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ORIGINAL ARTICLE

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Thioacidolytic analysis of residual lignins isolated from the chemical pulps of spruce (*Picea abies*) and beech (*Fagus sylvatica*) wood

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Abstract Eight different residual lignins isolated from unbleached chemical pulps [sulfite, kraft, alkaline sulfite-anthraquinone-methanol (ASAM), soda/AQ/MeOH] of spruce and beech were characterized using gel permeation chromatography (GPC), thioacidolysis, and desulfurization to determine average molecular weight, amounts of uncondensed β-*O*-4 linkages, and dimeric linkage patterns, respectively. The total amounts of G-CHSEt-CHSEt-CH_2SEt and S-CHSEt-CHSEt-CH_2SEt were markedly reduced in residual lignins to 40% to 80% of the values for the corresponding milled wood lignins (MWLs). The number of dimeric units determined by thioacidolysis and desulfurization of the residual lignins was decreased by one-half to one-fifth compared to the MWLs. Among the diverse types of dimeric units, reduction of β-1 and β-5 units was significant in most of the residual lignins, with the exception of beech ASAM residual lignin. Compared to beech MWL, 40% more C6-C3 main monomers were detected, whereas the relative composition of the dimeric units in the beech residual lignin was very similar to that in the beech MWL. The average molecular weights of residual lignins were less than those of the MWLs. However, the average molecular weights of the spruce kraft and soda residual lignins were determined to be higher than those of the corresponding MWLs.

Key words Residual lignin · Thioacidolysis · Desulfuration · β-*O*-4 linkage · S/G ratio

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Introduction

The enzymatic isolation procedure for residual lignins from four unbleached chemical pulps [sulfite, kraft, alkaline sulfite-anthraquinone-methanol (ASAM), and soda/AQ/MeOH], their chemical characterization by analytical pyrolysis and potassium permanganate oxidation, and the presence of lignin–carbohydrate complexes were discussed in depth in previous papers.[1–3]

One of the results of the enzymatic isolation of residual lignins was the presence of proteins in the isolated residual lignins, evidenced by the high nitrogen content and the high yield of indole estimated by analytical pyrolysis.[2] The attachment of the proteins to the lignins could be explained by hydrophobic interaction between the enzyme and lignin during the isolation procedure. The proteins in the residual lignins interfered with the interpretation of the results from analytical pyrolysis and potassium permanganate oxidation. Nevertheless, the ASAM residual lignin, especially when isolated from beech wood, was clearly distinguished from the other residual lignins isolated from the kraft and soda/AQ/MeOH pulps. There is a clear distinction in the syringyl unit in beech ASAM lignin. In comparison with the other lignins, analytical pyrolysis and potassium permanganate oxidation produced much higher syringyl/guaiacyl (S/G) ratios in the ASAM residual lignin. In particular, a high yield of the C6-C3 form of sinapylaldehyde was identified in the lignin. The presence of this form of sinapylaldehyde indicated that propane side chains (C3) were still intact in the aromatic rings (C6) of beech wood during ASAM pulping. The C6-C3 forms of coniferyl and synapyl alcohol/aldehyde are rarely found in technical lignins as a consequence of the cleavage of α-*O*-4 or β-*O*-4 linkages during chemical pulping.[4,5]

Even though the structural features of residual lignins produced by analytical pyrolysis and potassium permanganate oxidation are known, there is still insufficient information to explain the structural features in the ASAM residual lignin, especially for the abundance of S units and the C6-C3 form of sinapylaldehyde.

We analyzed the isolated residual lignins using the thioacidolytic method to determine the C6-C3 monomers released only by the β-*O*-4 linkages in the residual lignins and quantitative S/G ratios in an effort to explain in depth the mysteries of the ASAM residual lignin. The relative frequencies of diverse dimeric patterns were also evaluated in the residual lignins by desulfuration. In addition, information about the molecular weight of the residual lignins was determined using GPC analysis.

Materials and methods

Preparation of unbleached chemical pulping

Kraft, ASAM, and soda/AQ/MeOH pulping was performed in the laboratory. The sulfite pulp was of industrial origin. The kraft pulping was run for 90 min with 22% active alkali (based on oven-dried wood as NaOH) and 30% sulfidity at 175°C for spruce and 165°C for beech wood. The alkaline sulfite pulping with anthraquinone and MeOH (ASAM) was carried out at 180°C with 6.3% NaOH, 18.7% sodium sulfite, 0.1% anthraquinone (AQ), and 10% methanol (v/v) for 150 min for spruce and 90 min for beech wood. The soda/AQ/methanol pulping process used 22% NaOH, 0.1% AQ, and 20% methanol for 120 min at 165°C for spruce and 160°C for beech wood. The liquor-to-wood ratio was 4:1 for all pulping processes. The kappa number and lignin content of each unbleached pulp are listed in Table 1.

Enzymatic hydrolysis and isolation of residual lignins

The wet, unbleached pulps were ground in a Jokro mill for approximately 20–30 min, and the resulting fiber suspension was stirred at a 5% consistency with an acetate buffer (0.01 M, pH 4.5). Celluclast enzymes (Novo Nordisk, Denmark) were added at a concentration equivalent to 750 NCU/g (based on O.D. pulp), and hydrolysis was performed for 3 days at 40°C in a rotating autoclave. The insoluble fractions were separated from the hydrolyzate by centrifugation and then were freeze dried after being washed thoroughly with distilled water. The hydrolysates were acidified to a pH of 2 using HCl to obtain other solid fractions, which were also freeze dried after being washed with distilled water. The two solid fractions were dissolved in dimethyl acetamide (DMAC), and the insoluble materials were removed by centrifugation. The supernatants were poured into diethyl ether. First the purified solid fractions were dissolved in 0.5 M NaOH (stirred for 1 h at room temperature) and precipitated by acidification to a pH of 2 using HCl. The samples were washed with distilled water until they reached a pH of 6 to 7 and then were freeze dried.[6] The final product obtained from the insoluble residues of enzymatic hydrolysis was abbreviated as residual lignin 1 (RL 1), and the final product isolated from the solubles was abbreviated as residual lignin 2 (RL 2).

Chemical analysis of residual lignins

The content and composition of carbohydrates were determined by sulfuric acid hydrolysis and by a subsequent analysis of the sugar composition by HPLC according to Puls.[7] Elemental analysis (C, H, N, and S determination) was done in a Heraeus elemental analyzer, and the methoxyl group determination was performed according to the method by Vieböck and Schwappach[8] in a Zeisel apparatus (double determination). Each residual lignin (20–30 mg) was reacted with HI in a round-bottomed flask at 140°C, and then the gas form of CH_3I was produced from the methoxyl groups. The produced CH_3I was converted to HIO_3 by the reaction of acetic acid containing sodium acetate and a few drops of bromine. Finally, methoxyl content in the residual lignins was calculated by titration of I_2 with $Na_2S_2O_3$, which was released by reaction of the HIO_3 with KI. In this method, starch was added as a color indicator.

Thioacidolysis

The residual lignins were subjected to thioacidolysis in accordance with the conditions described by Lapierre et al. and Rolando et al.[9,10] About 30–40 mg lignin was added to the thioacidolysis reagent [15 ml, 0.2 M BF_3 etherate in dioxane/ethanethiol (8.75:1, v/v)] in a glass tube with a Teflon-lined screw-cap. The thioacidolysis mixture was heated for 4 h at 100°C in an oil bath with N_2 and occasional

Table 1. Characterization of unbleached chemical pulps

Wood	Unbleached chemical pulp	Kappa number	Lignin determined by sulfuric acid hydrolysis (%)		
			Acid insoluble	Acid soluble	Total
Spruce	Sulfite	14.3	1.01	1.31	2.32
	Kraft	33.5	6.22	0.51	6.73
	ASAM	26.2	4.98	1.15	6.13
	Soda/AQ/MeOH	42.0	6.32	0.46	6.78
Beech	Sulfite	11.0	1.23	1.27	2.50
	Kraft	18.3	1.36	0.93	2.29
	ASAM	20.9	2.77	1.21	3.98
	Soda/AQ/MeOH	14.6	4.30	0.85	5.25

ASAM, Alkaline sulfite-anthraquinone-methanol; AQ, anthraquinone

shaking. After being cooled in ice water, the thioacidolytic products were poured into dichloromethane (20 ml) and the glass tube was rinsed with distilled water. After the internal standard (docosane, C_{22}) was added, the pH of the aqueous phase was adjusted with sodium bicarbonate (0.4 M) to a value of 3 to 4. The organic phase was extracted using dichloromethane (30 ml×3) and then evaporated to dryness. The residues were redissolved in dichloromethane (1 ml) and stored in a refrigerator (–4°C) before injection into the gas chromatograph.

Desulfuration

Desulfuration was performed according to the specifications of Lapierre et al.[11] An aliquot of the thioacidolytic products was mixed with Raney nickel and methanol in a glass tube with a Teflon-lined screw-cap, and the desulfuration reaction was conducted for 4 h at 80°C in an oil bath with nitrogen.

Silylation and GC and gas chromatography/mass spectroscopy (GC/MS) analysis

The thioacidolysis products (30 µl) and desulphurization products (30 µl) were sylilated with 100 µl *N*,*O*-bis-(trimethylsilyl)-trifluoroacetamide (BSTFA) and 50 µl GC grade pyridine in a 200-µl GC vial, respectively. The silylated products were separated by gas chromatography in the capillary column DB-1701 (60 m × 0.25 mm × 0.25 mm), and each peak was identified by GC/MS. The temperature program of the GC increased the temperature at a rate of 3°C/min from 100° to 280°C, and then the final temperature was maintained for 60 min.

Gel permeation chromatography

The molecular weight distribution of the residual lignins was determined by gel permeation chromatography (GPC; Kontron) using a Chrompack Microgel column (styrene-divinylbenzene copolymer; 250 mm × 7.7 mm) and two detector systems: a UV detector at 280 nm and a RI (refractive index) detector. Each residual lignin was acetylated overnight with acetic anhydride and pyridine at 75°C for GPC analysis. The acetylated samples were dissolved in tetrahydrofuran (THF; 1 mg at 6 ml) and then filtered with a 0.2-µm polytetrafluoroethylene (PTFE) membrane to remove the insolubles. An aliquot (100 µl) was injected into the GPC system and eluted with THF at a flow rate of 1.0 ml/min. The calibration curves were created with a different molecular weight range than the polystyrene standards.

Results and discussion

Determining arylglycerol-β-aryl ether linkages in residual lignins

The yields of residual lignins isolated from four different chemical pulps and their elemental analysis data are displayed in Table 2. In general, the nitrogen contents of all the residual lignins were regarded as a parameter for protein contaminants, which can be produced during the enzymatic hydrolysis of pulps. Although a NaOH treatment was performed to eliminate protein impurities during the final stage of the purification of the residual lignin, 2%–66% of the protein contaminants [N (%) × 6.25] and 1%–12% of the carbohydrates remained in the residual lignins.

Table 2. Yields of residual lignins and their elemental analysis

Wood	Unbleached chemical pulp	Degree of enzyme hydrolysis (%)[a]	Yield of residual lignin (%)[a]		Elemental analysis (%)				
			RL 1 / RL 2	Total	C	H	O	N	S
Spruce	Sulfite	93.7	1.2	1.8	51.9	6.95	27.5	11.9	1.64
			0.6		54.4	6.45	26.91	9.82	2.41
	Kraft	94.4	4.6	4.7	57.9	6.07	30.6	3.88	1.53
			0.06		51.6	5.85	38.1	3.18	1.31
	ASAM	95.0	1.5	5.4	53.1	6.97	27.9	10.5	1.49
			3.9		55.4	6.20	30.4	6.31	1.69
	Soda/AQ/MeOH	79.2	8.8	8.8	59.1	6.27	30.5	3.72	0.33
			0.01		–	–	–	–	–
Beech	Sulfite	95.7	0.4	2.4	54.3	6.54	29.6	7.87	1.73
			2.0		52.3	6.63	30.0	9.17	1.88
	Kraft	96.6	1.1	1.7	58.3	5.73	31.3	2.17	2.46
			0.6		47.0	6.26	42.3	3.08	1.36
	ASAM	97.6	1.0	5.5	58.36	6.33	34.3	0.34	0.69
			4.5		56.4	6.25	33.1	3.26	1.01
	Soda/AQ/MeOH	94.8	0.7	0.8	59.2	6.20	31.4	2.89	0.31
			0.1		56.3	6.45	34.8	2.32	0.15

RL, Residual lignin
[a] Based on the dry weight of unbleached chemical pulps

It was proven in previous studies[1,2] that the analytical pyrolysis of residual lignins produced unexpectedly high amounts of phenol/cresol because of the existence of protein impurities. Similar to the results of analytical pyrolysis, anisic acid was overestimated by $KMnO_4$ oxidation in highly contaminated residual lignins. Anisic acid can only be derived from an uncondensed H-unit.

Arylglycerol-β-aryl ether linkage(β-*O*-4 linkage), which was the most frequent interunit linkage type in the lignin polymer, was degraded during the thioacidolysis reaction and produced several thioethylated monomers. G-CHSEt-CHSEt-CH_2SEt and S-CHSEt-CHSEt-CH_2SEt were produced as the C6-C3 main monomers (Fig. 1). In general, the amounts of β-*O*-4 linkages were approximately 50%–60%

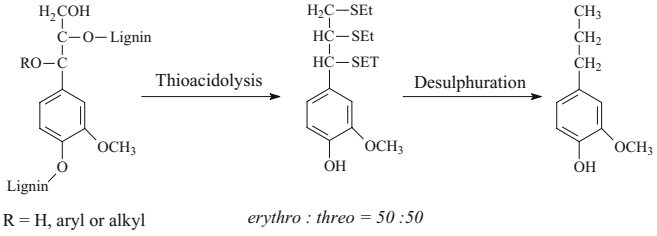


Fig. 1. Formation of thioacidolytic products from arylglycerol-β-aryl ether units (from Lapierre et al.[11])

of those in native lignins.[12] Therefore, the aim of the pulping process was focused on the cleavage of the β-*O*-4 linkages under either alkaline or acidic conditions. However, a certain amount of residual lignin always remained in the unbleached pulps, and the β-*O*-4 linkages in the residual lignin could be quantitatively evaluated by thioacidolysis.

Tables 3 and 4 show the structures of thioethylated monomers and the quantitative yields of those monomers obtained from spruce and beech residual lignins, respectively, as well as spruce and beech milled wood lignin (MWL). Experimental results indicated that the structure of MWL, especially the phenolic hydroxyl group and β-*O*-4 linkages, can be modified by milling conditions so that the lignin in the cell wall is no longer completely composed of MWL.[13] Nevertheless, for reasons of lack of reference, the spruce and beech MWLs purified by the traditional Björkman procedure[14] were subjected to thioacidolysis, and the results are included in the tables.

The results of the gas chromatographic separation of thioacidolytic monomers from spruce kraft and beech ASAM residual lignins are displayed in Fig. 2. The diastereoisomers caused each C6-C3 main monomer to appear as twin peaks on the GC chromatogram. Several minor signals also appeared together with the main peaks, and these were also included in this evaluation. In contrast to analytical pyrolysis and $KMnO_4$ oxidation, the analytical data from

Table 3. Yields of the thioethylated monomers of spruce residual lignins (μmol/g lignin)

No.	Thioethylated monomers (TMS derivatives)	MWL	Sulfite RL 2	Kraft RL 1	ASAM RL 2	Soda[a] RL 1
T1	**G**-CH_2-CH_2-CH_2OH	17.8	5.2	4.6	5.1	6.2
T2	**G**-CH$(SEt)_2$	36.8	7.3	4.2	6.0	6.3
T3	**G**-CH_2CH$(SEt)_2$	22.6	8.5	8.6	25.2	18.6
T6	**G**-CHSEt-CH_2-CH_2SEt	13.1	10.7	8.1	–	10.5
T7	**G**-CSEt = CHSEt	7.9	6.2	4.2	4.4	10.9
T10	**G**-CHSEt-CH_2-CH$(SEt)_2$	24.9	3.1	2.7	1.7	1.8
T11	**G**-CHSEt-CHSEt-CH_2SEt[b]	517.4	77.3	100.4	214.8	194.1
T13	**G**-CHSEt-CH_2-**G**	19.2	3.3	2.4	2.7	4.2
T14	β-β (THF form)	8.4	3.8	5.7	5.3	7.2
	Total	668.1	125.4	141.0	275.1	259.8

MWL, Milled wood lignins; THF, tetrahydrofuran; TMS, trimethyl silylated
[a] Soda pulping with AQ and methanol
[b] Sum of *erythro* and *threo* forms

Table 4. Yields of the thioethylated monomers of beech residual lignins (μmol/g lignin)

No.	Thioethylated monomers (TMS derivatives)	MWL	Sulfite RL 2	Kraft RL 1	ASAM RL 1	ASAM RL 2	Soda[a] RL 1
T2	**G**-CH$(SEt)_2$	10.9	7.2	4.1	–	–	5.0
T3	**G**-CH_2CH$(SEt)_2$	20.4	15.2	5.3	9.4	10.8	8.2
T4	**S**-CH$(SEt)_2$	25.9	14.5	6.6	5.9	5.4	8.2
T5	**S**-CH=CH-CH_2SEt	7.4	14.2	3.2	7.4	4.7	2.0
T6	**G**-CHSEt-CH_2-CH_2SEt	6.2	11.9	4.2	4.9	4.7	2.5
T7	**G**-CSEt=CHSEt	–	13.5	5.0	2.7	1.9	6.2
T8	**S**-CH_2CH$(SEt)_2$	32.9	29.4	8.0	24.3	30.5	8.6
T9	**S**-CHSEt-CH_2-CH_2SEt	12.0	25.2	7.0	9.4	9.8	2.4
T11	**G**-CHSEt-CHSEt-CH_2SEt[b]	356.8	114.5	103.7	280.8	229.0	77.2
T12	**S**-CHSEt-CHSEt-CH_2SEt[b]	698.0	331.5	153.6	1293.8	1023.7	124.7
	Total	1170.5	576.9	300.7	1638.6	1320.5	244.9

[a] Soda pulping with AQ and methanol
[b] Sum of *erythro* and *threo* forms

thioacidolysis were not influenced by protein contaminants, because the thioethylated monomers of the residual lignins were only derived from the G and S units, but not the H unit.

The contents of the C6-C3 main monomers varied from 77 to 214 (μmol/g lignin, T11) in the spruce residual lignins and from 202 to 1575 (μmol/g lignin, T11 + T12) in the beech residual lignins. Compared to the reference MWLs (spruce, 517 μmol/g lignin; beech, 1055 μmol/g lignin), a significant reduction was observed in all the residual lignins (except the beech ASAM lignin) in the residual lignins. The reduction of C-C3 main monomers can be logically explained by the continual cleavage of *β-O*-4 linkages during the pulping procedures.

The high amounts of C6-C3 main monomers, particularly S monomers, in the beech ASAM residual lignin was the unexpected result of this experiment. As noted in Table 4, the amounts of C6-C3 main monomers (average value of the sum of T11 and T12: 1408 μmol/g in beech ASAM-RL 1 and -RL 2) were about 40% higher than those in the beech MWL (sum of T11 and T12: 1054 μmol/g). Considering that more than 90% of lignins were dissolved during the chemical pulping irrespective of diverse pulping processes, a persuasive explanation for this peculiar result in the ASAM residual lignin was very difficult to find with the knowledge of pulping chemistry and related references. To make an in-depth discussion about the results, it is necessary to study a reaction mechanism of lignin model compounds as well as natural lignin polymers during ASAM conditions.

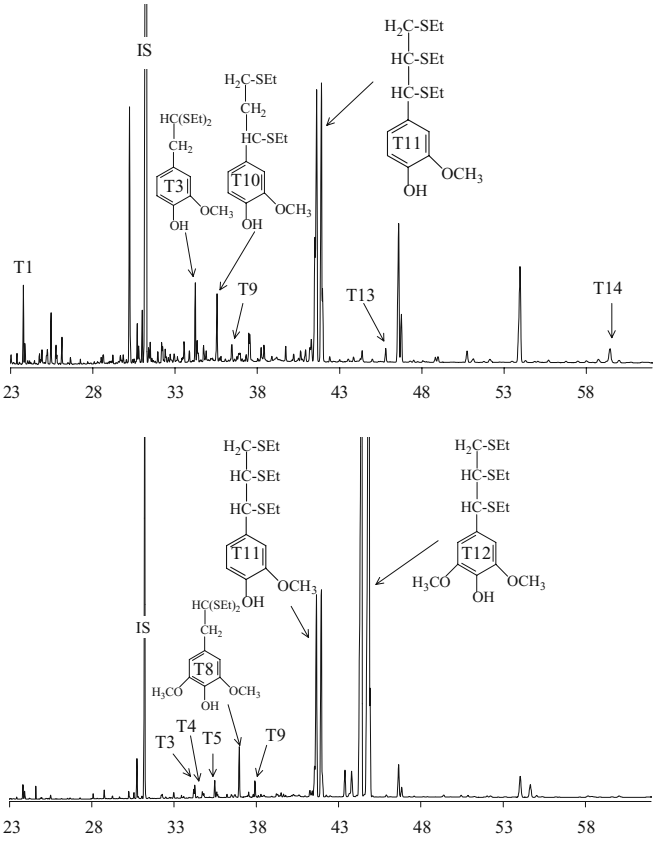


Fig. 2. Chromatograms of the thioacidolytic products from residual lignins (RL) (upper: spruce-kraft-RL 1; and lower: beech-ASAM-RL 1). Besides the main products, several thioacidolytic products were also created from the phenylpropanoid structures as well as carbohydrates

S/G ratio of beech residual lignins

The S/G ratios in the beech residual lignins, evaluated using the sum of the G and S types of thioethylated monomers, are listed without corrections in Table 5 together with those values determined by analytical pyrolysis[1] and $KMnO_4$ oxidation.[2] To clarify the results, the methoxyl group contents of the residual lignins were also listed. The S/G ratios of the beech residual lignins determined by thioacidolysis were in the range of 1.5 to 4.5. The S/G ratios determined by analytical pyrolysis were between 0.6 and 1.1, and those determined by $KMnO_4$ oxidation were between 1.7 and 2.2. It is a well-known fact that the S/G ratios are overestimated by analytical pyrolysis.[15] Therefore, S/G ratios by analytical pyrolysis were corrected using an equation developed by Boettcher in 1993: corrected S (%) = –5.9 + 0.704 × S (% obtained by analytical pyrolysis).[16] However, because of the lack of correction equations for the S/G ratios determined by $KMnO_4$ oxidation and thioacidolysis, those results were directly evaluated using aromatic carboxylic acid products and thioethylated products, respectively.

The S/G ratios in residual sulfite and ASAM lignins determined by the three methodologies were higher than those in the reference MWLs, whereas that of the kraft and soda residual lignins was slightly lower. These results could be potentially explained by the solubility of the G and S units in the pulping liquors. When sulfite was present (sulfite

Table 5. S/G ratios of beech residual lignins

Beech wood lignins	Methoxyl groups (%)[a]	S/G ratio		
		Thioacidolysis	Analytical pyrolysis[a]	$KMnO_4$ oxidation[b]
MWL	21.7	1.92	0.75	0.74
Sulfite RL 2	17.9	2.56	0.69	1.73
Kraft RL 1	17.4	1.46	0.68	2.00
ASAM RL 1	26.0	4.49	1.12	1.68
ASAM RL 2	23.7	4.35	1.02	2.24
Soda/AQ/MeOH	18.8	1.48	0.66	2.01

S/G, Syringyl/guaiacyl
[a] Choi et al.[1]
[b] Choi and Faix[2]

Table 6. Total yields of dimeric units and relative distribution of the dimeric linkage patterns after the desulfuration of residual lignins

Residual lignins	Total yields (μmol/g lignin)	Frequencies of dimeric units (μmol/g lignin)						
		5-5	4-O-5	β-1	β-5	β-6	β-β THF	β-β resinol
Spruce MWL	326.8	68.3	12.7	75.8	126.5	24.2	19.3	–
Sulfite RL 2	60.7	21.1	8.6	16.6	6.4	3.6	4.5	–
Kraft RL 1	89.3	24.9	3.4	17.7	27.1	6.5	9.6	–
ASAM RL 2	119.3	36.7	6.4	18.3	38.8	13.2	5.7	–
Soda/AQ/MeOH-RL 1	100.9	32.6	5.1	19.6	29.3	5.8	8.7	–
Beech MWL	232.1	19.0	9.3	121.4	49.2	6.5	–	27.4
Sulfite RL 2	124.4	12.1	4.4	60.1	13.6	14.2	–	20.2
Kraft RL 1	105.6	10.9	4.2	49.9	18.2	9.0	–	13.4
ASAM RL 1	224.3	11.7	3.6	102.7	28.5	37.7	–	40.4
ASAM RL 2	207.0	11.2	3.7	101.2	24.6	28.8	–	37.3
Soda/AQ/MeOH-RL 1	74.3	8.8	3.9	36.7	12.6	5.2	–	7.1

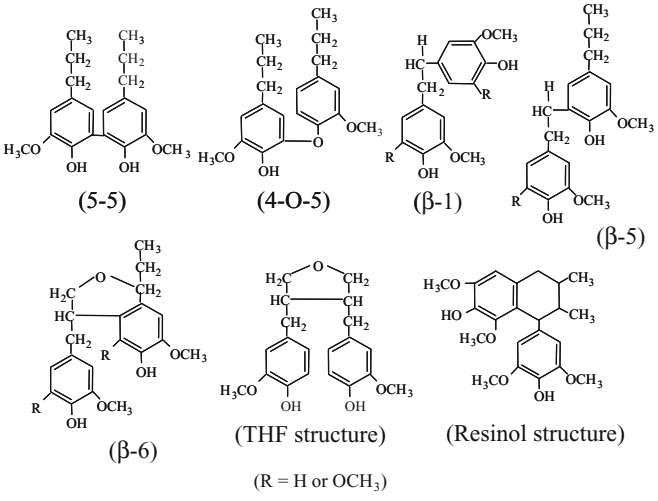


Fig. 3. Essential types of dimeric units recovered from desulfuration followed by thioacidolysis of residual lignins. *THF*, tetrahydrofuran

and ASAM cooking), the G units seemed to be susceptible to the pulping liquor, which may explain why a high level of S units can exist in the sulfite and ASAM residual lignins.[17] This explanation fit especially well with the S/G ratio and contents of the methoxyl groups in the ASAM residual lignins. However, the reverse phenomenon was applicable in the kraft and soda residual lignins, in which the G unit was more stable.

Determination of dimer structures

The thioethylated products were subjected to a desulfuration reaction in the presence of Raney nickel and methanol to obtain detailed information about the quantitative frequencies of dimeric units in the residual lignins.[11,15] The structures of the dimeric units and their quantitative frequencies in the residual lignins are illustrated in Fig. 3 and Table 6. The same dimeric structures, without terminal CH_2OH in the side chains, were also identified in this experiment, but those are not evaluated in Table 6.

The main dimeric linkages in spruce residual lignins were identified as β-5, 5-5, and β-1, similar to the spruce MWL. The β-β type (tetrahydrofuran, THF) was typical in the softwood lignins. The beech residual lignins were constructed with β-1, β-5, and β-β (resinol form). The total yields of dimeric units in the spruce and beech MWL were determined to be 327 and 232 μmol/g lignin, respectively. In general, the softwood lignins have a higher potential for the formation of condensed structures than hardwood lignins as a result of the free C5 in the guaiacyl unit. Therefore, the spruce MWL gave rise to higher relative yields of the dimeric units than the beech MWL. In contrast, hardwood lignins can be constructed of more β-*O*-4 linkages because of the existence of the syringyl unit.[18,19] In spruce residual lignins, the yields of dimeric units amounted to between 60 and 120 μmol/g lignin, which were reduced by 1/3 to 1/5 of the reference MWL. The reduction of dimeric units was also observed in beech, except for in the ASAM residual lignin. In contrast to the other residual lignins, there were few changes in the total yields and frequencies of the dimeric units between the beech MWL and ASAM residual lignin, indicating that few modifications of the lignin occurred during the ASAM pulping of the beech wood. Another feature observed in the beech ASAM residual lignin was the relative stability of the β-β (resinol) and the higher frequency of the β-6 units than in the beech MWL. Previously, the $KMnO_4$ oxidation also suggested that large amounts of C6 condensed units such as β-6 were present in the ASAM residual lignin.[2]

Gel permeation chromatography of acetylated residual lignins

Spruce and beech residual lignins and the corresponding MWLs were acetylated with acetic anhydride and pyridine to improve the lignin solubility as well as to prevent the hydrogen bond type interaction of the phenolic hydroxyl groups in the lignins with tetrahydrofuran (THF).[20] In the acetylated lignin and the THF system, hydrophobic interactions between the aromatic structure of the lignins and the phenyl groups of the column packing gel (styrene-divinylbenzene copolymer) still exist and can affect the elution time during the GPC analysis. Therefore, the relative molecular weights of the residual lignins could be determined based on the calibration curves obtained from the polystyrene standards in this study, which might represent

Table 7. Determination of the average molecular weights and polydispersity (D) of the residual lignins

Residual lignins	UV detector			RI detector		
	M_w	M_n	D (M_w/M_n)	M_w	M_n	D (M_w/M_n)
Spruce-MWL	5500	3000	1.8	5720	3270	1.8
Sulfite-RL 2	2800	1700	1.6	2690	1600	1.7
Kraft-RL 1	7600	3200	2.4	7920	3170	2.5
ASAM-RL 2	3900	1950	2.0	3900	2050	1.9
Soda/AQ/MeOH-RL 1	8400	3830	2.2	8710	4020	2.2
Beech-MWL	10100	4200	2.4	10680	4900	2.2
Sulfite-RL 1	3770	2030	1.9	3750	2140	1.5
Kraft-RL 1	7440	2700	2.8	7910	3020	2.6
ASAM-RL 1	9200	3760	2.4	9360	4140	2.3
Soda/AQ/MeOH-RL 1	5730	2830	2.0	5980	3010	2.0

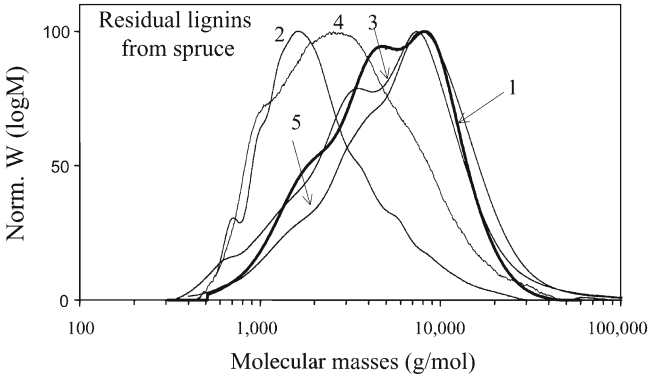
a similar interaction to that with the column packing gel.[21,22]

Table 7 illustrates the GPC data for residual lignins and the corresponding MWLs as determined by the refractive index (RI) and the UV detector, respectively. Figure 4 shows the elution profiles of the acetylated lignin samples as determined by the RI detector. In comparison, there were no significant differences in the elution profiles and the apparent average molecular weights determined by both detector systems.

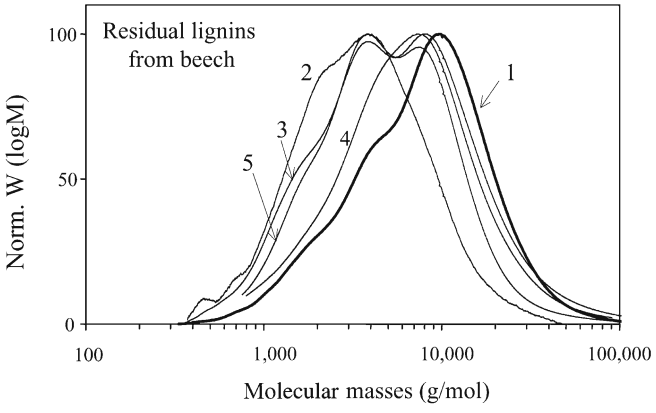
The elution profile of the spruce MWL was distinguished by a bimodal shape, and the average molecular weight (M_w) and polydispersity were determined to be 5500 and 1.8, respectively. However, the elution profiles of the residual lignins after sulfite and ASAM pulping were transformed into a unimodal distribution and shifted to a lower molecular weight region. The average M_w of the sulfite and ASAM residual lignins was reduced to 2800 and 3900, respectively. A significant finding of the GPC analysis was that the estimated M_w was ~1.4 to 1.5 fold higher in kraft and soda residual lignins as compared to the M_w in the MWL. As shown in Fig. 4, the elution profiles of the kraft and soda residual lignins were shifted to a higher molecular weight region.

According to a report by Jiang and Argyropoulos,[23] the increase in molecular weight of residual lignin isolated from pine kraft pulp may be closely associated with the prevalence of the condensation reaction in the guaiacyl type fragments in an alkaline atmosphere.

As shown in Fig. 4 (in beech wood, lower panel), the elution curves of the kraft (no. 3) and soda residual lignins (no. 5) were also clearly distinguishable. Both the residual lignins exhibited a bimodal distribution profile in contrast to the unimodal curve of the MWL. A unique characteristic of the ASAM residual lignin was also observed in the molecular weight. The average molecular weight of the ASAM residual lignin was determined to be between 9200 and 9440, which was almost comparable to the beech MWL (M_w, 10100). Considering the high reactivity of the G unit in the ASAM pulping and unfavorable conditions for a condensation reaction resulting from the lack of G units, the high molecular weight of the ASAM residual lignin could be solely a consequence of the lesser fragmentation of the lignin during the pulping process.



1: spruce MWL, 2: Sulfite-RL2, 3: Kraft-RL1, 4: ASAM-RL2, 5:Soda/AQ/MeOH-RL1



1: beech MWL, 2: Sulfite-RL1, 3: Kraft-RL1, 4: ASAM-RL1, 5:Soda/AQ/MeOH-RL1

Fig. 4. Molecular weight distribution profiles of residual lignins

Conclusions

Although residual lignins isolated from spruce and beech unbleached chemical pulps (sulfite, kraft, ASAM, and soda/AQ/MeOH) by enzymatic hydrolysis were contaminated to some extent with protein impurities, the thioacidolysis analysis of those residual lignins was not influenced by those impurities because the main thioethylated products (G-

CHSEt-CHSEt-CH_2SEt and its S derivative) could not be formed by these proteins. Yields of those derivatives were diminished by as much as 85% in the spruce residual lignins and as much as 80% in the beech residual lignins. A unique characteristic of the beech ASAM residual lignin was discovered by thioacidolytic analysis, in which the ASAM residual lignin gave rise to ~50% higher yields of main thioethylated monomers than the beech MWL. Desulfuration revealed that the relative frequencies of the dimeric units in the residual lignins were also lowered significantly, to 1/3 to 1/5 of the yields of the corresponding MWLs. In spruce residual lignins, the essential dimeric units were identified as 5-5, β-1, and β-5 bonds, whereas the beech residual lignins were mainly associated with the β-1 and β-6 and β-β (resinol form) bonds. GPC analysis revealed that kraft and soda residual lignins showed higher molecular weights compared to spruce MWL, whereas sulfite and ASAM residual lignins suffered a marked decrease of their molecular weights. These results may be explained by chemical reactions such as splitting or condensation between the lignin fragments formed during the pulping procedures.

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References

1. Choi JW, Faix O, Meier D (2001) Characterization of residual lignins from chemical pulps of spruce (*Picea abies* L.) and beech (*Fagus sylvatica* L.) by analytical pyrolysis-gas chromatography/mass spectrometry. Holzforschung 55:185–192
2. Choi JW, Faix O (2003) Characterization of residual lignins from chemical pulps of spruce (*Picea abies*) and beech (*Fagus sylvatica*) by $KMnO_4$ oxidation. J Korean Wood Sci Technol 31:31–39
3. Choi JW, Choi DH, Faix O (2007) Characterization of lignin–carbohydrate linkages between residual carbohydrates and lignin fractions in the residual lignins isolated from chemical pulps of spruce (*Picea abies*) and beech wood (*Fagus sylvatica*). J Wood Sci 53:309–313
4. Kleen M, Gellerstedt G (1991) Characterization of chemical and mechanical pulps by pyrolysis-gas chromatography/mass spectrometry. J Anal Appl Pyrolysis 19:139–152
5. Hage ERE, Mulder MM, Boon JJ (1993) Structural characterization of lignin polymers by temperature resolved in-source pyrolysis-mass spectrometry and Curie-point pyrolysis-gas chromatography/mass spectrometry. J Anal Appl Pyrolysis 25: 149–183
6. Horlting B, Turunen E, Sundquist J (1992) Investigation of residual lignin in chemical pulps. Part 2. Purification and characterization of residual lignin after enzymatic hydrolysis of pulps. Nord Pulp Paper Res J 7:144–151
7. Puls J (1982) Chemical analysis of lignocellulosic residues. In: Strub A, Chartier P, Schleser G (eds) Energy from biomass. Applied Science, London, pp 863–867
8. Vieböck F, Schwappach A (1930) Eine neue Methode zur nassanalytischen Bestimmung der Methoxyl- und Aethoxylgruppe. Chem Ber 63:2818–2823
9. Lapierre C, Monties B, Rolando C (1985) Thioacidolysis of lignin: comparison with acidolysis. J Wood Chem Technol 5:277–292
10. Rolando C, Monties B, Lapierre C (1992) Thioacidolysis. In: Lin SY, Dence CW (eds) Methods in lignin chemistry. Springer-Verlag, Berlin, pp 334–349
11. Lapierre C, Pollet B, Monties B, Rolando C (1991) Thioacidolysis of spruce lignin: GC-MS analysis of the main dimers recovered after Raney nickel desulphuration. Holzforschung 45:61–68
12. Adler E (1977) Lignin chemistry: past, present and future. Wood Sci Technol 11:69–218
13. Fujimoto A, Matsumoto Y, Chang HM, Meshitsuka G (2005) Quantitative evaluation of milling effects on lignin structure during the isolation process of milled wood lignin. J Wood Sci 51:89–91
14. Björkman A (1956) Studies on finely divided wood. I. Extraction of lignin with neutral solvents. Sven Papperstidn 59:477–485
15. Genuit WJ, Boon JJ, Faix O (1987) Characterization of beech milled wood lignin by pyrolysis-gas-chromatography-photoionization mass spectrometry. Anal Chem 59:508–513
16. Boettcher JH (1993) Quantitative Analyse von Holz und Holz-Komponenten mittels FTIR-Spectroskopie unter Anwendung multivariater statistischer Verfahren. Dessertation, Universitaet Hamburg
17. Gellerstedt G, Gustafsson K, Northey RA (1988) Structural changes in lignin during kraft cooking. Part 8. Birch lignins. Nord Pulp Paper Res J 3:87–94
18. Gellerstedt G, Wafa Al-Dajani W, Zhang L (1999) On the structure of residual lignins in alkaline pulps. In: Proceedings, 10th international symposium of wood pulp and chemistry, June 7–10, Yokohama, Japan, vol 1, pp 346–349
19. Lapierre C, Pollet B, Tollier MT, Chabbert B, Monties B, Rolando C (1993) Molecular profiling of lignins by thioacidolysis. In: Proceedings, 7th international symposium of wood pulp and chemistry, May 25–28, Beijing, China, vol 2, pp 818–828
20. Nimz HH (1974) Das Lignin der Buche: Einwurf eines Konstitutionsschema. Angew Chem 86:336–344
21. Faix O, Lange W, Salud EC (1981) The use of HPLC for the determination of average molecular weight distribution of milled wood lignins from *Shorea polysperma* (Blco.). Holzforschung 35:3–9
22. Himmel ME, Mlnar J, Sakarnen S (1995) Size exclusion chromatography of lignin derivatives. In: Wu CS (ed) Handbook of size exclusion chromatography. Dekker, New York, pp 353–379
23. Jiang ZH, Argyropoulos DS (1997) Isolation and characterization of residual lignin in kraft pulps. In: Proceedings, 9th international symposium of wood pulp and chemistry, June 9–12, Montreal, Canada, vol 1, J2-1–J2-6