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NMR study on residual lignins isolated from chemical pulps of beech wood by enzymatic hydrolysis

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ABSTRACT

Two residual lignins isolated from kraft and ASAM pulps of beech wood (*Fagus sylvatica* L.) were analyzed by ^{1}H and ^{13}C NMR spectra to confirm the structural features previously obtained from wet chemical degradation methods. ^{1}H NMR spectra revealed that the most distinct features of ASAM lignin was the signal at δ_H 5.9 ppm and δ_H 6.6 ppm for Hα in β-O-4 linkage and protons for syringyl units. The abundance of syringyl units in ASAM lignin was also evidenced by the signals at δ_C 154 ppm and δ_C 104 ppm in the ^{13}C NMR spectra, responsible for aromatic C3/C5 and C2/C6 of syringyl units. The important functional groups, such as phenolic OH, aliphatic OH and methoxyl groups, were indirectly evaluated by ^{13}C NMR spectra of acetylated residual lignin. In particularly, the increment of phenolic OH, one of the parameter for cleavage of β-O-4 linkages, was determined to relative low in of β-O-4 linkages in ASAM residual lignin as compared to kraft residual lignin, indicating that ASAM procedures may run by quite different pathway for delignification unlike the fragmentation of β-O-4 linkages and cleavage of side chain in the general alkaline pulpings.

1. Introduction

All terrestrial plant cell walls are constructed with three essential organic materials: cellulose, hemicellulose and lignin. Since cellulose and hemicelluloses amount to ca. 70% of biomass weight and are the carbohydrate based macromolecules, those are the major raw materials in diverse fields including pulping and bioethanol industries. Lignin, next to cellulose, is a second most abundant (20–30% of dried biomass weight) natural organic resource in nature, and initially synthesized to phenol macromolecules via oxidative coupling of three hydrocinnamyl alcohols (p-coumaryl (H), coniferyl (G) and sinapyl alcohol (S)) in the presence of peroxidase and H_2O_2 (Fig. 1) [1].

In spite of its abundance the utilization potential of lignin polymer is quite underestimated due to its rigid and recalcitrant properties. So far, lignin polymers are mainly produced in the pulping processes as waste forms. In future, it will be expected that enormous amount of lignins are additionally generated when the lignocellulosic biomasses are utilized as raw materials for bioethanol production.

Recently, lignins are newly evaluated as environmental friendly and biodegradable materials due to their potentials for replacement of phenol based chemicals derived from oil refinery industries.

In the previous series of studies on residual lignins [2–4] we have showed that beech ASAM residual lignin had very distinguishable structural moieties in comparison with other residual lignins. In spite of pulping procedure, it showed close structural similarity with beech MWL. Thioacidolysis of beech ASAM lignin suggested that the lignin was still built with high contents of β-O-4 linkages and its frequency/C_9-unit as even higher than MWL. The increments of phenolic hydroxyl groups, which was one of the major parameters for cleavage of β-O-4 linkages during pulping, were only few in the ASAM residual lignin. However, the ASAM residual lignin contained higher amount of methoxyl groups than the corresponding MWL, indicating different reactivity between guaiacyl and syringyl units under the delignification conditions during the ASAM pulping. Elimination of CγH_2OH, demethylation and *O*-demethoxylation could be insignificant during the ASAM pulping of beech wood.

Nuclear magnetic resonance spectroscopic techniques (^{1}H, ^{13}C NMR and 2D-, 3D-NMR) were employed for structural study on the lignin macromolecules. These instrumental methods allowed us to elucidate the whole structure of lignins without complexity of sample preparation. Comparative studies on kraft lignin between

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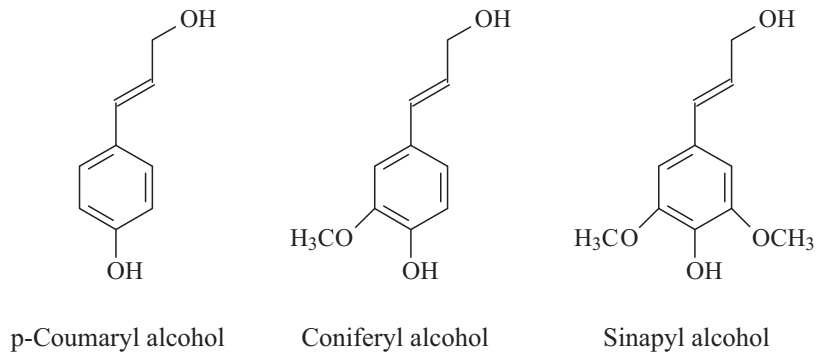

Fig. 1. Three hydroxycinnamyl alcohols as lignin precursors.

modified and conventional processes were performed by NMR spectroscopic methods [5,6]. They addressed the structural differences of lignins during both pulping procedures. Some functional groups of lignins can be evaluated by ^{13}C NMR spectroscopy [7,8]. A series of studies by Lundquist [9–11] and Lundquist et al. [12] showed the possibility that bonding types of lignin polymers and their frequencies could be elucidated by ^{1}H NMR spectroscopy.

In order to verify the structural moieties in the ASAM residual lignin determined by chemical degradation methods, two fractions of ASAM residual lignin after acetylation were further characterized by ^{1}H and ^{13}C NMR spectroscopies. Additionally, quantitative studies of several functional groups, e.g. primary, secondary and phenolic OH as well as methoxyl groups were also evaluated.

2. Experimental

2.1. Preparation of unbleached chemical pulping

Unbleached chemical pulps (kraft and ASAM pulps) were prepared with beech wood (*Fagus sylvatica*) at the laboratory according to standard method. Kraft pulping was run for 90 min with 22% active alkali (based on oven dried wood as NaOH) and 30% sulfidity at 165 °C. 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 90 min. The liquor to wood ratios was 4:1 for the both pulping processes. The kappa numbers of unbleached kraft and ASAM pulps were determined to 18.3 and 20.9, respectively.

2.2. Isolation of residual lignins

The wet pulps were ground with Jorkomuhle (Germany) for 20–30 min and then subjected to enzymatic hydrolysis of the pulps with cellulase cocktail (750 FPU/g, Celluclast 1.5 L, Novo Nordisk, Denmark) in 0.05 M sodium acetate buffer (pH 4.6) for 3 days at 40 °C. The solid fractions were separated from the hydrolyzate by centrifugation and then were freeze dried after being washed thoroughly with distilled water. From the solid fractions residual lignins were purified using aprotic (dimethyl acetamide) and protic solvent (alkali) [13]. In the purified kraft and ASAM residual lignins nitrogen content was determined to 2.17% and 0.34% by elemental analysis and the carbohydrate contents were ca. 9.9% and 0.8% by HPLC (Dionex Sunnyvale, USA) analysis, respectively.

2.3. Acetylation

Each residual lignin was acetylated with pyridine and acetic anhydride (1:1, v/v, 100 mg/2 ml) for 24 h at 50 °C. The liquid part was poured into the cold water and the precipitates were washed thoroughly with distilled water and dried *in vacuo* over P_2O_5. The acetylated lignins were examined by FT-IR for completion of acetylation of lignin.

2.4. Quantitative NMR spectral analysis

^{1}H and ^{13}C NMR spectra were recorded at 80 °C, using Varian Spectrometer at 400 MHz. For ^{13}C NMR study ca. 500 mg of acetylated residual lignins were dissolved in 3 ml of DMSO-d_6. ^{13}C NMR spectra were accomplished according to the method of Robert [8]. ^{1}H NMR spectral analysis was performed with 50–60 mg of acetylated samples in 1 ml of DMSO-d_6 [11].

3. Results and discussion

3.1. ^{1}H NMR spectral analysis of residual lignins

Fig. 2 illustrated ^{1}H NMR spectra of ASAM and kraft residual lignins of beech wood. Interestingly, NMR spectrum of ASAM residual lignin was very similar with that of beech MWL. Signal for methoxyl groups was observed in the region between δ_H 4.0 and 3.3 ppm. The most distinct features of ASAM spectrum were signal at δ_H 5.9, which were assigned to Hα for β-O-4 linkage [11] and signal at δ_H 6.6 was corresponding to aromatic proton in syringyl protons. However, signal for Hα was hardly visible and proton signal for syringyl units was also insignificant in ^{1}H NMR spectrum of kraft residual lignin. On the basis of the chemical shifts of Hα signal, the β-O-4 linkages were elucidated to *threo* form [14,15]. Signals between δ_H 2.5 and 2.0 were corresponding to be methyl

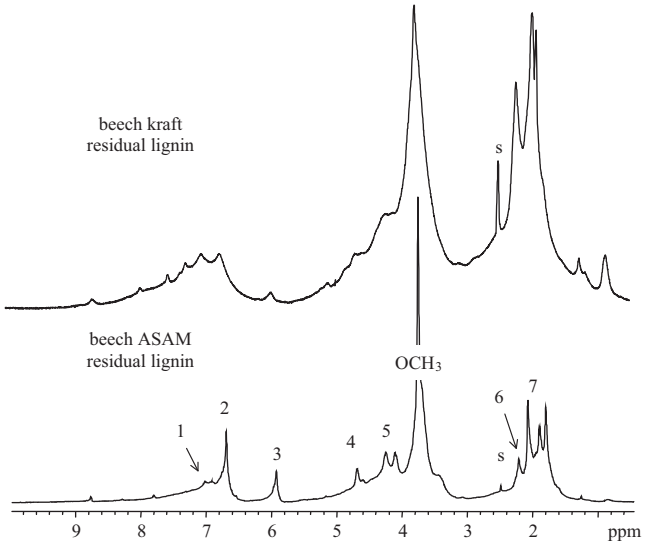

Fig. 2. ^{1}H NMR spectrum of acetylated kraft and ASAM residual lignins from beech wood (s: DMSO-d_6).