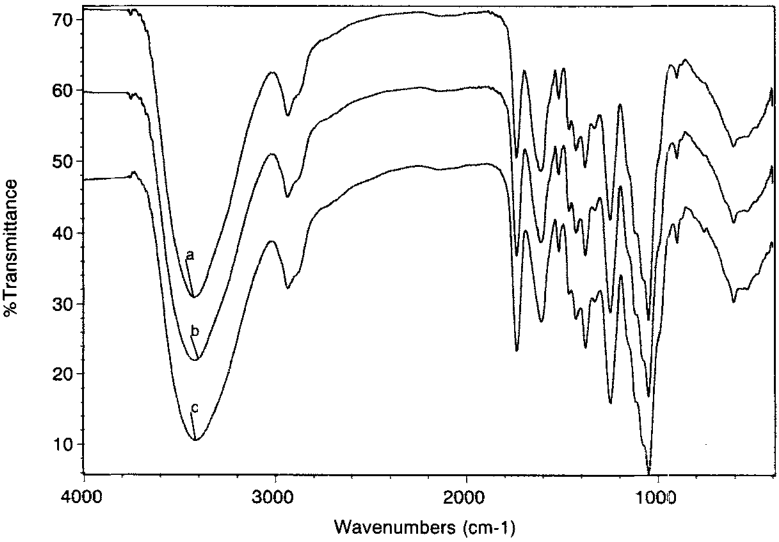

FIGURE 2 FT-IR spectra of hemicellulosic fractions obtained from aspen by steam treatment at 180°C for 10 min (a), 190°C for 10 min (b), and 200°C for 10 min (c).

The bands in the region of 1125–1000 cm^{-1} are typical of xylans. Two small bands at 1126 and 1078 cm^{-1} indicate the C–O and C–C stretching, and ring vibration and COH bending in xylans, respectively.[27] Additional evidence for the presence of carbonyl absorbance comes from the absorbance at 1249 cm^{-1}, which can be assigned to carboxylic acid O–H deformations. The low intensities of the bands at 1461, 1420, and 1375 cm^{-1} represent CH_2, CH and OH bending, and CH_2 and OH bending, respectively.[28] The occurrence of a small band at 1510 cm^{-1} in all three hemicellulosic spectra corresponds to the aromatic skeleton vibrations in associated lignin. The small band at 1335 cm^{-1} represents the syringyl ring breathing with CO stretching in lignin molecules. This confirmed again that all three isolated hemicellulosic preparations are contaminated with associated lignins, which corresponds to the results obtained by alkaline nitrobenzene oxidation.

Figure 3 shows the FT-IR spectra of two hemicellulosic fractions, extracted with water from steam-exploded birchwood. As mentioned above, the absorbances at 1734, 1627, 1467, 1422, 1375, 1249, 1056, and 897 cm^{-1} seen in the two spectra are assigned to hemicelluloses.

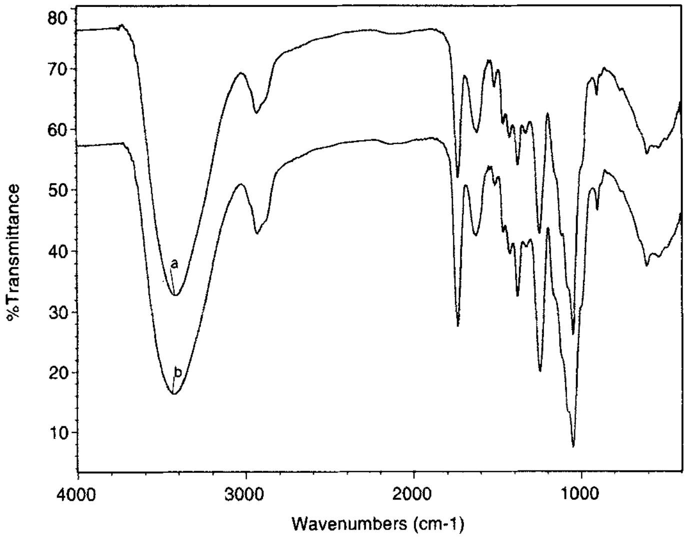

FIGURE 3 FT-IR spectra of hemicellulosic fraction 4 (a) and hemicellulosic fraction 5 (b) obtained by steam-explosion from birchwood.

The associated lignin absorbances appear at 1516 and 1328 cm^{-1}. A slightly higher intensity of these two bands in Spectrum a as compared to in Spectrum b, indicates a relatively higher amount of associated lignin in the hemicellulosic fraction, extracted from steam-exploded birchwood at 190°C, than in the fraction isolated at 210°C for 10 min.

CONCLUSION

The above results indicated that the three hemicellulosic fractions, obtained from steamed aspen, were more branched, as shown by the relatively higher content of mannose, glucose, galactose, and arabinose. In comparison, the extractions of steam-exploded birchwood yielded two hemicellulosic preparations, which were more linear as shown by a relatively higher content of xylose (72.9–81.3%). All five hemicellulosic fractions appeared to have a low molecular size (6610–9390) and associated relatively higher amounts of lignin (6.2–26.6%). A relatively higher amount of associated lignin (14.6–26.6%) was found in the isolated three steamed aspen hemicelluloses; whereas a relatively lower content of associated lignin (6.2–9.5%) appeared in the isolated two steam-exploded birchwood hemicelluloses. Further studies showed that lignin in the cell walls of aspen is associated with hemicelluloses mainly via syringyl and guaiacyl units, while in the cell walls of birchwood, lignin is associated to hemicelluloses mainly via syringyl units.

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