

Effect of autohydrolysis on the lignin structure and the kinetics of delignification of birch wood

Tiina Rauhala, Alistair W. T. King, Gerhard Zuckerstätter, Simopekka Suuronen, and Herbert Sixta

KEYWORDS: Birch, Autohydrolysis, Kraft pulping, Delignification kinetics, Lignin structure, ^{13}C -NMR, ^{31}P -NMR, MWL

SUMMARY: The first aim of this study was to investigate the delignification kinetics of alkaline cooking for autohydrolyzed birch wood (*Betula pendula*). A series of cooking trials, at temperatures ranging between 130°C and 170°C were performed on untreated (P-factor 0) and autohydrolyzed (P-factor 200) wood. The results of carbohydrate and lignin analyses were fitted to a kinetic model and it was observed that autohydrolysis doubles the delignification rate, during bulk delignification. However, a slight decrease was noticed in the delignification rate during residual pulping. Thus, the second aim of this study was to elucidate the reasons behind accelerated delignification. For this purpose two different autohydrolysis treatments (P-factors 200 and 750) were applied to birch wood. Lignin was isolated from both the native and autohydrolyzed wood and subjected to elemental analysis, methoxyl group determination, carbohydrate analysis and quantitative ^1H , ^{13}C and ^{31}P nuclear magnetic resonance (NMR) spectroscopy. The results indicate that, during autohydrolysis, β -O-4 linkages were cleaved, the methoxyl group content was reduced and the syringyl/guaiacyl (S/G) ratio decreased. In addition, a decrease in primary and secondary aliphatic hydroxyl groups and an increase in phenolic hydroxyl groups were detected. It could be concluded that condensation reactions were also occurring during autohydrolysis. Although lignin reactions such as the cleavage of β -O-4-cleavages and the increase in phenolic hydroxyl groups partly account for the pulping behaviour the reason behind the significantly accelerated bulk delignification is not entirely explained through this study. More research concerning reactions during autohydrolysis is needed.

ADDRESSES OF THE AUTHORS: **Tiina Rauhala** (tiina.rauhala@tkk.fi), **Simopekka Suuronen**, and **Herbert Sixta** (herbert.sixta@tkk.fi): Aalto University, School of Science and Technology, Department of Forest Products Technology, PO Box 16300, 00076 Espoo, Finland, **Alistair W T King** (alistair.king@helsinki.fi): University of Helsinki, Department of Chemistry, Laboratory of Organic Chemistry, PO Box 55, 00014 Helsinki, Finland, **Gerhard Zuckerstätter** (g.zuckerstaetter@lenzing.com): Kompetenzzentrum Holz GmbH (Wood K plus), A-4021 Linz, Austria.
Corresponding author: Herbert Sixta

Pre-hydrolysis remarkably improves delignification efficiency during subsequent alkaline cooking processes. Kinetic investigations have confirmed that the bulk delignification rate of kraft cooking is significantly

enhanced when *Eucalyptus saligna* was subjected to autohydrolysis, with a pre-hydrolysis factor (P-factor) of 620 (Schild et al. 1996). Similar effects have also been reported for soda, soda-AQ and kraft cooking of sweetgum (*Liquidambar styraciflua* L.) (Lin 1979). In practice, this is expressed by both lower H-factors and kappa numbers (Lin 1979; Sixta 2006). The rate of delignification during the kraft pulping stage increases with pre-autohydrolysis intensity and reaches a maximum for P-factors of 600 – 1000, depending on the wood species. After extensive autohydrolysis, exceeding P-factors of 1000, the delignification rate in a subsequent alkaline cooking step decreases due to the precipitation of condensed lignin degradation products on the wood fibre surface, thus preventing solution-phase mass transport (Leschinsky et al. 2008b).

The acceleration of the delignification rate of pre-hydrolysed wood chips mainly applies to hardwoods. However, as recently shown, the Kraft pulping efficiency of softwood was also noticeably improved by autohydrolysis pre-treatment (Trogen 2010). Under the given cooking conditions (18% EA-charge, 40% ‘sulfidity’ and 160°C cooking temperature) the H-factor could be reduced from 1450 to 1050, to achieve the kappa number target of 33, when spruce wood was pre-treated by autohydrolysis using an intensity of P-factor 200. Aiming at a kappa number of 18, after oxygen delignification, the H-factor could be further reduced to 850 for the autohydrolyzed spruce, due to its higher delignification efficiency as compared to the reference kraft pulp.

The reason for the enhanced delignification after autohydrolysis has been suggested to be associated with the cleavage of aryl-ether bonds, resulting in lignin depolymerisation and the formation of new phenolic hydroxyl groups (Leschinsky et al. 2008b). This leads to an increase in the pore volume of the wood, thus improving the diffusion of pulping chemicals and degraded lignin (Smiljanski, Stankovic 1974). In addition, it may be assumed that the cleavage of covalent bonds, between carbohydrates and lignin, accounts for an enhanced delignification rate, particularly during oxygen delignification. It has also been suggested that the formation of non-lignin derived chromophoric structures is impeded, because of the lower content of residual carbohydrate structures. This has been demonstrated for pre-hydrolysed kraft pulps, by the presence of considerably lower amounts of non-lignin and hexenuronic acid (HexA) structures contributing to the kappa numbers, when compared to conventional paper-grade kraft pulps (Antonsson et al. 2003).

In order to further our understanding of chemical phenomena occurring during autohydrolysis, further studies are required on different species incorporating traditional and advanced analytical techniques. This study is comprised of two parts: a kinetic study of

autohydrolyzed wood, during kraft pulping and the detailed analysis of lignin, before and after autohydrolysis. Furthermore, the aim of the first part is to compare the cooking kinetics of birch autohydrolyzed and untreated wood. The aim of the second part is to study lignin structural changes during autohydrolysis in order to understand the enhancement of delignification.

Materials and Methods

Delignification kinetic studies

Autohydrolysis conditions

Birch chips (*Betula pendula*) were used as raw material. Frozen chips were thawed for 2 days prior the experiments. Autohydrolysis was conducted in a rotating batch air-bath digester carrying six two litre-autoclaves. 300 g of wood was placed inside each autoclave together with deionized water to achieve a liquor-to-wood ratio of 4:1. The reactions were stopped once P-factors of 200 (150°C) or 750 (170°C) were reached (Sixta 2006). The P-factor expresses the prehydrolysis time and temperature as a single variable based on the Arrhenius equation analogous to the H-factor but using an activation energy of 125.6 kJ/mol (Sixta 2006). The rather mild conditions were chosen to ensure sufficiently high yield and mechanical properties, as required for the production of a paper-grade pulp. Untreated and autohydrolyzed wood were analysed for insoluble (Klason) lignin, acid soluble lignin and carbohydrate content, according to the NREL standard (Sluiter et al. 2008). The quantification of the carbohydrates was carried out with the Dionex ICS 3000 HPAEC-PAD (Sixta et al. 2001). The method was modified for the new CarboPac PA20 column (Dionex).

Kraft pulping conditions

Both untreated and autohydrolyzed birch chips were thoroughly washed with deionized water, at 80°C, air dried for 24 h, milled using a Wiley mill and sieved through a 1 mm hole screen. The kinetic studies were conducted in a 2 l oil-heated Büchi reactor (Suuronen 2010). A high liquor-to-wood ratio of 100:1 was selected to ensure a relatively constant concentration of the active cooking chemicals throughout the reaction. The reactor, filled with cooking liquor containing the wood substrate, was purged twice (0.5 MPa) with nitrogen to remove oxygen from the reactor. The reactor contents were stirred at 200 rpm. At least eight time steps were conducted at four different temperature levels: 130°C, 140°C, 155°C, and 170°C. The range of the time steps was from 3.5 min to 22 h. The emphasis of the time steps was to encompass the beginning of bulk-phase and the advanced residual-phase delignification. In all experiments, the concentrations of the active cooking chemicals were kept constant: [OH⁻]=0.6 M, [HS⁻]=0.2 M and [Na⁺]=1.5 M. The reaction was terminated by turning on the cooling circuit and discharging the contents of the reactor. The reactor was flushed with deionized water, while stirring was increased to 400 rpm, to ensure thorough washing. A sample of the undiluted black liquor was collected for further analysis. The washed pulp was filtered on a Büchner funnel and dried at room temperature for two days. After drying, the moisture content of the materials

was analysed and thus the yield was determined. Insoluble lignin, acid soluble lignin and carbohydrates were also analysed with the same methods as for the raw material. More detailed information about the analytical methods can be found elsewhere (Suuronen 2010).

Kinetic model

The general structure of the kinetic model follows the Purdue model further developed by Smith (1974) and Christensen et al. (1983). In this model, delignification is described by three parallel pseudo-first order reactions, assuming that the three different lignin species react simultaneously. The chosen experimental conditions did not enable us to follow the delignification kinetics of the initial lignin species. Thus, delignification was modelled with two parallel pseudo-first order reactions representing the bulk (L_2) and residual lignin (L_3) species.

The corresponding rate equation is expressed in Eq 1,

$$L = L_2 \cdot e^{-k_2 t} + L_3 \cdot e^{-k_3 t} \quad [1]$$

here L is the sum of L_2 and L_3 remaining after time t . L_2 and L_3 represent the bulk and residual lignin species. k_2 and k_3 are the corresponding delignification rate constants.

The rate constants for the removal of the two different forms of lignin can be expressed as an Arrhenius-type equation, Eq 2,

$$k_j = A_j \cdot e^{\left(\frac{E_{A_j}}{RT}\right)} \quad [2]$$

where j refers to lignin species 2 or 3. A is the pre-exponential factor and E_A is the activation energy for the delignification reactions.

The fitting of the models to the experimental data was performed by minimizing the residuals between the calculated and the experimental data, according to the least squares method available in Scientist® for Windows 2.0, from Micromath Research.

Lignin analysis

Autohydrolysis for lignin analysis

Autohydrolysis was performed on birch chips in a rotating batch oil-bath digester, with eight autoclaves of 220 ml each. Two different intensities were applied: P-factor 200 at 150°C and P-factor 750 at 170°C. The liquid-to-wood ratio used was 4:1. After completion of autohydrolysis, autoclaves were cooled rapidly in ice water. The wood residue was washed manually using washing bags at the temperature of 70°C. After autohydrolysis the wood chips were milled with a Wiley mill to mesh 30 and extracted with a mixture of acetone/water (9:1) for 24 h in a Soxhlet apparatus.

Lignin isolation

Milled wood lignin (MWL) was isolated and purified from the residual untreated wood (P-factor 0) as well as from autohydrolyzed residual wood (P-factors 200 and 750), following the protocol of (Bjorkman 1956) with some modifications: During ball milling, toluene was replaced by nitrogen and during the dioxane/water extraction an ultrasonic bath was used to enhance the extraction.

MWL analyses

Elemental analysis was carried out by the Fraunhofer Institut für Angewandte Polymerforschung, Potsdam, Germany. The carbohydrate content was analysed after a two stage total hydrolysis procedure, by anion exchange chromatography (AEC), with pulsed amperometric (PAD) detection, after total hydrolysis with H₂SO₄ (Sixta et al. 2001). The methoxyl group (OCH₃) content was quantified according to a literature method (Chen 1992).

Prior to quantitative ¹H and ¹³C (Q-¹³C) NMR measurements, all MWL samples were acetylated following a literature protocol (Lundquist 1992). NMR measurements were performed according to a published method (Leschinsky et al. 2008a) with the following exceptions: Additional ¹³C Q-DEPT+ (Jiang, Xiao et al. 2008) NMR scans were recorded with 5 s relaxation delay, 0.54 s acquisition time, 200 ppm spectral width and 40,000 – 60,000 scans. 2D ¹H/¹³C HSQC NMR data was collected without addition of chromium(III)-acetylacetonate (Cr(acac)₃). Each spectral dataset comprised 2048 data points in the ¹H-dimension and 512 time domain increments, in the ¹³C dimension. 512 scans were accumulated per transient with 0.5 s relaxation delay and 0.21 s acquisition time at 16 ppm (¹H) and 165 ppm (¹³C) spectral width. 2D time domain data were multiplied with a shifted square sine bell and exponential window functions (¹³C dimension: WDW=QSINE, SSB=2; ¹H dimension: WDW=EM, LB=3 Hz) in both dimensions and zero-filled to 2048 * 2048 real data points, prior to Fourier transform.

Prior to quantitative ³¹P (Q-³¹P) NMR measurements, samples were phosphitylated according to a modified procedure by (Guerra et al. 2006): MWL (25 mg) was phosphitylated and diluted with Cr(acac)₃/CDCl₃ solution (1 mg/ml, 1 ml). Quantitative ³¹P spectra (243 MHz) were recorded at 27°C using an inverse gated proton decoupling sequence on a Varian Unity Inova 600 spectrometer equipped with a 5 mm direct detection broadband probe-head. Quantitative ³¹P spectra were collected with 512 scans using 80° pulse-flip angle, 80000 Hz spectral width, 1 s acquisition time, and 10 s relaxation delay. Publication data was processed using iNMR, from Mestralab Research. The fid files were Fourier transformed with 64 K zero-filling and a 3 Hz exponential line broadening factor. Phasing was performed manually between the internal standard region (152.0 ppm) and symmetrical phosphite anhydride region (132.2 ppm). Baseline correction was performed using a polynomial with 0° of correction and a 128 K filter.

Results and Discussion

Kraft cooking kinetics

The results of the fitting of the parameters to the experimental data are presented in *Table 1*.

The standard deviation of modeling the P-0 and the P-200-data was calculated to 1.80% and 1.95%, respectively. According to the model calculation, the fraction of the easily removable initial lignin species ($L_1 = 1 - (L_2 + L_3)$) increases significantly from 0.22 to 0.52, indicating that autohydrolysis causes substantial lignin fragmentation and thus the formation of low molecular

weight phenolic compounds, susceptible to alkali extraction. Both an increase in the phenolic hydroxyl group content and a decrease in the molecular weight of residual lignin fractions, isolated as MWL after autohydrolysis of *Eucalyptus globulus*, has been shown previously (Leschinsky et al. 2008a).

Table 2 reveals that the mild autohydrolysis pre-treatment of birch wood meal approximately doubles the bulk delignification rate constant (k_2) of both Klason lignin and acid soluble lignin (Suuronen 2010). The ratio of the bulk delignification rate constants of the autohydrolyzed (P-200) and the untreated (P-0) birch chips decreases with increasing temperature due to the significantly lower E_A of the former. The rather low E_A (100 kJ/mol) for the bulk delignification of autohydrolyzed wood, compared to that of untreated wood (113 kJ/mol), indicates slight changes in the overall delignification mechanisms. For beech wood it was already reported previously that the E_A of the bulk delignification decreases, as a result of the autohydrolysis pre-treatment (Schild et al. 1996).

Consequently, this effect would contribute to improved delignification selectivity provided that the E_{AS} for cellulose depolymerisation and carbohydrate degradation remain unchanged. However, more research, using model compounds, is necessary to elucidate the effect of autohydrolysis on the mechanism of alkaline delignification.

Interestingly, the residual delignification rate is about 20% lower for autohydrolyzed, as compared to the untreated birch wood (*Table 2*). The decrease in the residual lignin reactivity is even more pronounced for the acid soluble lignin where the delignification rate is only about 50% of that of untreated wood (Suuronen, 2010). This can be partly explained by an increase in the proportion of condensed lignin structures through autohydrolysis of wood as later shown in this study. The overall delignification kinetics, however, is not significantly affected when mild autohydrolysis conditions are applied (*Fig 1*).

Table 1. Delignification kinetics, based on the Klason lignin data from untreated (P-0) and pre-hydrolysed (P-200) birch chips: parameters obtained by fitting to the model represented by Eqs (1) and (2).

Coefficients	Unit	P-0	P-200
A ₂	min ⁻¹	2.51*10 ¹² ± 2.31*10 ¹¹	1.19*10 ¹¹ ± 1.78*10 ¹⁰
A ₃	min ⁻¹	8.88*10 ¹³ ± 4.83*10 ¹³	1.10*10 ¹⁴ ± 1.01*10 ¹⁴
EA ₂	kJ mol ⁻¹	112.9 ± 0.40	99.5 ± 0.64
EA ₃	kJ mol ⁻¹	133.9 ± 2.35	135.3 ± 3.90
L ₂		0.69 ± 0.03	0.42 ± 0.02
L ₃		0.09 ± 0.03	0.06 ± 0.01

Table 2. Calculated delignification rate constants k_2 and k_3 , based on the Klason lignin data.

Temp. (°C)	k_2 (min ⁻¹)		k_3 (min ⁻¹)		k_3 P-200 / P-0	
	P-0	P-200	P-0	P-200		
130	0.006	0.015	2.50	0.0004	0.0003	0.75
140	0.013	0.031	2.39	0.0010	0.0009	0.90
155	0.042	0.087	2.07	0.0041	0.0034	0.83
170	0.123	0.224	1.82	0.0146	0.0122	0.84

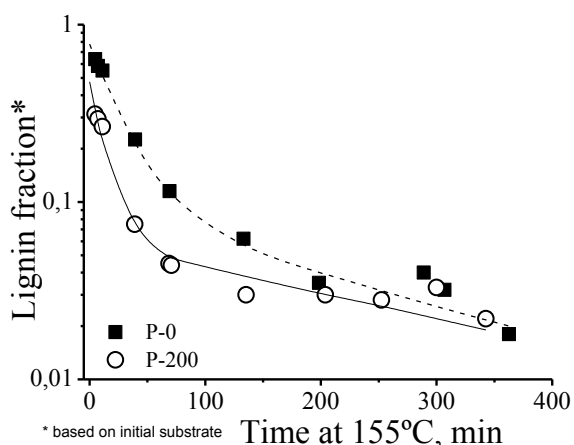


Fig 1. Effect of autohydrolysis (P-200) on the delignification kinetics of birch wood under comparable conditions: liquid:solid = 100:1, $[OH^-] = 0.6$ M, $[HS^-] = 0.2$ M, $[Na^+] = 1.5$ M, 155°C .

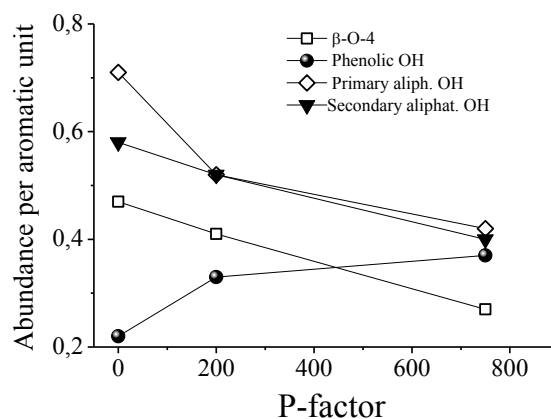


Fig 2. The relationship between aliphatic and phenolic hydroxyl (OH) groups (^{13}C NMR) compared to β -O-4 linkages (Q-DEPT+) in acetylated MWL samples.

Table 3. Elemental composition, methoxyl group content and sugar content of the MWL samples.

P-factor	Impurities (%)		Elemental analysis (%)			Methoxy group content (%)	Empirical C_{900} formula
	Sugar content	Ash content	C	H	O		
0	4.20	2.36	59.00	5.94	34.85	22.46	$\text{C}_{900}\text{H}_{781}\text{O}_{297}(\text{OCH}_3)_{158}$
200	3.00	1.62	59.63	5.91	34.15	21.71	$\text{C}_{900}\text{H}_{802}\text{O}_{305}(\text{OCH}_3)_{152}$
750	2.20	1.12	59.78	5.67	34.21	21.48	$\text{C}_{900}\text{H}_{813}\text{O}_{311}(\text{OCH}_3)_{147}$

Values are adjusted to account for sugar and ash impurities

Residual lignin

Elemental compositions, carbohydrate contents and molecular weight distributions

The carbohydrate analysis of the MWLs revealed that the overall sugar content decreases with increasing autohydrolysis intensity (Table 3) indicating a partial cleavage of lignin carbohydrate (LCC) bonds. The decrease in carbohydrate content during autohydrolysis is attributable to the removal of xylan. This finding is expected since xylan rich hemicelluloses are heavily degraded and depolymerised during autohydrolysis. The elemental composition of the autohydrolyzed wood reveals slightly higher carbon percentages along side lower hydrogen and oxygen percentages. The methoxyl group content in MWL isolated from autohydrolyzed wood was also lower than in MWL isolated from native (untreated) wood. The changes in the elemental analyses however were not significant to any extent.

MWL analyses

Quantification of the most important structural units of MWL as obtained from the ^{13}C NMR and ^{13}C Q-DEPT+ measurements presented in Fig 2 and Table 4.

The processed spectra from the ^{31}P NMR measurements, as a method of quantifying aliphatic vs shows little change in substitution (Ar-C and Ar-O) patterns in the aromatic rings but yet a decrease in S/G phenolic and carboxylic hydroxyl groups, are illustrated in Fig 3. The data from ^{31}P NMR measurements are presented in Table 5 and the values are given in mmol/g of hydroxyls. Birch wood contains mainly syringyl (S) and guaiacyl (G) phenolic structures. S and condensed

Table 4. ^{13}C NMR and Q-DEPT+ data for the corresponding acetylated MWL samples.

P-factor	S/G ratio	Methoxy/Ar ratio
0	1.98	1.51
200	1.68	1.18
750	1.57	1.07

(C) phenolic regions are grouped together as the peaks are broad and not fully resolved. Increases in G-type phenolic resonances, with increasing P-factor, can indicate either cleavage of β -O-4 linkages or demethylations. This is accompanied by an increase in the combined S-type (142-143 ppm) and C-type phenolic (140.5-144.5 ppm) resonance regions (Figure 3), although it is qualitatively obvious from visual inspection of the spectra that a significant increase in C-type phenolic resonances is occurring and not necessarily S-type phenolic resonances. This could indicate condensation of G-type phenolic residues. This is also accompanied by a decrease in the S/G ratio by Q- ^{13}C (Table 4) and a slight increase in G-type residues. These observations could be explained by a significant dealkylation (through demethylation or β -O-4 cleavage) of G-type residues, followed by condensation (Ar-Ar) of a large proportion of those residues. This condensation is not supported by the Q- ^{13}C and Q-DEPT data, which residue ratios. A further explanation for these changes could be as a result of extraction of non-condensed syringyl-rich lignin, during the pre-hydrolysis treatment. A decrease in S/G residue ratios would also be supported by dealkoxylation of S residues. Again this requires a significant change in Ar-O resonances, by ^{13}C NMR,

which is not evident at this resolution. A modest decrease in aliphatic (A) resonances are also observed from both the ^{31}P and ^1H data (Table 5 and Fig 2), with the ^{13}C data indicating the major decrease from secondary alcohols. This suggests that if condensation reactions are occurring, they at least involve consumption of benzylic positions, possibly brought about by conversion of phenolic structures into reactive intermediate quinone methides and further complexation with alcohols. An increase in condensed structures upon autohydrolysis of *Eucalyptus globulus* has also been reported previously (Leschinsky et al. 2008b).

The value of 1.51 methoxyl groups per aromatic unit, determined for the untreated birch MWL (Table 4), corresponds rather well to the value of 158 per 100 C_9 units in Table 3. However, the ^{13}C NMR values of the P-200 and P-750 MWL differ significantly from the corresponding empirical formulas of Table 4. It is possible that C_{900} formulas do not describe the structure of degraded lignin correctly because of the changes occurring in lignin structure during autohydrolysis. Regardless of this, both methods show a decrease in the amount of methoxyl groups for autohydrolyzed wood. As mentioned previously (Table 4), the S/G ratio decreased during autohydrolysis. Again these results indicate that the lignin enriches in G-type units due to the preferential degradation of the S-type lignin units, although not all the data supports this conclusion.

The lignin degradation during autohydrolysis is clearly demonstrated by the decrease in the $\beta\text{-O-4}$ content (Figure 2). The increase of the phenolic hydroxyl group content, observed in both ^{13}C and ^{31}P data (Fig 2 and Table 5), also indicates the occurrence of an extensive cleavage of the aryl-ether bonds. An increase of the phenolic OH groups is catalysed by acid and it has been attributed to the cleavage of $\beta\text{-O-4}$ bonds (Klemola 1968). At the same time the content of aliphatic primary and secondary OH groups is lower for the hydrolysed wood (Figure 2 and Table 5). Similar results have been found with autohydrolyzed *Eucalyptus globulus* (Leschinsky et al. 2008b). It can be assumed that the elimination of aliphatic OH groups is a result of the aryl-ether cleavage mechanism. This is in agreement with a previous model compound study (Li, Lundquist 2000).

Table 5. ^{31}P NMR data quantifying free hydroxyls in the corresponding MWL samples.

P-factor	Free Hydroxyl Functionalities (mmol/g)			
	Aliphatic (A)	Phenolic (S and C)	(G)	Carboxylic Acid (COOH)
0	5.43	0.74	0.42	0.18
200	5.10	1.12	0.52	0.18
750	4.35	1.66	0.66	0.23

Conclusions

The results show that even a mild autohydrolysis (P-factor 200) causes a significant increase in the delignification rate during kraft pulping conditions, especially during the bulk delignification phase. Native lignin in *Betula pendula* experiences structural alterations and degradation during autohydrolysis. The main reaction causing the lignin degradation is the cleavage of $\beta\text{-O-4}$ linkages. Also, a decrease in primary and secondary aliphatic hydroxyl groups and an increase in phenolic hydroxyl groups can be detected. It may be concluded that lignin degradation proceeds via homolytic cleavage of aryl-ether bonds. In addition, evidence suggests that condensation reactions are also occurring, potentially involving condensation at the benzylic position. The results here are not conclusive and the apparent increase in degree of condensation and reduction in S/G ratio may also occur due to selective removal of low molecular weight (uncondensed) syringyl-rich lignin, during the autohydrolysis treatment. Despite the attempts to expose the reactivity of lignin under these conditions, these results do not entirely explain the increase in the delignification rate during kraft pulping of autohydrolyzed wood, other than the potential breakage of lignin carbohydrate complexes. In addition, improved NMR analyses and a more comprehensive analysis of extracted lignins would be desirable for future studies to achieve more solid conclusions.

Acknowledgements

We would like to acknowledge TEKES, Andritz Oy, Danisco Sweeteners Oy, Oy Metsä-Botnia Ab, Stora Enso Oyj and UPM Kymmene for financial support of the project.

Literature

- Antonsson, S., Lindström, M. E. and Ragnar, M. (2003): A comparative study of the impact of the cooking process on oxygen delignification, Nord. Pulp Pap. Res. J., 18 (4), 388-394.
- Bjorkman, A. (1956): Finely divided wood. I. Extraction of lignin with neutral solvents, Sven. Papperstidn., 59, 477-85.
- Chen, C.-L. (1992): Determination of Methoxyl Groups. Methods in Lignin Chemistry. S. Y. Lin and C. W. Dence. Germany, Springer-Verlag Berlin Heidelberg, 578.
- Christensen, T., Albright, L. F., and Williams, T. (1983): A kinetic mathematical model for the kraft pulping of wood, TAPPI Annual Meeting, Atlanta, Georgia, USA, 239-246.
- Guerra, A., Filpponen, I., Lucia, L., Saquing, C., Baumberger, S., and Argyropoulos, D. (2006): Toward a Better Understanding of the Lignin Isolation Process from Wood, J. Agric. Food Chem., 54 (16), 5939-5947.
- Jiang, B., Xiao, N., Liu, H., Zhou, Z., Mao, X., and Lui, M. (2008): Optimized Quantitative DEPT and Quantitative POMMIE Experiments for ^{13}C NMR, Anal. Chem., 80 (21), 8293-8298.
- Klemola, A. (1968): Investigations of birchwood (*Betula pubescens*) lignin degraded by steam hydrolysis, Suomen Kemistilehti A, 41 (7-8), 166-180.

Leschinsky, M., Zuckerstätter, G., Weber, H., Patt, R., and Sixta H. (2008a): Effect of autohydrolysis of Eucalyptus globulus wood on lignin structure. Part 1: comparison of different lignin fractions formed during water prehydrolysis, *Holzforschung*, 62 (6), 645-652.

Leschinsky, M., Zuckerstätter, G., Weber, H., Patt, R., and Sixta H. (2008b): Effect of autohydrolysis of Eucalyptus globulus wood on lignin structure. Part 2: influence of autohydrolysis intensity, *Holzforschung*, 62 (6), 653-658.

Li, S. and Lundquist, K. (2000): Cleavage of arylglycerol β -aryl ethers under neutral and acid conditions, *Nordic Pulp and Paper Research Journal*, 15 (4), 292-299.

Lin, C. K. (1979): Prehydrolysis-alkaline pulping of sweetgum. Department of Wood and Paper Science. Raleigh, NC 27650, USA, PhD Thesis at North Carolina State University. PhD-Thesis: 121.

Lundquist, K. (1992): Proton (^1H) NMR spectroscopy [of lignin in solution]. *Methods Lignin Chem.* S. Y. Lin and C. W. Dence. Berlin, Springer, 242-249.

Schild, G., Mueller, W. and Sixta, H. (1996): Prehydrolysis kraft and ASAM paper grade pulping of eucalypt wood. A kinetic study, *Papier (Darmstadt)*, 50 (1), 10-22.

Sixta, H. (2006): Multistage kraft pulping, In: Sixta, H. (ed): *Handbook of Pulp*. Weinheim, Wiley-VCH, 325-365.

Sixta, H., Schelosky, N., Milacher, W., Baldinger, T., and Röder, Th. (2001): Characterization of Alkali-Soluble Pulp Fractions by Chromatography. 11th ISWPC. Nice, France. 3, 655-658.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., and Crocker, D. (2008): <http://www.nrel.gov/biomass/pdfs/42618.pdf>.

Smiljanski, S. and S. Stankovic (1974): Beech wood glucuronoxylan in the prehydrolysis kraft process, *Cellul. Chem. Technol.*, 8 (3), 283-94.

Smith, C. (1974): Studies of the mathematical modelling, simulation and control of the operation of a Kamy continuous digester for the Kraft process. West Lafayette, Indiana, USA, Purdue University. **PhD Thesis**.

Suuronen, S. (2010): Effect of autohydrolysis on the kinetics of alkaline cooking. Department of Forest Products Technology. Espoo, Finland, Aalto University. **MSc Thesis: 98**.

Trogen, M. (2010): Autohydrolysis of softwood prior to alkaline pulping. Department of Forest Products Technology. Espoo, Finland, Aalto University, School of Science and Technology. **MSc Thesis**.

Manuscript received February 22, 2011

Accepted June 8, 2011

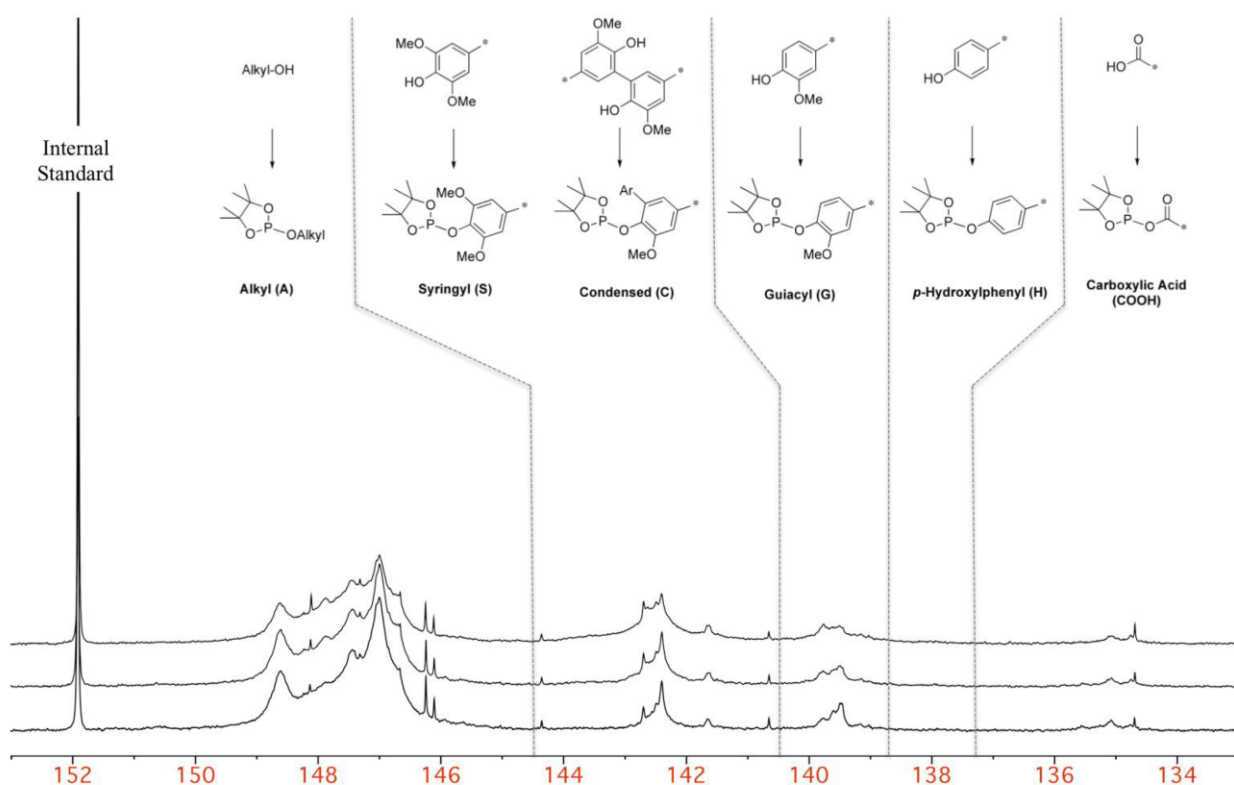


Fig 3. ^{31}P NMR spectra of MWLs; P-factor 0 (bottom), P-factor 200 (middle), and P-factor 750 (top).