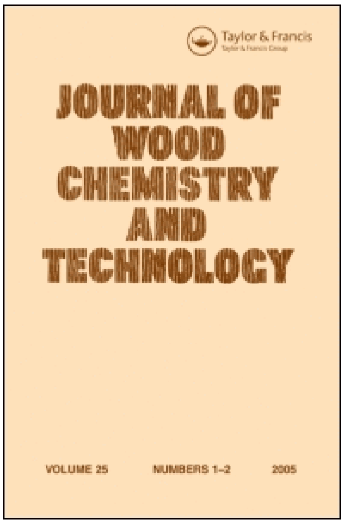

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Abstract: Dissolved aromatic substances in water suspensions of unbleached and peroxide-bleached spruce thermomechanical pulp (TMP) were isolated by sorption on XAD-8 resin and fractionated into lignin and oligomeric aromatic substances (OAS). The fractions were characterized by chromatographic (GC and HP-SEC) and spectrometric (FT-IR, ^{13}C-NMR, GC-MS) methods, as well as by GC after direct on-line pyrolysis and thermally assisted hydrolysis and methylation. The dissolved lignin in unbleached TMP water was structurally similar to spruce milled-wood lignin (MWL), but its average molar-mass was lower and the mass distribution more narrow. The oligomeric aromatic substances included phenolic dimers, trimers, and tetramers that were structurally different from those of MWL. After peroxide bleaching the amount of dissolved semipolar (MTBE) extractives and oligomeric aromatic substances was

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much lower. The amount of dissolved lignin from wood fibers was, on the contrary, much higher. The lignin released from TMP after bleaching was extensively oxidized and had a slightly higher average molar-mass than lignin released from unbleached TMP.

Keywords: Dissolved lignin, oligomeric aromatic substances, isolation, thermomechanical pulp, Norway spruce, chromatography, spectrometry

INTRODUCTION

A trend in mechanical pulping is going toward closed water systems; this is stimulated by both economical and ecological incentives. Normally, the amount of wood material released into the process water during mechanical pulping and bleaching is relatively small (2–5%). However, these organics, commonly called dissolved and colloidal substances (DCS or DisCo's), accumulate in the process waters upon re-circulation and cause problems regarding both production efficiency and product quality in papermaking.[1–5]

Major constituents of DCS from spruce TMP are hemicelluloses, acetic and pectic acids, and various lipophilic and semipolar extractives.[5,6]

Lipophilic extractives (fatty and resin acids, steryl esters, and triglycerides) can be extracted from TMP fiber and water samples with hexane. Semipolar extractives, mainly comprising aromatic compounds such as lignans and other low-molar-mass substances, can be isolated from TMP water by extraction with aprotic polar solvents (MTBE or diethyl ether). The composition of lignans in spruce wood and DCS, that is, mainly C_β-C_β coupled dimers of guaiacylpropanoid derivatives, is well documented.[7–9] Two diastereoisomers of hydroxymatairesinol are the predominating lignans in TMP water from Norway spruce.

The nature of other semipolar aromatic substances that are non-extractable with MTBE is still not quite clear. In previous reports,[10–11] these substances were ambiguously twisted between "lignin-like substances" and "lignin." Some researchers, however, concluded that there is no lignin similar to milled-wood lignin in TMP water, and it was called hydrophilic lignin[12] or lignosaccharides and lignan-related substances.[13] Lignan-related substances seem to exist in wood in a wide molecular mass range from oligomers to polymers. The polymer fraction of lignans may correspond to Brauns lignin (termed polylignan by the authors).[14] Very recently β-O-4-linked guaiacylglyceryl ethers of many different lignans, called oligolignans, were identified in spruce and pine knotwood extracts.[15] The optical activity of lignans is a "corner stone" in the hypothesis considering their different biogenesis from that of optically inactive "normal" lignin.[9] It has been suggested that the lignin biopolymer has cell wall structural roles, whereas oligomeric lignans are non-structural components.[9]

During peroxide bleaching of mechanical pulps the amount of lignans in TMP water is substantially decreased.[11,16] On the other hand, peroxide bleaching results in higher overall loss of fiber material, probably also due to lignin material leaching.[6,11] Recently it was shown that aromatic substances are released from spruce TMP fibers into water in considerable amounts (up to 10 mg/g TMP at alkaline conditions, with and without hydrogen peroxide).[17]

It is essential to use a selective procedure for quantitative isolation of lignin in pure and unaltered form. One way to isolate dissolved aromatics in TMP water selectively is using adsorption chromatography.[1,13,18–19] Based on the hydrophobic properties of dissolved aromatics, this method allows concentration of aromatic substances from dilute process waters, and their separation from other, more hydrophilic solutes, such as hemicelluloses, pectins, low molecular weight acids, and inorganic salts.

A C_8 stationary phase bound to silica gel can be used as an adsorbent to isolate hydrophobic substances from TMP process waters.[13] Solid-phase extraction (SPE) with C_{18} cartridges has also been tested for the isolation of dissolved lignin from various natural waters (fresh, estuarine, and marine).[20] In many other related publications, organic macroporous resins are reported to be the best column material for the isolation of water-soluble aromatics on a preparative scale.[1,18–19]

Here we report on the isolation and detailed characterization of lignin and other oligomeric aromatic substances dissolved in mechanical pulping and alkaline peroxide bleaching of spruce TMP. Comparison was made to MWL and Brauns lignin extracted from unbleached spruce TMP.

RESULTS AND DISCUSSION

Isolation and Fractionation of Aromatic Substances

As much as 10 mg/g TMP of the lipophilic extractives were extracted from the TMP (Figure 1). Almost no colloidal substances were released upon suspension of pre-extracted (lipophilic-free) TMP. Extraction of water from suspended unbleached TMP (UTMP) with MTBE gave 2.9 mg/g TMP of semipolar extractives, which were mainly lignans (about 80% w/w of the extract) (Figures 1 and 2). Lignans are known to be degraded in hydrogen peroxide bleaching.[11] Accordingly, peroxide-bleached TMP (BTMP) gave only 1.0 mg/g TMP of MTBE extractives.

The amount of hydrophobic substances isolated by XAD-8 resin from BTMP water (5.8 mg/g TMP) was twice the amount obtained from UTMP water (2.7 mg/g TMP) (Figure 1). Both samples of hydrophobic substances were further fractionated into a carbohydrate-rich fraction (substances insoluble in methanol), a fraction containing mainly oligomeric aromatic substances (OAS), and a fraction containing predominantly water-soluble low-molar-mass lignin.

The total amount of semipolar substances (i.e., the sum of MTBE extract and XAD-sorbed hydrophobic material) was 5.6 mg/g TMP for the UTMP

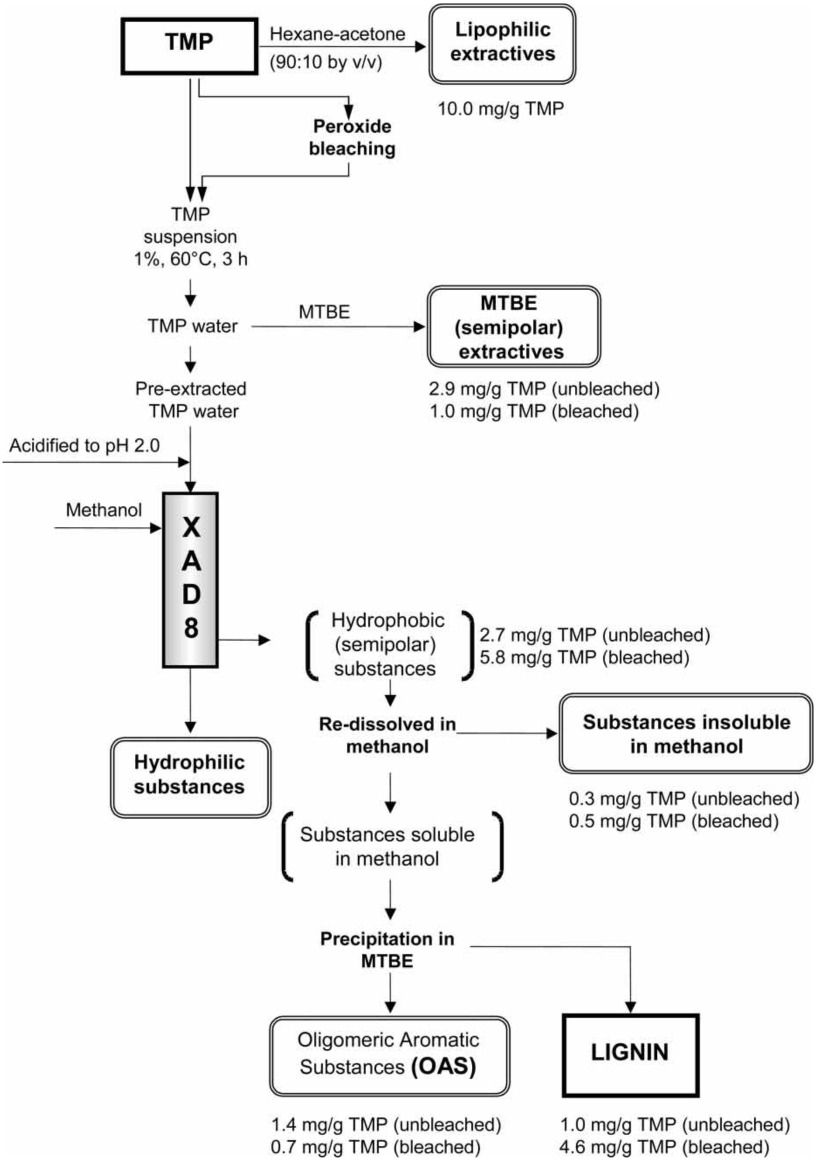

Figure 1. TMP-water preparation, and isolation and fractionation of aromatic substances from unbleached and bleached TMP.

water, and 6.8 mg/g TMP for the BTMP water. However, much more lignin was obtained from the BTMP water (4.6 mg/g TMP) than from the UTMP water (1.0 mg/g TMP) (Figure 1). The lignan composition in the OAS fraction was essentially the same as in the MTBE extractives from

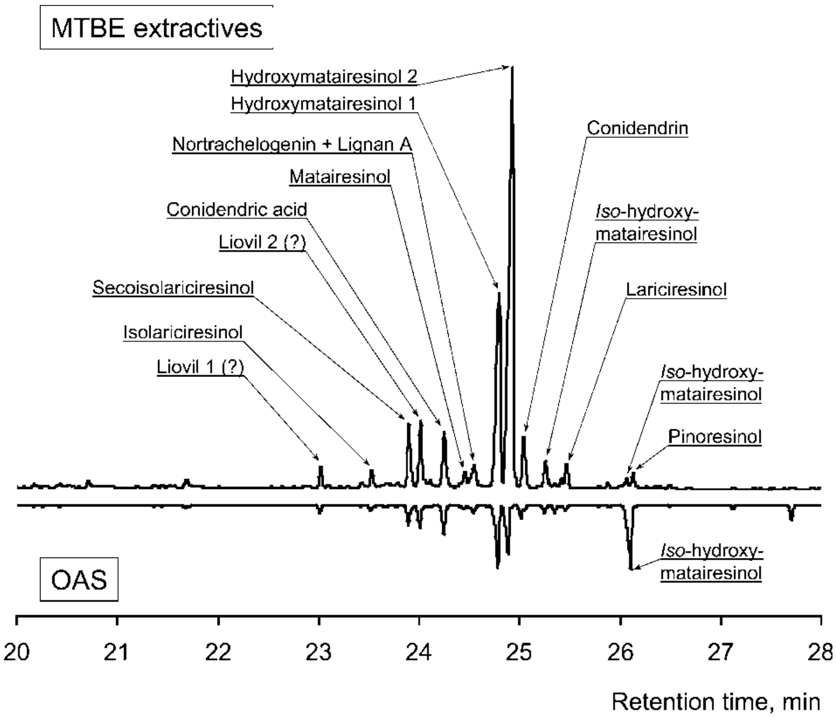

Figure 2. GC of lignans in the MTBE-extract and the fraction of oligomeric aromatic substances from unbleached TMP water.

unbleached TMP water (Figure 2). However, the proportion of the main lignans constituents was different, particularly regarding the iso-hydroxymatairesinols (identified by GC-MS[21]).

Purity of Lignin and OAS Fractions

Purification of the lignin preparations from both UTMP and BTMP waters (named unbleached lignin and bleached lignin) by multiple precipitation in MTBE resulted in low amounts of residual extractives (about 3% of the lignin preparation), consisting predominantly of lignans. In the OAS fractions, mainly low-oligomeric compounds were accumulated. Both OAS (unbleached OAS and bleached OAS) fractions contained about 8% of lignans (Figure 2).

Analysis by methanolysis and GC demonstrated that the lignin and OAS fractions contained only small amounts of carbohydrates. Unbleached lignin contained 1.6% carbohydrates (mostly glucose units), whereas bleached lignin contained 0.5% of carbohydrates (almost only glucose units). The

OAS fractions contained even less carbohydrates. The glucose in the lignin and OAS fractions most probably originated from glucosides. Indeed, trace amounts of a few stilbene glucosides (for instance, isorhapontin) were detected in unbleached OAS by GC-MS after their concentration on TLC plates. Unbleached lignin contained, in addition to the predominant glucose, also mannose and galactose units. The lignin and OAS fractions from BTMP water contained much less carbohydrates than the corresponding fractions from UTMP, and included almost only glucose. This indicates the carbohydrates in unbleached lignin and unbleached OAS fractions are linked by alkali-sensitive bonds.

A small fraction of carbohydrate-rich material, which was adsorbed on the XAD-8 column and eluted by methanol, but which was not soluble in methanol after freeze-drying (see Figure 1), contained 11% of carbohydrates with mannose, glucose and galactose in the ratio 4.8 : 1.3 : 1.

The spruce MWL contained 0.9% carbohydrates with mannose (0.2%), galactose (0.3%), and glucose (0.4%) sugar units.

Size-Exclusion Chromatography

The lignin preparations differed from MWL in molar-mass average and polydispersity (Table 1, Figure 3). Unbleached lignin exhibited a lower weight-average mass ($\overline{Mw}$) than bleached lignin, with $\overline{Mw}$ values of 1470 Daltons and 1770 Daltons, respectively, corresponding to an average number of phenyl propane units in the range of 5–8. These $\overline{Mw}$ values were much lower than for MWL (5320 Daltons) and even for Brauns lignin (2290 Daltons),

Table 1. Molar-mass characteristics of lignins from SEC analyses of acetylated samples

	MWL	Brauns lignin	Unbleached lignin	Bleached lignin	Unbleached OAS	Bleached OAS
Weight average mass, $\overline{Mw}$	5320	2290	1470	1770	820	680
Number average mass, $\overline{Mn}$	2110	1750	1140	1120	670	580
$\overline{Mw}/\overline{Mn}$	2.52	1.31	1.28	1.58	1.21	1.17
C_9-units, weight average	24	10	7	8	4	3
C_9-units, number average	10	8	5	5	3	3

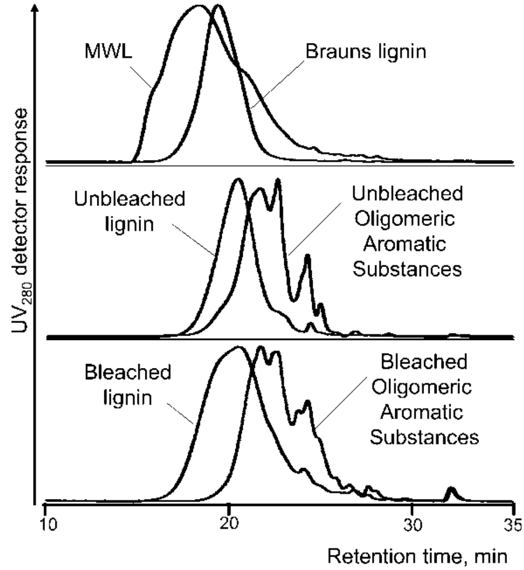

Figure 3. HP-SEC of acetylated lignins and oligomeric aromatic substances.

but higher than for the OAS fractions (820 Daltons for unbleached OAS and 680 Daltons for bleached OAS). The OAS fractions were composed mainly of dimeric, trimeric, and tetrameric phenolic compounds.

The MWL exhibited a much broader molar-mass distribution than the other lignin preparations (Figures 3 and 4). However, the unbleached lignin had a more narrow mass distribution than the bleached lignin (Table 1).

It has been shown that lignin material is readily released from pre-extracted and thoroughly water-washed TMP fibers at mildly alkaline conditions (pH higher than 10 and temperature 50–60°C).[17] For instance, a 1-h treatment of "clean" TMP at pH 11 and 60°C released 3.1 mg/g TMP lignin into water and; after 6 h the release was 7.3 mg/g TMP. Cleavage of the most alkali-sensitive bonds (such as esters in lignin-carbohydrate complexes (LCC) or esters at C_α in the side chains of phenyl propane units) can partly explain the alkali-induced lignin dissolution.[22–24] Probably lignin dissolution from TMP fibers is also governed by diffusion limitations, which are related to swelling of the fiber material.[25] At alkaline conditions larger lignin molecules can migrate into the water phase.

The effect of hydrogen peroxide on lignin dissolution is even more drastic than that of only alkali. The yields after 1 h of treatment were almost doubled with hydrogen peroxide compared to those with mere alkali.[17] Peroxide probably cleaves the lignin macromolecule.[26] However, the effect of the formed carboxylic groups on additional fiber swelling and, hence, on the dissolution of larger lignin molecules should also be taken into consideration.

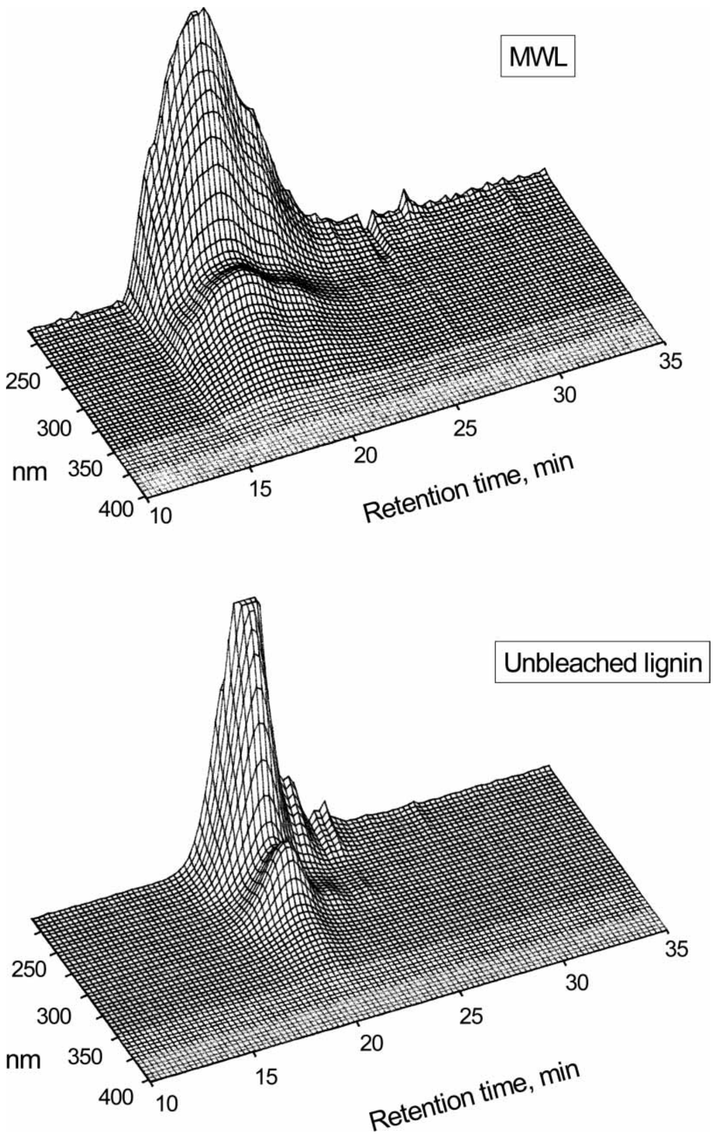

Figure 4. 3D HP-SEC of acetylated lignins (diode-array detector).

This is probably a reason why the bleached lignin exhibited an additional shoulder on the main peak in the higher molar-mass region of the SEC chromatogram (Figure 3). This causes notable broadening of the mass distribution compared to that of unbleached lignin.

The main peak in the SEC chromatogram of unbleached lignin (Figures 3, 4) is close to the low-molar-mass shoulder on the dominating peak in the chromatogram of MWL. Probably, the low-molar-mass constituents of MWL correspond to the hot-water-soluble fraction, that is, unbleached lignin.

IR Analysis

The IR spectra of all studied lignin preparations were rather similar (Figure 5). The only differences were found in the region 1600–1750 cm^{-1} and about 1400 cm^{-1}, particularly in the absorption bands of carbonyl groups. The absorption band about 1600 cm^{-1}, which is assigned to aromatic skeletal vibration and carbonyl stretch,[27] is much broader and abundant for bleached lignin versus other lignin preparations. This is probably due to strongly overlapped intensive bands assigned to carbonyl originating from carboxylate ion (asymmetrical stretching band).[8,28] The presence of carboxylic groups in bleached lignin is also supported by notable broadening of the band at about 1400 cm^{-1}, which is due to an increase in the intensity of symmetrical stretching band.

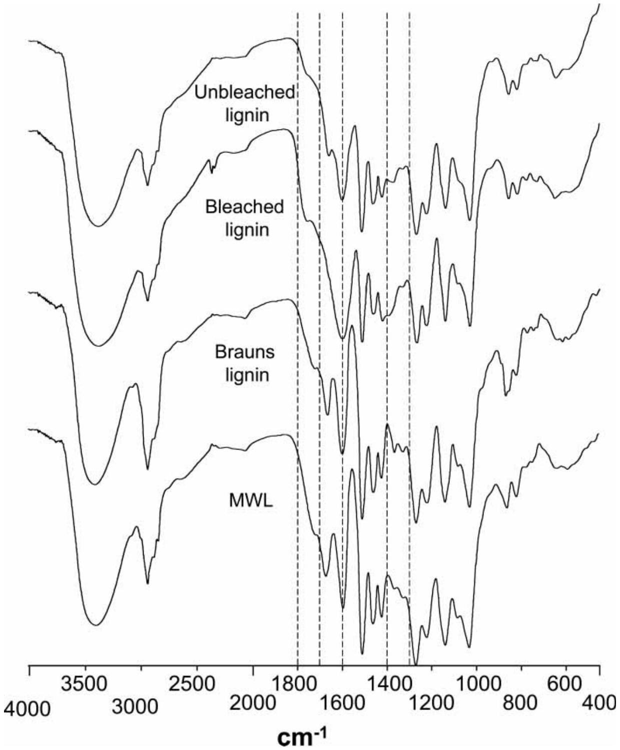

Figure 5. IR-spectra of lignins.

In all IR spectra, except for that of bleached lignin, a more or less pronounced band exists in the carbonyl region around 1660 cm^{-1} assigned to carbonyl stretch, particularly in conjugated p-substituted aryl ketones.[27] Most probably that band disappears from the spectrum of lignin from BTMP waters due to oxidation of the lignin at the carbonyl group, which is known to be one of the main mechanisms, in hydrogen peroxide bleaching.[26]

Interestingly, the band related to unconjugated carbonyl at 1720 cm^{-1} is seen only in spectra of MWL and Brauns lignin. On the other hand, a low intensity absorption band (as a shoulder) due to γ-lactone[14] is observed in spectra of both unbleached and bleached lignins at 1750 cm^{-1}, but not in the spectra of MWL and Brauns lignin. These γ-lactone structures probably originate from the lignan impurities in the isolated lignin preparations. The γ-lactone structure occurs in hydroxymatairesinol, which is the most abundant lignan in spruce.[7] At mildly alkaline conditions it will be transformed to α-conidendrin.

^{13}C NMR Analysis

All ^{13}C NMR spectra of lignin preparations were quite similar (Figure 6). Contrary to the spectrum of MWL, however, some additional sharp peaks could be observed in the spectra of both unbleached and bleached lignins, probably due to residual lignans. The presence of lignans is confirmed by

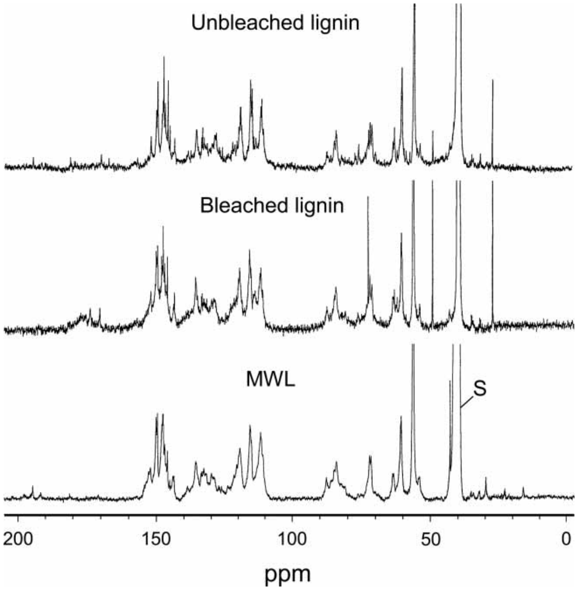

Figure 6. ^{13}C-NMR spectra of lignins.

sharp peaks at 27 ppm (probably, C_α in β-β units), at 47 ppm (C_β in β-β units), 72 ppm (C_γ in β-β units), and 75 ppm (C_α with C_α-OH in β-β units).[29–30] The broad signal in the range 170–180 ppm in the spectrum of the bleached lignin can be assigned to carboxylic groups.[29]

The most specific feature for lignin substances is the β-O-4 interunit linkage.[31] The signals in the range 60 to 90 ppm (assigned predominantly to the C_β, C_α, and C_γ carbons in β-O-4 units[29]) were similar in all spectra.

Quantification of ^{13}C-NMR signals showed that bleached lignin contained less methoxyl groups and aromatic units and much more carbonyl groups, especially carboxyl groups than MWL and unbleached lignin (Figure 7).

According to Pan et al., who studied the behavior of spruce thermomechanical pulp lignin during hydrogen peroxide bleaching, bleaching seems not to affect the macromolecular structure of lignin to any noticeable extent.[32] The observed discrepancy to our results is probably due to the fact that dissolved lignin (lignin dissolution is known to be extensive at alkaline conditions) is more accessible to the bleaching reagents than lignin in TMP fibers. On the other hand, the oxidation of TMP lignin in hydrogen peroxide bleaching will result in the formation of new hydroxylic and carboxylic groups. In spite of the significance of peroxide-induced lignin depolymerization, some low-molecular part of the oxidized lignin moiety probably becomes more soluble in the water phase. Also, that part of the oxidized lignin may be preferably washed out from the fibers and accumulate in the bleach water.

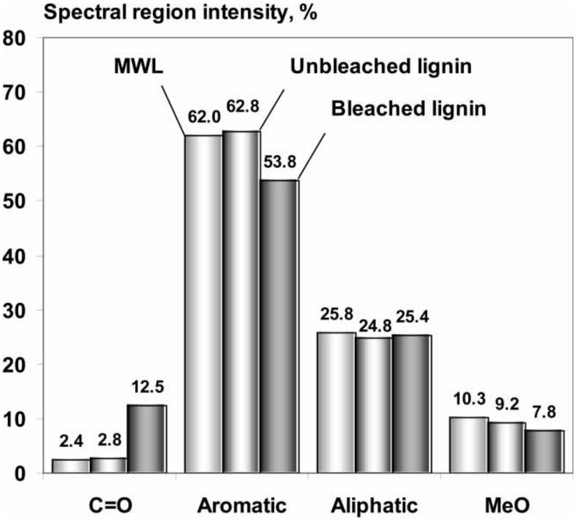

Figure 7. Relative ^{13}C-NMR intensities of lignins within different spectral regions.

Pyrolysis-Gas Chromatography-Mass Spectrometry

Even if the lignin degradation mechanism during pyrolysis (thermolysis) is not yet fully understood, the method has proven useful for comparison of lignins of different origin.[33] Direct "on-line" Py-GC-MS analysis confirmed similarities of MWL and both lignins from UTMP and BTMP waters, giving guaiacol, methylguaiacol, ethylguaiacol, vinylguaiacol, and iso-eugenols as major pyrolysis products (Figure 8). In addition to the pyrolysis products from unbleached lignin, the bleached lignin gave more vanillin and homovanillin compounds. This probably reflects oxidative changes in bleached lignin, particularly side-chain cleavage with formation of aromatic aldehydes.[26] Brauns lignin produced a more complex pyrogram, clearly differing in pattern from those of MWL and unbleached lignin (Figure 8), containing larger peaks for methylguaiacol, eugenol, vanillin, homovanillin, and acetoguaiacone than other lignins.

It is relevant to note that some effects of inorganic moieties on the pyrolysis pathways has been recognized and become of critical importance when the method is employed as an analytical tool.[10,12] In order to reduce or eliminate the effects of inorganic salts, and probably to stabilize the low-molar-mass lignin, acetylation of isolated lignin preparations has been recommended.[10]

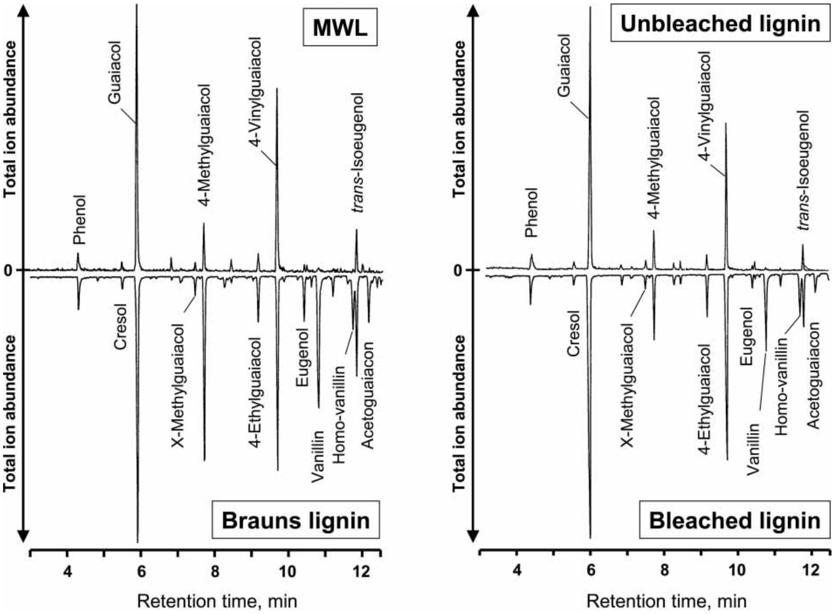

Figure 8. Py-GC-MS (TIC) of different lignins.

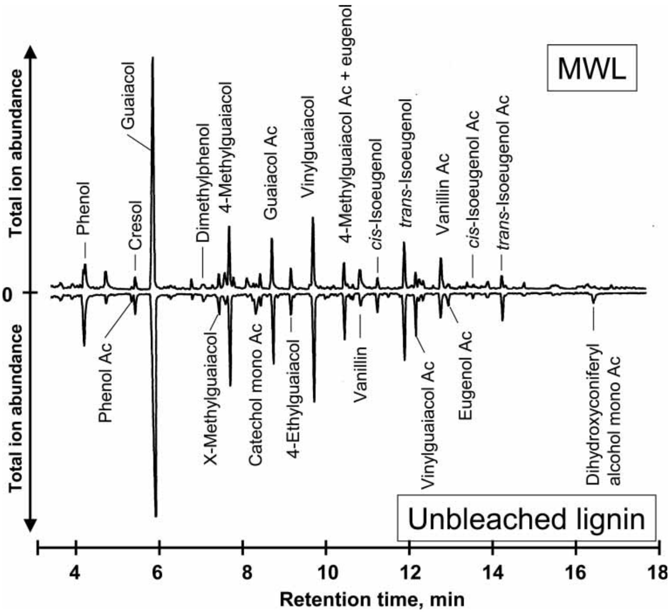

Figure 9. Py-GC-MS (TIC) of acetylated MWL and acetylated lignin from unbleached TMP water.

The pyrograms of acetylated lignins exhibited several structural similarities between MWL and unbleached lignin (Figure 9), but were somewhat different from those of unbleached OAS. In spite of the simplicity and semiquantitative character of the data obtained from pyrograms by the normalization method, it is still possible to draw some tentative conclusions about structural differences in different samples (Table 2). Particularly, based on the assumptions that acetyl groups do not cleave from the volatile pyrolysis product at the applied conditions, and that the percentage of acetylated products in pyrolysates is proportional to the number of free phenolic hydroxyl groups in the starting material, it can be possible also to tentatively examine the origin of lignin and OAS preparations.

For acetylated MWL the ratio of acetylated phenolic pyrolysis products to free phenolic pyrolysis products was 23/77, whereas it was 29/71 for acetylated unbleached lignin (Table 2). Assuming that both dissolved lignin and MWL are polymerized mainly by aryl ether bonds, the finding that the high-molar-mass MWL had less acetyl groups than the low-molar-mass dissolved lignin is logical.[10] Accordingly, the corresponding ratio was found to be highest for acetylated unbleached OAS, and estimated to be 41/59.

The origin of p-hydroxyphenyl structures, particularly of phenol and cresol, among the products is still unclear.[10] Their appearance varies in the range 4–8% based on the sum of eluted compounds and may be explained by thermal demethoxylation of guaiacyl units, or/and by the fact that they

Table 2. The peak assignment and their proportion in the chromatogram from Py-GC-MS of different acetylated lignins and oligmeric aromatic substances

	MWL, %		Unbleached lignin, %		Unbleached OAS, %	
	Acetylated	Non-acetylated	Acetylated	Non-acetylated	Acetylated	Non-acetylated
Phenol	0.3	2.2	0.8	5.3	0.6	2.1
Cresol		1.5		2.1		1.7
Guaiacol	5.8	27	7.1	22.6	4.7	11.5
Dimethylphenol		0.5		0.8		0.6
x-Methylguaiacol		1.9		2		1.7
4-Methylguaiacol		7.4		9.3		6.5
Catechol mono-Ac	1.3		1.3		1	
4-Ethylguaiacol		2.6		2.2		1.9
4-Vinylguaiacol	1.9	8.3	4.5	11.2	2.7	5.5
Methylcatechol mono-Ac			0.3		1.3	
4-Methylguaiacol-Ac + eugenol	2.9		4.7		8.3	
Eugenol Ac	0.5		1.4			
Vanillin	3.5	2.2	2.5	1.4	3.3	1.1
cis-Isoeugenol	0.2	1.3	0.6	2	0.7	1
trans-Isoeugenol	1.4	5.4	2.8	6.8	2.7	3.6
Dehydroconiferyl alcohol mono-Ac	0.3		1.1		0.1	
SUM	18.1	60.4	27.1	65.7	25.4	37.2
Unknown	21.4		7.3		37.4	
Acetylated/Non-acetylated, %	**23**	**77**	**29**	**71**	**41**	**59**

originate from compression wood tissues, known to be enriched in p-hydroxyphenyl structures (Table 2).[34]

Thermally Assisted Hydrolysis and Methylation with Combined Gas-Chromatography and Mass-Spectrometry

Because the structural information obtained from lignin by direct pyrolysis is quite limited, a modified analytical pyrolysis (thermolysis) at lower temperature and in the presence of tetramethylammonium hydroxide (TMAH), that is, so-called thermally assisted hydrolysis and methylation (THM),[35,36] was also applied in this study (Figure 10). It verifies the similarity in chemical structure of MWL and unbleached lignin. Differences were observed in the proportion of degradation products of different lignin and OAS preparations (Table 3). By normalization of peaks in the chromatograms it was found that the sum of two diastereomeric 1-veratryl-1,2,3-trimethoxy propane derivatives **20** and **21** from both unbleached and bleached lignins as well as MWL were 6.9–7.6% of the products formed. In both unbleached and bleached OAS these two diastereomers were found in much smaller quantities, that is, 1.6% and 0.9%, respectively.

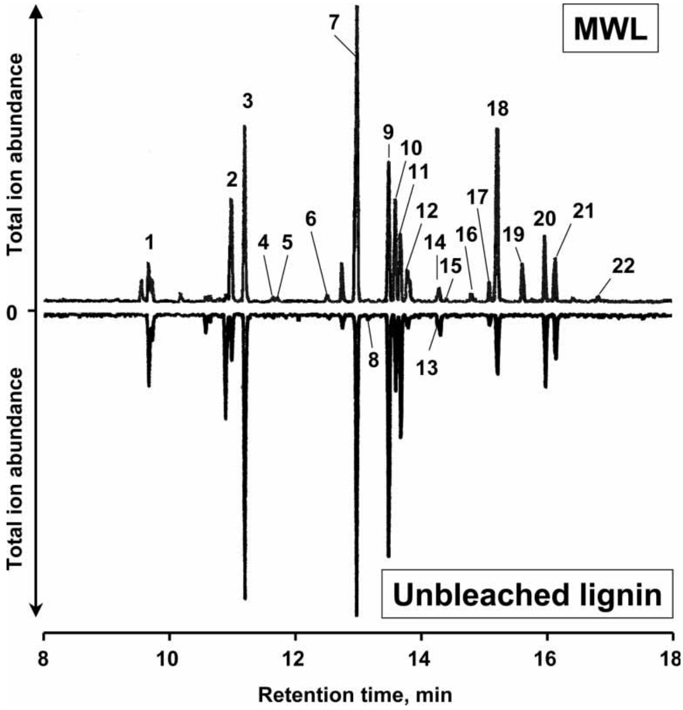

Figure 10. THM-GC-MS (TIC) of MWL and lignin from unbleached TMP water.

Table 3. Identities of the main peaks and their proportion in chromatograms from THM-GC-MS of different lignins and oligomeric aromatic substances

		% of total peaks area				
Peak number	Formula[a]	MWL	Unbl. lignin	Bleached lignin	Unbl. OAS	Bleached OAS
1	$\mathrm{V{-}CH{=}CH_2}$	2.7	4.6	5.7	10.3	9.5
2	$\mathrm{V{-}CH_2OMe}$	7.2	3.0	4.1	5.7	2.1
3	$\mathrm{V{-}CHO}$	12.2	18.1	9.0	14.2	8.1
4	$\mathrm{V{-}CH_2{-}CHO}$	0.2	0.0	1.1	1.5	1.1
5	$\mathrm{V{-}CH{=}CH{-}CH_3}$ (trans)	0.2	0.3	0.0	0.0	0.0
6	$\mathrm{V{-}CO{-}CH_3}$	0.5	0.4	1.1	0.6	1.8
7	$\mathrm{V{-}COOMe}$	20.4	19.4	26.7	15.0	18.9
8	$\mathrm{V{-}CH{=}COMe{-}CH_3}$	0.0	0.3	0.0	0.2	0.3
9	$\mathrm{V{-}CH{=}CHOMe}$ (cis)	9.7	15.3	6.3	7.4	6.9
10	$\mathrm{V{-}CH_2{-}CH_2{-}CH_2OMe}$	7.1	4.9	7.1	7.9	13.2
11	$\mathrm{V{-}CH{=}CHOMe}$ (trans)	4.7	7.9	3.5	4.3	3.2
12	$\mathrm{V{-}CHOMe{-}CH_2OMe}$	2.3	0.9	1.4	3.1	3.5
13	$\mathrm{V{-}COMe{=}CH{-}CH3}$ (cis)	0.0	0.7	0.0	0.0	0.0
14	$\mathrm{V{-}COMe{=}CH{-}CH3}$ (trans)	0.9	1.5	1.1	1.4	1.3
15	$\mathrm{V{-}CH{=}COMe{-}CH2OMe}$	0.5	0.0	0.0	0.7	1.8
16	$\mathrm{V{-}CHOMe{-}CH_2{-}CH_2OMe}$	0.7	0.0	0.4	0.5	0.4
17	$\mathrm{V{-}CH_2{-}CHOMe{-}CH_2OMe}$	1.4	0.7	1.9	0.9	1.3
18	$\mathrm{V{-}CHOMe{-}COOMe}$	12.0	3.8	6.1	11.3	3.5
19	$\mathrm{V{-}CH(CH_3)OMe{-}COOMe}$	2.7	0.0	5.0	3.8	3.5
20	$\mathrm{V{-}CHOMe{-}CHOMe{-}CH_2OMe}$	**4.5**	**4.7**	**4.2**	**0.9**	**0.4**
21	$\mathrm{V{-}CHOMe{-}CHOMe{-}CH_2OMe}$	**2.9**	**2.9**	**2.7**	**0.7**	**0.5**
22	$\mathrm{V{-}CH{=}CH{-}COOMe}$	0.5	0.0	0.0	0.0	0.0
	Unknown compounds	6.8	10.6	12.6	9.7	18.6

[a] V-veratryl (3,4-dimethoxyphenyl).

Hardell and Nilvebrandt suggested that these diastereomeric glycerol derivatives are formed from β-ether-linked guaiacylpropane units under THM conditions.[36] Thus, the similar yield of diastereomeric glycerol derivatives confirmed the structural similarity between MWL and both unbleached and bleached lignins, in respect to β-O-4 bond occurrence. In bleached lignin these diastereomers constituted about 6.9% of the products formed, which is marginally less than for the other lignins. This is probably due to marginal cleavage of β-ether links during peroxide bleaching.

Comparison of lignins and OAS preparations clearly showed their different origin.

Veratric acid methyl ester, that is, the methylated derivative of vanillic acid ester, was the pre-dominant degradation product for all studied samples during the THM process (Table 3). Higher contents of veratric acid methyl ester among the corresponding degradation products in bleached lignin/OAS compared to unbleached lignin/OAS can be due to peroxide-induced oxidation of side chains in aromatic moieties.

The THM technique, even in the case of MWL analysis, yields a very high proportion of carboxylic-acid-containing degradation products **7**, **18**, **19**, comprising 35% of all degradation products formed. According to well-established statements[31] and our own analyses, the native MWL preparation did not contain such large amounts of carboxyl groups. This phenomenon has been recognized earlier and suggests formation of carboxylic acids by autoxidation reactions, especially at sub-optimal conditions.[36] The autoxidation is probably promoted by the strong alkaline medium during thermally assisted hydrolysis and methylation. In conventional pyrolysis, however, those carboxylic acids are generally not detected due to decarboxylation.[37] It is also possible that guaiacol, the most dominant degradation product in conventional pyrolysis (see Figures 8 and 9), is derived from vanillic acid intermediate. It seems that autoxidation is one of the main reaction pathways during thermo (chemo)lysis of aromatics. However, these carboxylic acids (originally present or formed) were trapped by methylation and could be detected using THM-GC-MS.

CONCLUSIONS

During TMP production from Norway spruce about 6 mg/g TMP (based on dried pulp) of aromatic substances were released into water. Half of the aromatic moiety were extractable with MTBE and comprised predominantly lignans. After MTBE extraction of the monomeric phenolics and dimeric lignan-type aromatics the water still contained semipolar compounds, particularly oligomeric aromatic substances, and about 1 mg/g TMP of dissolved lignin. That lignin, that is, unbleached lignin, was structurally similar to MWL, but had a lower molar mass. The average number of phenyl propane units was predominantly 5–8. Unbleached lignin contained small amounts of carbohydrates (1.6% of lignin fraction), comprising predominantly glucose with some mannose and galactose units. It also contained about 3% of extractives contaminants, comprising mainly lignans.

The oligomeric aromatic substances dissolved in TMP-waters consisted mainly of dimeric, trimeric and tetrameric phenolics, which were structurally different from those in MWL. This unbleached OAS fraction contained about 0.6% of carbohydrates, of which 0.5% were glucose units, and about 8% of residual lignans.

About 7 mg/g TMP (based on dried pulp) of aromatic substances were found in the water after production and hydrogen peroxide bleaching of TMP fibers. Compared to the TMP process alone, peroxide bleaching reduced the amount of MTBE extractives to one third, and the amount of OAS to one half, but lead further up to fivefold dissolution of lignin. This bleached lignin was structurally close to MWL. It was oxidized to some extent, and exhibited a somewhat higher molar-mass average than dissolved lignin from unbleached TMP. It also contained less carbohydrate impurities than unbleached lignin.

The oligomeric aromatic substances dissolved in alkaline peroxide bleaching of TMP were also of low molar mass, and contained the same amount of lignans (based on fraction amount), but much less carbohydrates than those of unbleached TMP.

Changes in both the composition and the amount of the carbohydrate impurities during peroxide bleaching suggested that lignins and OAS dissolved in TMP water contain alkali-sensitive bonds between the aromatic moieties and the carbohydrates.

EXPERIMENTAL

Materials

Thermomechanical pulp from Norway spruce (*Picea abies* L.) was sampled during the winter in a Finnish mill, after the second stage refiner. The pulp consistency was about 35%, and the Canadian standard freeness (SCAN-M4) about 140 mL. The pulp was stored in a freezer at −24°C until needed.

Glass fiber filters GF 50 were obtained from Schleicher and Schuell GmbH (Dassel, Germany). Macroporous polyacrylate resin, Amberlite XAD-8, 20–50 mesh and tetramethylammonium hydroxide (25% in methanol, approx. 2.2 M) (TMAH) was obtained from Fluka Chemie AG (Buchs, Switzerland). Mono-disperse polystyrene standards Kit for molecular mass determination (Mw from 600 to 1460000 Daltons) was obtained from Perkin Elmer, Inc. (Norwalk, Connecticut, USA). HPLC-grade methyl-t-butyl ether (MTBE) and tetrahydrofuran (THF) were obtained from LabScan Ltd. (Dublin, Ireland). Other chemicals were of *pro analysis* grade. The spruce Milled Wood Lignin (MWL) was a gift from Dr. J. Gravitis (Latvian State Institute of Wood Chemistry, Riga).

Methods

TMP Extraction

TMP was freeze-dried and extracted in a Soxhlet apparatus with hexane-acetone (90:10 v/v) for 24 h to remove all lipophilic material.

Bleaching

Extractive-free TMP was bleached in the laboratory with alkaline hydrogen peroxide in polyethylene bags (20 g o.d. pulp, 10% consistency, 3% H_2O_2, initial pH 11.1–11.3) at 60°C during 90 min. 0.2% DTPA, 0.05% $MgSO_4$ and 3% sodium silicate were also added. After bleaching the suspension was acidified with SO_2-water to pH 5.5. In order to obtain a representative sample of bleached TMP, five separate bleachings were performed. After bleaching and acidification, the five pulp suspensions were combined into one.

TMP Water

The pre-extracted TMP (Figure 1) was suspended in distilled water (pH 5.5) at 60°C at 1% consistency and agitated for 3 h with a square-blade impeller (about 150 rpm). The TMP water, containing dissolved substances, was pipetted off after the centrifugation of the suspension at 1500 rpm during 30 min. The TMP was re-suspended in distilled water and the agitation procedure was repeated as explained earlier. The centrifuged TMP waters were then combined.

The same procedure was used to produce TMP water from peroxide-bleached TMP.

For easier handling of the combined water sample, it was concentrated about 15 times by evaporation at reduced pressure (water pump) in a water bath at 40°C. To remove micro-fines, the concentrate was passed through a glass fiber filter (GF 50).

To remove most of the lignans the concentrate was extracted five times with MTBE (1:1 v/v). The combined MTBE extract was dried with anhydrous sodium sulfate, then concentrated in a rotary-evaporator, and finally the extractives were dried in a vacuum oven at 40°C.

After extraction, MTBE was purged from the concentrate with nitrogen. The complete of purging was verified by measuring the TOC value of the concentrate.

Brauns Lignin

Brauns lignin was isolated from pre-extracted (with cold water and diethyl ether) TMP by extraction with 95% ethanol in a flask at room temperature for 10 days. The extract was then purified according to the original description.[38]

Preparation of XAD-8 Resin Column

The XAD-8 resin was washed and extracted to remove microparticles and low-molar-mass contaminants according to Sjöström.[1]

About 220 mL of dry XAD-8 was suspended in 0.1 M NaOH and stirred with a Teflon-coated magnetic bar at room temperature for 1 h. The solution together with microparticles was decanted off and discarded. The alkaline washing procedure was repeated twice, and then the resin was quickly washed three times with distilled water. The same washing procedure was repeated three times with 0.1 M HCl. The XAD-8 resin was rinsed five times with distilled water and then twice with methanol.

The washed resin was sequentially extracted in a Soxhlet apparatus for 24 h with each of the following solvents: methanol, acetone, diethyl ether, and again, methanol. The resin was kept in methanol until needed.

Before packing into the column, the washed and extracted resin was rinsed five times with distilled water and three times with 0.01 M HCl. The resin was kept overnight in 0.01 M HCl and on the next day packed into a 30 mm i.d. × 300 mm length glass column. On the top, the column was equipped with a 2-L separation funnel as a reservoir for the mobile phase.

Prior to analysis, the column was conditioned by elution in cycles with 0.01 M HCl, 0.1 M HCl, distilled water, 0.1 M NaOH and distilled water until the Total Organic Carbon (TOC) released into 0.01 M HCl was below 1 mg/L.

Isolation of Aromatic Substances

The concentrate of pre-extracted TMP water was acidified to pH 2.0 with diluted HCl and passed immediately through the column of XAD-8 in 0.01 M HCl (Figure 1). The acidic effluent contained mainly carbohydrates. The column was washed with about 10 bed volumes of acidified (precisely adjusted to pH 2.0 with HCl) distilled water. Finally, aromatic substances were desorbed from the resin with 5 bed volumes of methanol. Because the pH value was low, isolation of aromatic substances was performed in as short time as possible.

Distilled water was added to the methanol effluent to about one third of the initial volume. The solution was neutralized with 0.01 M NaOH to pH 6. The neutralized methanol-water solution was evaporated at 40°C under vacuum and dried in a vacuum desiccator at 40°C. pH was measured during evaporation and was adjusted, if needed.

Fractionation of Aromatic Substances

The dried product was re-dissolved in methanol to a 10% solution, and the solution was centrifuged and filtered through a GF 50 glass fiber filter. Solids on the filter, containing carbohydrate-rich material, were washed out with distilled water into a separate flask and freeze-dried. The clear methanol solution was dropped slowly into 19 times its volume of MTBE with vigorous stirring and left overnight to coagulate. The clear solution was decanted and the precipitate placed into a centrifuge tube with MTBE. After centrifugation at 1500 rpm during 30 min, the precipitate was carefully dried in a vacuum oven at 40°C. The dried product was re-dissolved in methanol to a 10% solution and precipitated into MTBE as expalined earlier. The precipitation was repeated (five to seven times) until the content of extractives in lignin was reduced to about 3% (determined by GC). The lignin material was dried in a vacuum desiccator at 40°C and stored in the dark at −24°C.

All supernatants were combined and MTBE evaporated under vacuum at 30°C. OAS were dried in a vacuum desiccator at 40°C and stored in the dark at −24°C.

Acetylation

Lignins and OAS were acetylated with freshly distilled acetic anhydride and pyridine (4.7:4.0 v/v) according to Zakis.[39]

Analyses

Total Organic Carbon (TOC) was measured with a TOC-5050 Analyzer (Shimadzu Corp., Kyoto, Japan).

Carbohydrates in isolated products was analyzed by GC of sugar monomers obtained by acid methanolysis.[40,41]

The composition of low-molar-mass extractives in isolated products was determined by GC analysis of their TMS derivatives using a short (7.5 m × 0.53 mm i.d., film thickness 0.15 μm, with a temperature from 100°C to 340°C)[42] and a long (25 m × 0.2 mm i.d., film thickness 0.11 μm, with a temperature from 100°C to 290°C)[43] HP-1 capillary columns.

Molar-mass characteristics of isolated products were determined after acetylation by high-performance size-exclusion chromatography (HPSEC) on a HP1090 Liquid Chromatograph (Hewlett-Packard Comp., USA) equipped with a TSK gel G3000 HXL, G2500 HXL, and G1000 HXL columns (three columns: 7.8 mm i.d. × 300 mm length, in series) plus a guard column TSK gel HXL-L (6 mm i.d. × 40 mm length) (Toyo Soda Manufacturing Co., Ltd, Tokyo, Japan). A diode-array detector and a LKB 2142 Differential Refractive Index Meter (LKB-Produkter AB, Bromma, Sweden), were connected in series. THF was used as mobile phase; the flow rate was 1 mL/min. The injection volume was 100 μL (2–4 mg of sample in 1 mL THF). Calibration was done using polystyrene standards and the acetylated lignin dimeric model compound guaiacylglycerol-β-guaiacyl ether.

GC-MS analysis was performed on a HP 5890 GC (Hewlett-Packard Comp., USA) and a HP 5970 quadrupole mass selective detector (EI, 70 eV) using a HP-1 capillary column (25 m × 0.2 mm i.d.; film thickness 0.11 μm) and helium as a carrier gas. Temperature profile: initial temperature 60°C, final temperature 290°C, temperature increase rate 8°C/min. The injector temperature was 260°C; the temperature of the GC-MS transfer line was 290°C, and that of the ion source was 230°C.

Pyrolysis-gas chromatography-mass spectrometry analyses (Py-GC-MS) were performed on a filament pulse Pyrola 85 pyrolyzer (Pyrol AB, Lund, Sweden) connected to a GC-MS as described earlier. Acetylated and original (with free hydroxyl groups) samples were pyrolyzed at 650°C for 2 s. For thermally assisted thermolysis and methylation (THM) 10% aqueous TMAH was added[35,36] and the filament was heated at 360°C for 2 s.

^{13}C-NMR spectra were recorded on a JEOL GX-400 NMR spectrometer (JEOL, Tokyo, Japan) at 100 MHz with DMSO-d_6 as solvent in quantitative mode (pulse angle = 45°, PD = 13 s, a gated pulse sequence, SGNNE).

FT-IR spectra of samples on KBr pellets were recorded with a Perkin-Elmer Spectrum 1000 (Perkin Elmer, Inc., Norwalk, Connecticut, USA) equipped with a DTGS detector, resolution 2 cm^{-1}.

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