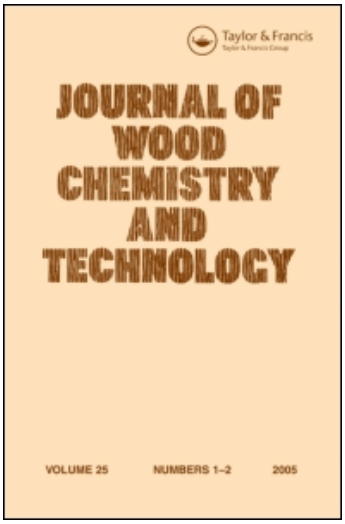

Journal of Wood Chemistry and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597282

Characterization of Lignins from Organosolv Pulping According to the Organocell Process, Part 2. Residual Lignins

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To cite this Article Lindner, Albert and Wegener, Gerd(1989) 'Characterization of Lignins from Organosolv Pulping According to the Organocell Process, Part 2. Residual Lignins', Journal of Wood Chemistry and Technology, 9: 4, 443 — 465
To link to this Article: DOI: 10.1080/02773818908050310
URL: http://dx.doi.org/10.1080/02773818908050310

JOURNAL OF WOOD CHEMISTRY AND TECHNOLOGY, 9(4), 443-465 (1989)

CHARACTERIZATION OF LIGNINS FROM ORGANOSOLV PULPING ACCORDING TO THE ORGANOCELL PROCESS

PART 2. RESIDUAL LIGNINS

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ABSTRACT

Residual lignins were isolated from cooking residues obtained from 20 l batch cookings of spruce chips by either ball-milling or enzymatic treatment. The lignins were characterized in terms of yield, elemental composition, polysaccharide moieties and functional groups and compared with the corresponding dissolved lignins.

The residual lignins generally proved to be less changed than the dissolved lignins. They show considerable adherence to cellulose and xylose portions. Aliphatic OH groups were found to decrease remarkably towards the end of the cook, which was explained by the formation of new lignin-lignin and lignin-polysaccharide bonds as well as by side chain degradation.

Lignin methylation by methanolic liquor was almost negligible.

INTRODUCTION

The first part of this series[1] discussed the chemical properties of lignins isolated from the spent liquor of cookings according to the Organocell process[2]. Apart from laboratory samples from a 20 l batch digester, the chemical characterization included lignins from the Organocell pilot plant, which were found to have properties comparable to those of the laboratory lignins. In order to gain further insight into the chemistry of this new pulping process, our investigations were extended to include residual lignins.

In the past few years research on residual lignins has been considerably intensified. In a comprehensive study of residual lignins from NSSC pulps, Glasser[3] described changes concerning lignin's chemical structure and its association with carbohydrates. Residual lignins from various sulfite pulps were recently investigated by Pelzer and Krause[4].

A major point of interest has been the nature of the lignin-carbohydrate bonds, which impede complete delignification during digestion. Yamasaki et al.[5] considered chemical linkages between lignin and carbohydrates, especially galactoglucomannans, the most plausible reason for the resistance of residual lignin to further delignification during kraft pulping. Chemical bonds to polyoses and/or cellulose have also been discussed by other authors[3,4,6,7]. Other factors such as lignin's impeded diffusion through the fibre pores or changes in its structural composition may

also contribute towards incomplete delignification[8,9]. In the present study two residual spruce lignins from each cooking stage obtained from 20 l batch cookings were isolated by ball-milling and compared to the corresponding lignins from the spent liquor. An alternative isolation procedure involving enzymatic treatment was applied for the second stage samples. On the basis of the analytical data obtained the behavior of the residual lignins during the cooking is discussed below.

EXPERIMENTAL

The cookings were performed in a 20 l batch digester with liquor circulation. The cooking conditions have already been described in the first part of this series[1]. Spruce chips (Picea abies L. Karst.) of industrial size were used for the experiments. The following samples were isolated:

Isolation via ball-milling

MWL : reference sample
1S/20 R : first stage 20 min
1S/40 R : first stage 40 min
2S/20 R : first stage 40 min, second stage 20 min
2S/40 R : first stage 40 min, second stage 40 min

Isolation via enzymatic treatment

2S/20 RE: first stage 40 min, second stage 20 min
2S/40 RE: first stage 40 min, second stage 40 min

Isolation of the Ball-Milled Lignins

The air-dried cooking residues were ground to particle sizes between 50 and 200 μm and thoroughly washed with water. Starting with ball-milling they were further processed according to reference 10.

Isolation of the Enzymatically Liberated Lignins

The isolation, with minor variations, was carried out as described by Pelzer[11]:

20 g of dry cooking residue were suspended in 250 ml water and ground in a Jokro mill for 40 min. After that 750 ml water, 160 ml buffer concentrate (see below) and 190 ml aqueous solution containing 2 g cellulase (Cellulase TC from Trichoderma reesei 0.86 U/mg, Serva) were added. The hydrolyses were carried out under shaking at 50°C. After a reaction time of 96 h the mixture was centrifuged. The undissolved rest was suspended in 100 ml buffer containing 1 g cellulase and exposed to another hydrolysis under the conditions mentioned above. This procedure was repeated once with 2S/40 RE and twice with 2S/20 RE. After a final centrifugation step the undissolved rest was thoroughly washed with water and further processed according to the method described for the ball-milled samples[10].

Buffer composition: One part buffer concentrate (70.4 g NaOH, 184.9 g citric acid-1-hydrate and 120 ml 1 n HCl in 1 l of an aqueous solution) is diluted with nine parts distilled water.

Determination of the Lignin Concentrations

- Cooking Residues
  The lignin contents were determined by calculating the difference between starting material (27 % lignin) and lignin isolated from the spent liquor

- Residues 3 (see Figure 1)
  The lignin contents were calculated by subtracting the polysaccharide content from 100 %

- Extractable Lignin of Residues 3
  This was determined by UV at 200 nm in hexafluoropropanol : water = 1 : 1 [12].

Functional Groups

The methods are given in part one of this series[1]. The phenolic OH groups were determined by gas chromatography (GC) according to Mansson[13].

^{1}H-NMR Spectroscopy

The acetylated lignins were dissolved in $CDCl_3$ with TMS as standard. The spectra were recorded on a 270 MHz instrument (JNM-GX 270, Jeol). The quantitative estimates were performed according to Faix and Schweers[14].

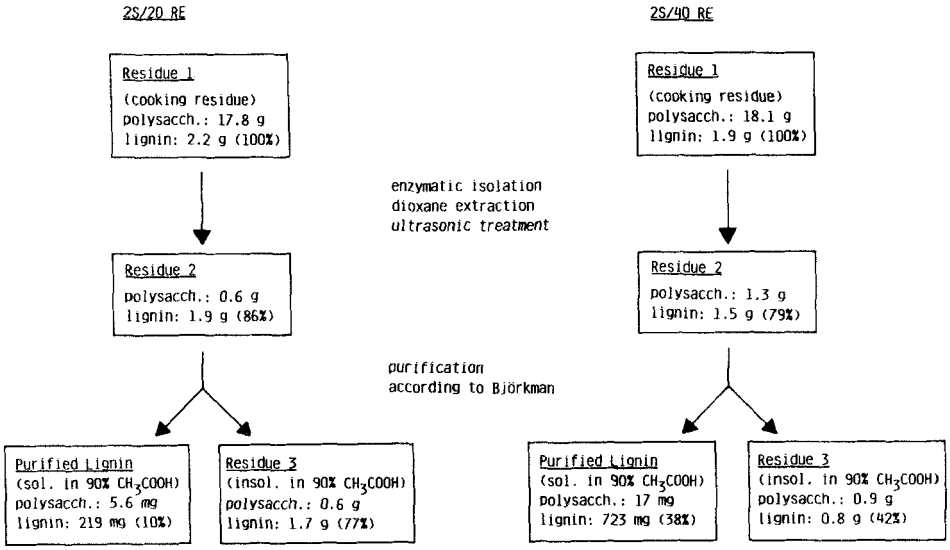

FIGURE 1: Scheme of the enzymatic isolation (2S/40 RE: 383 mg of polysaccharides remain in aqueous solution during purification)

RESULTS AND DISCUSSION

Yields and Composition

The yields of the crude, ball-milled lignin preparations, given in Table 1, drop from 67.5 % of the reference sample (MWL) to a mere 27.2 % at the end of stage 2. This distinct yield decline could be caused by

- a reduction of lignin's solubility on account of structural changes during digestion
- an increased chemical or physical adhesion of lignin to carbohydrate portions
- a selective removal of easily soluble lignin fractions.

As will be discussed later, changes in lignin's structure and its adhesion to carbohydrates seem indeed to play a major part in the yielding of low quantities of the second stage lignins.

The yields of purified ball-milled lignin preparations are of a slightly different kind, with the first stage lignins surpassing the reference MWL (Table 1). Percentages for the second stage samples, however, are again very low.

In order to improve yields and – as a consequence – the representativity of the purified residual lignins of the second stage, an alternative isolation procedure involving enzymatic treatment was applied. And indeed, sample 2S/40 RE yielded more than four times the amount of purified lignin than the corresponding 2S/40 R (Table 1). In the case of 20 minutes cooking time, however, no yield increase could be observed. Prolongation of the enzymatic treatment did not improve results. Figure 1 shows that high amounts of carbohydrate-rich material were dissolved during the dioxane extraction involving ultrasonic treatment (Residues 2). In the subsequent purification steps large portions of Residues 2, especially in the case of 2S/20 RE, proved to be insoluble in 90 % acetic acid. The insoluble portions (Residues 3) were subjected to sugar analysis. Table 2 reveals the substantial difference between 2S/20 RE and 2S/40 RE: While for the former most of Residue 2's lignin was insoluble and therefore remained in Residue 3 (74 %), a much higher solubility could be observed for the latter, which accounts for both a lower lignin content

TABLE 1

Yields of the Isolated Lignins

Substance	Total Lignin (%)	Crude Lignin (% total l.)	Purified Lignin (% total l.)
MWL	27.0	67.5	15.5
1S/20 R	23.5	53.8	25.8
1S/40 R	21.9	44.3	18.8
2S/20 R	11.1	31.3	9.7
2S/40 R	9.6	27.2	9.0
2S/20 RE	11.1	-	9.0
2S/40 RE	9.6	-	38.1

in Residue 3 and a much higher yield of purified lignin (Table 1, Figure 1).

In order to find out whether the high lignin concentration found in Residue 3 of 2S/20 RE is due to strong lignin-carbohydrate bonds or merely to lignin's poor solubility in 90 % acetic acid, both Residues 3 were extracted with 1 n NaOH. Although more lignin could be extracted from 2S/20 RE (10.0 % vs 2.3 % for 2S/40 RE), the portion of non-extractable lignin was nevertheless considerably higher for 2S/20 RE (64.0 %) than for 2S/40 RE (42.5 %) (Table 2).

TABLE 2

Lignin, Polysaccharide and Glucose Contents of Residues 3

Substance	Lignin Content (%)	Lignin Portions Extractable (%)	Lignin Portions Non Extractable (%)	Polysacch. Content (%)	Glucose Portion (%)
25/20 RE	74.0	10.0	64.0	26.0	21.8
25/40 RE	44.8	2.3	42.5	55.2	51.1

In their investigation of NSSC pulps, Glasser and Barnett observed an increased affinity of residual lignin towards cellulose with proceeding cooking time[15]. The sugar compositions of Residues 3 with glucose as the dominating unit seem to confirm this hypothesis (Table 2). Further evidence is found in the sugar analysis of the ball-milled samples. In contrast to the selective activity of enzymes, the mechanical, non-selective bond-cleaving of ball-milling creates a more representative picture of the lignin-carbohydrate bonds. It must be acknowledged, however, that the data obtained from the isolated lignin preparations cannot be directly transferred to the residual lignin in the pulp, but must be interpreted in terms of relative changes. As the isolation procedure was painstakingly kept constant for all samples, changes observed in the isolated lignins must be attributed to real changes of the lignin in the pulp. This hypothesis finds further support by the fact that these changes are logical and clearly directed. Table 3 shows that after an initial decrease in the sugar contents in the first stage there is a remarkable increase of the arabinose,

TABLE 3

Sugar Contents of Residual Lignins

Sugar		MWL	1S/20 R	1S/40 R	2S/20 R	2S/40 R	2S/20 RE	2S/40 RE
Rhamnose	%	0.05	0.02	0.02	0.12	0.07	-	-
Mannose	%	0.90	0.35	0.32	0.72	0.60	0.85	1.06
Arabinose	%	0.24	0.16	0.13	0.17	0.74	Traces	0.11
Galactose	%	0.38	0.25	0.22	0.37	0.43	0.15	0.30
Xylose	%	0.54	0.27	0.26	1.54	2.38	0.23	0.24
Glucose	%	0.77	0.38	0.36	1.66	1.42	1.46	1.82
Σ Sugars	%	2.88	1.43	1.30	4.58	5.64	2.69	3.92
Σ Poly-sacch.	%	2.58	1.28	1.17	4.11	5.06	2.37	3.45

xylose and glucose contents in the second stage. As softwood xylans are presumably linked to lignin through both arabinose and xylose[16], the rising concentrations of the first two sugars suggest an enrichment of linkages between lignin and xylan remainders in the second stage.

Galactoglucomannans, with a sugar composition of about 3:1:1 (Man:Glu:Gal) for spruce[17], seem improbable as a possible source for the rising amounts of glucose in the second stage, as the concentrations of the other building units mannose and galactose do not increase accordingly. Especially in the case of mannose, the concentration found in the reference MWL is not surpassed by any of the ball-milled samples (Table 3).

In contrast to the lignin-xylan bonds, the adherence to cellulose seems to be at least partially reversible, as is indicated by a lower glucose concentration of 2S/40 R as compared to 2S/20 R and a good lignin extractability of Residue 2 of 2S/40 RE: Lignin's enhanced affinity to xylanic and cellulosic units could be a prime reason for the resistance of residual lignin to further delignification in the Organocell process, all the more so as the cooking conditions of the second stage (alkalinity, absence of strongly nucleophilic molecules such as SH^-) obviously facilitate the formation of new lignin-carbohydrate bonds[18]. Covalent bonds between lignin and carbohydrates would provide a possible explanation for the loss of aliphatic OH groups at the end of the second stage (see below). Other factors, however, such as the above-mentioned impeded diffusion of lignin through fibre pores or changes in lignin's chemical structure might also contribute to the problem of delignification.

Elemental Composition and Methoxyl Groups

The elemental compositions of the residual lignins (RL) are subject to minor differences only (Table 4). They resemble MWL much more closely than the spent liquor lignins (SLL), which are characterized by higher carbon and lower oxygen contents[1]. The differences between ball-milled and enzymatically isolated samples are almost negligible.

Both isolation procedures indicate a reduction in methoxyl groups towards the end of the second stage.

This finding is in contrast to the results for SLL, where methoxyl contents remained constant throughout digestion. Table 4 shows that the drop of methoxyl groups between MWL and 2S/40 (R, RE) is less than 0.5 %, which is in accordance with Gierer's statement that alkaline pulping conditions without the interference of strongly nucleophilic agents such as SH^- do not cause considerable demethylation[19]. No definite explanation of the fact that the corresponding SLL (15.67 % OCH_3) is apparently spared demethylation can as yet be offered.

TABLE 4

Elemental Composition, Methoxyl Content, C_9-Formulae and C_9-Molecular Weight of Residual Lignins (RL). (Values are corrected for proteins and sugars)

Substance	C (%)	H (%)	O (%)	OCH_3 (%)[3]	C_9-formulae	Molecular weight
MWL	62.99	5.72	31.29	15.34	$C_9H_{7.93}O_{2.76}(OCH_3)_{0.94}$	189.46
1S/20 R	61.54	6.07	32.39	15.18	$C_9H_{8.83}O_{2.99}(OCH_3)_{0.95}$	194.18
1S/40 R	61.93	5.89	32.18	15.45	$C_9H_{8.40}O_{2.92}(OCH_3)_{0.96}$	193.19
2S/20 R	62.44	6.02	31.54	15.49	$C_9H_{8.58}O_{2.75}(OCH_3)_{0.95}$	191.50
2S/40 R	63.31	5.91	30.78	14.94	$C_9H_{8.42}O_{2.75}(OCH_3)_{0.90}$	190.79
2S/20 RE	63.83	6.13	30.04	15.78	$C_9H_{8.53}O_{2.56}(OCH_3)_{0.95}$	187.26
2S/40 RE	63.93	6.06	30.01	14.89	$C_9H_{8.49}O_{2.56}(OCH_3)_{0.89}$	185.83

Weakly Acidic Groups (Carboxylic Units)

The increase in weakly acidic groups, very moderate in the first stage, becomes more pronounced in stage 2 (Table 5, Figure 2). All values for RL, however, stay well below those for SLL. The initial

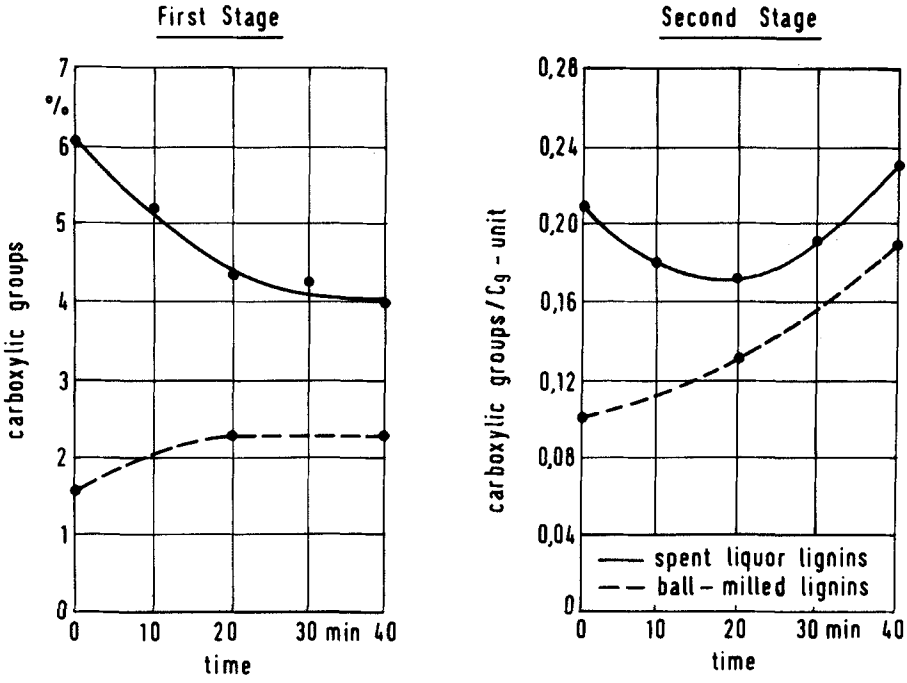

FIGURE 2: Carboxylic groups of residual and spent liquor lignins

decrease in SLL's weakly acidic groups in the first half of stage 2, explained on basis of solubility effects[1], cannot be observed in RL. This is mandatory for a substance that remains insoluble throughout the whole digestion. Besides there is another reason: RL's value at 0 min in Figure 2 ('2S/0 R'), which serves as starting point in the diagram, is identical to 1S/40 R. In constrast to the corresponding SLL, which was isolated at 0 min [1], it was therefore not yet exposed to the alkaline medium of the second stage. As a consequence the solubility effect discussed for SLL could not yet have set in.

TABLE 5

Phenolic and Aliphatic OH, Carboxylic and Carbonyl Groups of MWL and Residual Lignins (RL). (Values are corrected for sugars)

Substance	Phenolic OH			Aliphatic OH		COOH		CO	
	%	GC per C_9	NMR per C_9	%	per C_9	%	per C_9	%	per C_9
MWL	2.69	0.30	0.28	7.62	0.85	1.55	0.06	2.63	0.18
1S/20 R	2.73	0.31	0.30	7.82	0.89	2.28	0.10	1.98	0.14
1S/40 R	2.58	0.29	0.28	7.76	0.89	2.22	0.10	1.93	0.13
2S/20 R	3.18	0.36	0.40	7.22	0.81	3.08	0.13	2.13	0.15
2S/40 R	3.66	0.41	0.42	5.16	0.51	4.67	0.20	1.92	0.13
2S/20 RE	3.12	0.34	0.39	6.78	0.75	-	-	2.56	0.17
2S/40 RE	3.50	0.38	0.37	6.31	0.69	-	-	2.26	0.15

Carbonyl Groups

In their comprehensive investigation of kraft lignins, Marton [20] demonstrated that the labile coniferylaldehyde units (0.03 CO/OCH_3) are completely decomposed during the cook. This type of reaction presumably provokes the loss of about 0.04 CO/C_9 from RL in the first 20 min of stage 1 (Table 5). After that the CO contents remain constant. Values similarly low as those for SLL[1] were not detected. Again there is a striking coincidence between the two isolation procedures. As will be discussed later, acetal formation might also contribute to the decrease in CO groups, especially in the weakly acidic medium of stage 1.

OH Groups

The limited supply of residual lignins, especially for the enzymatically isolated samples made titration of phenolic OH groups impracticable. The application of Mansson's method plus the quantitative evaluation of ^{1}H-NMR spectra (Table 5) showed that in spite of a thorough test, the titration, used for the determination of SLL's phenolic contents, yielded too low values[1]. As all samples were affected to about the same degree relative differences between individual samples are nevertheless correct. Therefore the conclusions drawn from these results remain valid, except that the degree of ether cleavages is higher than originally suggested.

In the first stage, both the phenolic and the aliphatic OH contents are very close to MWL (Table 5). The relatively mild conditions of stage 1 apparently do not cause significant modifications of RL's structure.

The change to the alkaline medium of stage 2 triggers ether cleavages (ß-0-4) to a considerable degree, as can be seen by the substantial increase in RL's phenolic OH contents (Table 5, Figure 3).

Obviously, ball-milling causes some lignin fragmentation thus giving rise to both higher phenolic OH contents and lower molecular weights as will be discussed in Part 3 of this series.

So far changes in RL's functionality were less prominent than those for SLL. In the case of RL's aliphatic OH contents, however, rapid decomposition of this functional group was found in the second stage (Table 5, Figure 3). It is interesting to note that in their investigation of residual lignins from NSSC pulps Glasser et al.[21] also observed a significant, although reversible decrease in aliphatic OH contents, which was explained as formation and renewed cleavage of dialkylethers between different lignin units. As for the residual lignins according to the Organocell process, side chain degradation and formation or enrichment of covalent bonds to carbohydrates should also be considered possible alternatives to this hypothesis. This seems especially true as in contrast to the NSSC lignins, the loss of aliphatic OH groups is not reversible. At least in the case of the ball-milled samples, the latter alternative is confirmed by the rising sugar concentrations of the second stage samples. However, loss in aliphatic OH groups and concomitant increase in sugar concentrations are not sufficiently matched to be considered the sole reason, so that an interaction of all factors mentioned seems very likely.

Although the decrease of aliphatic OH groups is indicated by both isolation procedures, variations are larger than usual, particularly at the end of the second stage (2S/40 R, RE). However, this discrepancy can be attributed to the combination of extreme differences in yield and sugar concentration between 2S/40 R and 2S/40 RE on the one hand (Tables 1 and 4) and lignin's heterogeneity on the other hand.

^{1}H-NMR Spectra

Spectra of acetylated Brauns methanol acidolysis lignin show that methylation of lignin's α-C-OH group gives rise to signals in the region of δ3.3[22]. This finding was recently confirmed by Adler et al.[23]. As Organocell pulping is performed with methanolic liquors, partial methylation of lignin can be expected. Indeed, in contrast to MWL, all lignins isolated from the cooking residues signal at δ3.3 (Figure 4). However, the low signal intensity and an additional small maximum at δ6.1, which can be assigned to protons attached to acetylated α-carbons, suggest a very low degree of methylation of the α-C-OH group. This result is in accordance with the methoxyl group determination: since methylation necessarily raises lignin's methoxyl content, the amount of newly introduced methyl groups is obviously insignificant according to the data in Table 4.

Under the conditions of Organocell pulping acetal formation may also be possible, especially in the first stage. Adler at al.[23] stated that methoxyl groups from acetal structures may contribute to the δ3.3 peak. Also methoxyl groups from acetals of glyceraldehyde-2-aryl ethers were found to signal at about δ3.4. This signal, which is missing in the second stage RL (Figure 4), can be clearly separated from the one at δ3.3 in the case of first stage RL. Therefore, as far as stage 1 is concerned, the reduction of carbonyl groups may partly be caused by acetalization reactions.

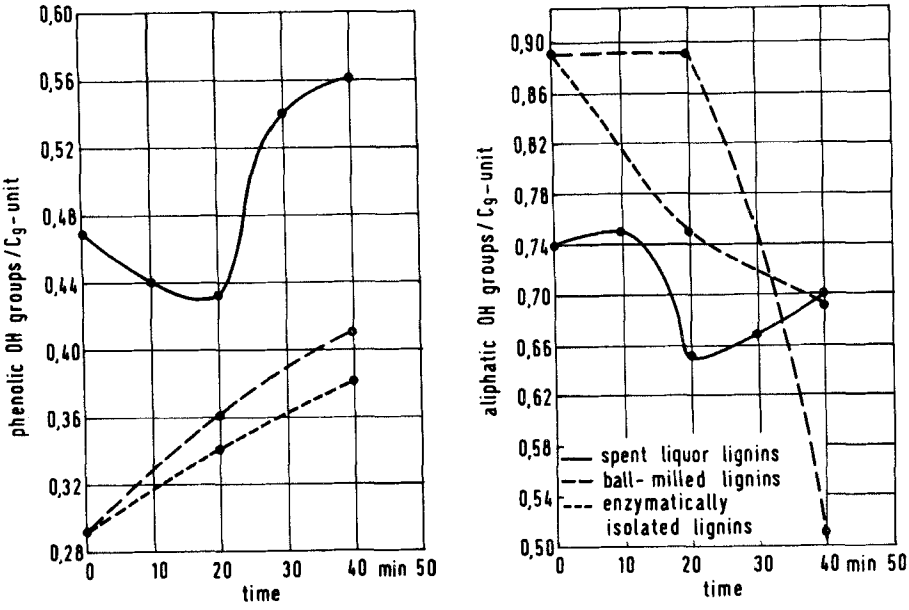

FIGURE 3: Phenolic and aliphatic OH groups of ball-milled, enzymatically isolated and spent liquor lignins from the second stage

As the aromatic signal range is relatively free from interference by other types of protons, estimates of the number of aromatic protons and, as a consequence, of the degree of condensation can be made[22]. Although these estimates are somewhat ambiguous for the second stage samples, as exposure to the alkaline medium of stage 2 certainly leads to partial side chain degradation, a clear trend towards more condensed structures with progressing reaction time can be observed. When calculating the aromatic protons of the second stage lignins - considering the above-mentioned side chain degradation - in terms of $H/C_{8.5}$[22], the formation of more condensed structures becomes even more evident (Table 6).

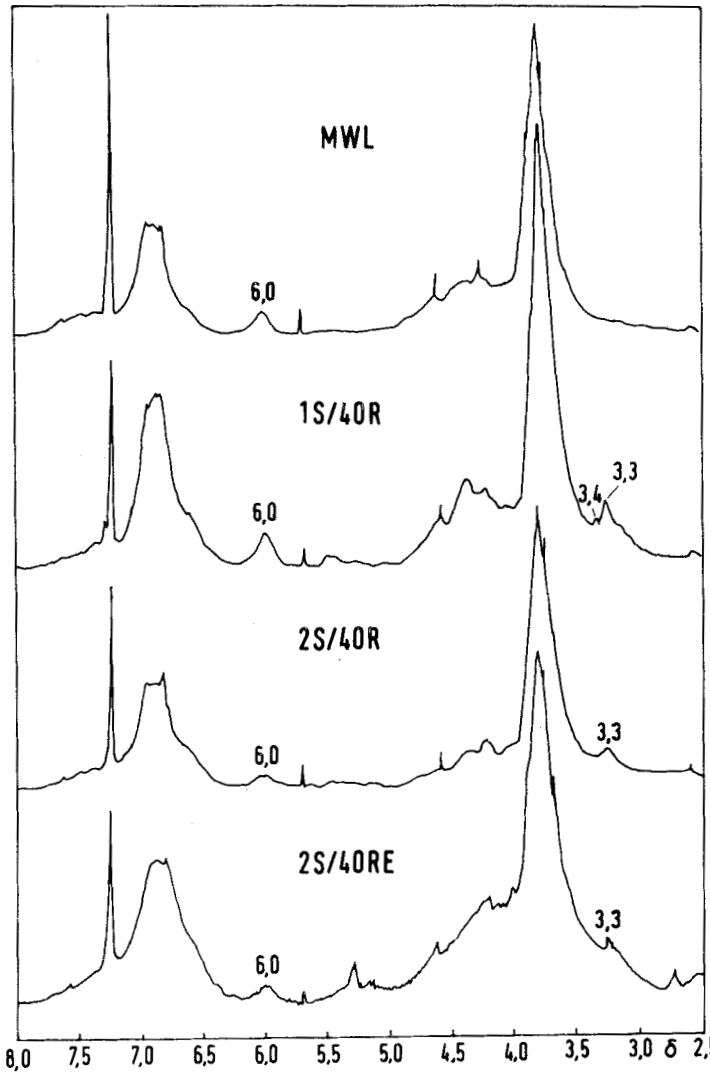

FIGURE 4: Sections of ^{1}H-NMR spectra of residual lignins

TABLE 6

Aromatic Protons of MWL and Residual Lignins

Substance	$H_{Arom.}/C_9$	$H_{Arom.}$/C 8.5
MWL	2.60	-
1S/20 R	2.75	-
1S/40 R	2.58	-
2S/20 R	2.28	2.15
2S/40 R	2.49	2.35
2S/20 RE	2.32	2.19
2S/40 RE	2.28	2.15

CONCLUSIONS

Lignins from cooking residues of 20 l batch digestions according to the Organocell process were isolated and characterized in terms of yields, sugars and functional groups. Two different isolation procedures involving either ball-milling or enzymatic treatment were applied for the second stage samples. While an increasing adherence to both xylanic and cellulosic units can be assumed for the ball-milled samples, the enzymatically isolated lignins are almost exclusively attached to cellulosic remainders.

No significant differences between the functional groups could be observed for the two isolation procedures. With the exception of aliphatic OH groups,

residual lignins are modified to a lesser extent than their counterparts precipitated from the liquor. The reduction of aliphatic OH groups at the end of the cook can be explained by the concomitant influence of several factors such as

- increase in covalent bonds to carbohydrates via aliphatic OH groups
- possible formation of dialkylether bonds between different lignin units
- side chain degradation.

The methylation of α-C-OH groups proved to be insignificant. For the first stage, a possible acetalization of aldehyde functions may be deduced from ^{1}H-NMR spectra. The reduction of aromatic protons indicates a more condensed structure of second stage lignins.

The final part of this series will deal with oxidative degradation and molecular weight determination of Organocell lignins.

ACKNOWLEDGEMENT

We wish to thank Professor Dr. Wabner, Angewandte Elektrochemie, Anorganisch-Chemisches Institut der Technischen Universität München, for providing us with the ^{1}H-NMR spectra and Organocell GmbH for its productive cooperation.

This work is part of the doctor's thesis of the first author and is supported by the Bundesministerium für Forschung und Technologie (BMFT, Bonn).

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