

Dissolution of Beech and Spruce Milled Woods in LiCl/DMSO

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A novel solvent system, lithium chloride/dimethyl sulfoxide (LiCl/DMSO), was developed for dissolving milled wood. This system completely dissolved beech and spruce milled woods prepared from the Wiley woods (coarse wood meals prepared by a Wiley mill) by 2 h planetary ball-milling under the milling conditions employed. The dependence of the structural change of lignin on the degree of milling was examined to evaluate the correlation between the dissolution and lignin structure. The nitrobenzene oxidation analyses showed that the 2 h milling caused almost no structural change in the aromatic part of lignin in the milled woods. The ozonation analyses suggested that the decrease of the *erythro* ratio [*erythro*/(*erythro* + *threo*)] obtained from β -O-4 structure in lignin is only 0.35% after the 2 h milling. Although the yield decrease of the ozonation products was 9.8% after the 2 h milling, this value was fairly smaller than that after a longer time milling. When it is taken into consideration that the other solvent systems for dissolution of wood meal, which are proposed by Lu and Ralph, require 5 h of milling under the same milling conditions to dissolve the milled woods, it is safely stated that the LiCl/DMSO solvent system completely dissolves milled wood, the lignin of which is structurally less altered and, thus, is expected to provide an improved method for structural analysis of the entire lignin fraction in wood cell wall.

KEYWORDS: Wood solvent; lithium chloride/dimethyl sulfoxide (LiCl/DMSO); dissolution of wood; lignin; ball-milling

INTRODUCTION

The main components of wood cell wall are cellulose, hemicellulose, and lignin. Because monolignols polymerize within the cell wall and are embedded in already accumulated polysaccharide gels, molecular associations and covalent bonds are possibly produced between lignin and carbohydrates (1). Phenylpropane units are linked by various types of ether and C–C bonds in a lignin macromolecule, and the frequency of each bonding type varies between cell walls. Such diversity of polymers in cell walls and the chemical and physical associations with carbohydrates make it impossible to isolate the entire lignin fraction without serious degradation (2–4). Consequently, analysis of complete lignin polymer has not been achieved by utilizing isolated lignin (3, 4). Alternatively, milled wood lignin (MWL) has commonly been used for the analysis of cell wall lignin. However, preparation of MWL requires rather extensive milling, causing noticeable changes in lignin structure depending on the degree of milling (5–8). Recently, we succeeded in quantitatively expressing the relationships between the degree of milling and structural change of lignin caused by the milling (7, 9). In addition, the chemical structure of MWL is strongly dependent on which portion of cell wall is its origin. It is known that the occupation of compound middle lamella lignin in extracted MWL is greater in the early stage of milling than in the later stage (4, 10). Therefore,

it should be emphasized that there are some limitations in discussing the structure of cell wall lignin on the basis of the structure of MWL.

One promising method to analyze the entire lignin fraction in cell wall is an application of complete wood solution. Spectroscopic analyses, for example, solution state NMR analysis, of such a solution may bring about new information on the entire lignin fraction, which can never be obtained by analyses of any isolated lignin. Kilpeläinen et al. demonstrated that both hardwoods and softwoods are readily dissolved in various imidazolium-based ionic liquids under gentle conditions. However, complete wood dissolution can be achieved at high temperature (80–120 °C) (11). Ionic liquids are complicated, which must prevent these solvents from direct utilization in spectroscopic analyses, such as NMR and UV absorbance. In addition to this, Kubo et al. reported that a nonphenolic β -O-4 type lignin model compound is almost completely converted into the corresponding enol ether compound during the dissolution process in imidazolium-based ionic liquids, which indicates that ionic liquids are not inert to lignin (12). Lu and Ralph described that finely ground plant cell wall can be dissolved in dimethyl sulfoxide and tetrabutylammonium fluoride (DMSO/TBAF) or dimethyl sulfoxide and *N*-methylimidazole (DMSO/NMI) binary solvent systems. An ability to dissolve wood meal without serious degradation of lignin would provide significantly improved methods for the analysis of the entire lignin fraction (13). However, the DMSO/NMI solvent system requires a rather long milling time to dissolve

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Table 1. Solvent Systems Examined for the Dissolution of Milled Woods from Beech with Different Milling Times

| | Milling time | | | | | | | | | | | | | | | | | | | | |
|----------------------|----------------|--------------------|--------------------|----------------|-------|-------|-----|-------|-------|-----|-------|-------|-----|-------|-------|-----|-------|-------|------|-------|-------|
| | 1 h | | | 2 h | | | 4 h | | | 5 h | | | 6 h | | | 8 h | | | 10 h | | |
| | S ^a | C ^b (%) | T ^c (h) | S | C (%) | T (h) | S | C (%) | T (h) | S | C (%) | T (h) | S | C (%) | T (h) | S | C (%) | T (h) | S | C (%) | T (h) |
| LiCl/DMSO (wt 6%) | N ^d | 1 | 24 | Y ^e | 10 | 24 | Y | 10 | 2 | Y | 10 | 2 | Y | 10 | 2 | Y | 10 | 2 | Y | 10 | 2 |
| LiCl/DMAc (wt 6%) | N | 1 | 24 | N | 1 | 24 | Y | 1 | 24 | Y | 1 | 24 | Y | 1 | 24 | Y | 1 | 24 | Y | 1 | 24 |
| DMSO/NMI (v/v = 2/1) | N | 1 | 24 | N | 1 | 24 | N | 1 | 24 | Y | 10 | 3 | Y | 10 | 3 | Y | 10 | 3 | Y | 10 | 3 |
| DMSO/EDA (v/v = 1/1) | N | 1 | 24 | N | 1 | 24 | N | 1 | 24 | N | 1 | 24 | N | 1 | 24 | N | 1 | 24 | Y | 1 | 24 |

^a S, solubility. ^b C, concentration. ^c T, time of stirring. ^d N, sample could not be completely dissolved into solvent system. ^e Y, sample could be completely dissolved into solvent system.

wood meal. It should be emphasized that milling of wood meal always causes structural change of lignin depending on the degree of milling (7,9). Therefore, we have been trying to develop a novel solvent system that can completely dissolve wood meal with as short a milling time as possible. It was reported recently that lithium chloride/dimethyl sulfoxide (LiCl/DMSO) can be used as a solvent system for the dissolution of regenerated cellulose (14). In this paper, we described the results when a LiCl/DMSO solvent system was applied to the dissolution of milled wood.

MATERIALS AND METHODS

Materials. Beech (*Fagus crenata* Blume) and spruce (*Picea abies*) woods were ground in a Wiley mill and extracted with ethanol/benzene (1:2, v/v) for 8 h. The extracted Wiley wood was dried under air and subsequently under vacuum. The dried Wiley wood (2 g) was milled in a planetary ball mill (Fritsch GMBH, Idar-Oberstein, Germany) for 1–10 h to give milled woods with different milling degrees. A zirconium dioxide bowl (45 mL) with 18 zirconium dioxide balls (1 cm diameter) was used in the milling. The milling frequency was 600 rpm. The milling was conducted in a cold room (–20 °C), and 5 min intervals were provided between every 15 min of milling to prevent overheating.

Determination of Lignin Content and Extractable Lignin Yield. The Klason method (15) was used for the determination of lignin content in the beech Wiley and milled woods. The yield of extractable lignin from the milled wood was determined according to the method described by Fujimoto et al. (7) and calculated as the weight percentage based on the total lignin content (Klason + acid soluble lignins) of the corresponding milled wood.

Alkaline Nitrobenzene Oxidation. Alkaline nitrobenzene oxidation analyses were applied to the Wiley and milled woods according to the common procedure (16). Nitrobenzene oxidation products were silylated with *N,O*-bis(trimethylsilyl)acetamide (BSA) at 100 °C for 10 min and, then, analyzed by gas chromatography under the following conditions: gas chromatograph, GC-17A with FID (Shimadzu Co., Kyoto, Japan); column, NB-1 (fused-silica capillary column, 30 m, 0.25 mm i.d.) (GL Science Inc., Tokyo, Japan); column program, kept for 15 min at 150 °C, raised by 3 °C/min to 180 °C and by 10 °C/min to 280 °C; injection temperature, 250 °C; detector temperature, 280 °C. Syringyl ratio [S/(S + V)], calculated on the basis of the formula

$$\frac{S}{S+V} = \frac{\text{(syringaldehyde+syringic acid)}}{\text{(vanillin+vanillic acid+syringaldehyde+syringic acid)}}$$

where syringaldehyde is 4-hydroxy-3,5-dimethoxybenzaldehyde, syringic acid is 4-hydroxy-3,5-dimethoxybenzoic acid, vanillin is 4-hydroxy-3-methoxybenzaldehyde, and vanillic acid is 4-hydroxy-3-methoxybenzoic acid.

Ozonation. Ozonation was performed according to the method of Akiyama et al. (17). Ozonation products were silylated with dimethyl sulfoxide (DMSO), hexamethyldisilazane (HMDS), and trimethylchlorosilane (TMCS) at 60 °C for 30 min and, then, analyzed by the GC-17A under the same conditions with those described above except the temperature program. The program was as follows: the initial temperature (120 °C) was kept for 5 min followed by the program rate of 4 °C/min to 170 °C, and consecutively, the other program rate of 10 °C/min to 280 °C.

Two ozonation products, erythronic acid (E) and threonic acid (T), obtained from *erythro* and *threo* forms of β -O-4 structure, respectively, were quantified. The proportion of E to the total amount of E and T gives the *erythro* ratio in β -O-4 structure [E/(E + T)], and the total yield of E and T (E + T) gives information on the content of β -O-4 structure.

X-ray Diffraction Analysis. The Wiley wood and milled woods from beech were converted into pellets using a disk apparatus for IR measurement and subjected to X-ray diffraction analysis from 5° to 35° diffraction angle 2θ using the reflection method by means of a Rigaku RINT 2000 with Ni-filtered Cu K α radiation ($\lambda = 0.1548$ nm) at 40 kV and 40 mA.

Dissolution of Milled Wood in Various Solvent Systems. Solvent systems examined in this paper were DMSO containing 1–6% LiCl (LiCl/DMSO), dimethylacetamide containing 1–6% of LiCl (LiCl/DMAc), mixtures of DMSO and *N*-methylimidazole (DMSO/NMI) (2/1, v/v), and a mixture of DMSO and ethylenediamine (DMSO/EDA) (1/1, v/v). The milled wood was suspended into the above solvent systems with different concentrations (1–10%, w/w) and stirred continuously at room temperature for 2–24 h. Detailed information is shown in Table 1.

RESULTS AND DISCUSSION

Dissolution of Milled Wood in LiCl/DMSO Solvent System. Beech and spruce woods were finely milled by planetary ball-milling. The particular milling conditions (milling intensity and temperature) with various milling times were applied to the preparation of milled woods with different degrees of milling. These milled woods were dissolved in many kinds of solvent system to evaluate the ability of each system as a solvent of milled wood. Among the systems examined, the 6% LiCl/DMSO system completely dissolved both beech and spruce milled woods prepared by using the 2 h milling. Other various milled woods, prepared from paulownia, eucalyptus, and even nonwood samples such as rice grass, were also completely dissolved in the 6% LiCl/DMSO system by using the 2 h milling. The DMSO/NMI system (13) also dissolved the milled woods completely, but 5 and 6 h millings were required for the beech and spruce woods, respectively. The results obtained for the beech wood are shown in Table 1 and Figure 1.

The concentration of the milled woods in the 6% LiCl/DMSO system was as high as 10%, which was higher than the value (8%) reported for solutions of some ionic liquids (11). As a role of LiCl in the LiCl/DMSO system in the dissolution of regenerated cellulose, Petrus et al. suggested that undissociated ion pairs of LiCl molecules in a polar aprotic solvent (DMSO) might interact with the oxygen atoms of hydroxyl groups and, thus, disrupt and prevent re-formation of hydrogen bonds between cellulose molecules, whereby the dissolution is facilitated. LiCl/DMSO, as a cellulose solvent system, can dissolve only regenerated cellulose and, thus, is not regarded as a general system (14). LiCl/DMAc is a better solvent system than LiCl/DMSO to dissolve cellulose. On the contrary, LiCl/DMSO, as a solvent system for milled wood, is much better than LiCl/DMAc, as shown in Table 1. In addition, the degree of lignin degradation during the milling of the milled woods was very small when the milling time was 2 h, as will be seen in the following section.



Figure 1. Photos of beech milled wood solution and suspension in 6% LiCl/DMSO with 1% concentration after 24 h of stirring. (Solution, milled wood with 2 h of milling could be completely dissolved in 6% LiCl/DMSO. Suspension, milled wood with 1 h of milling could not be completely dissolved in 6% LiCl/DMSO.)

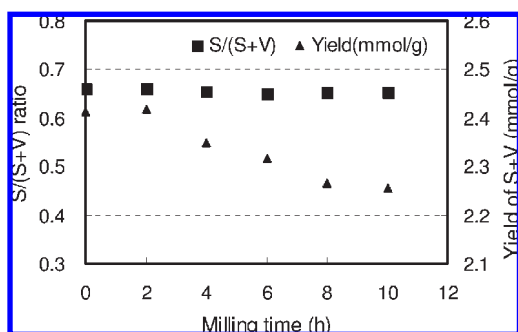


Figure 2. Changes of the syringyl ratio $S/(S + V)$ and yield of alkaline nitrobenzene oxidation products ($S + V$) from the beech milled wood prepared by different milling time. [$S/(S + V) = (\text{syringaldehyde} + \text{syringic acid})/(\text{vanillin} + \text{vanillic acid} + \text{syringaldehyde} + \text{syringic acid})$.]

Because the milled woods containing lignin with relatively small structural change are completely dissolved in the LiCl/DMSO system as shown here, there are many expected potential applications of this system, for example, to the structural analysis of the entire lignin fraction in wood cell wall and isolation of cell wall components.

Effect of Milling on the Structure of the Aromatic Part of Lignin.

The nitrobenzene oxidation was performed to examine the milling effect on the structure of aromatic part of lignin (Figure 2). The syringyl ratio, $S/(S + V)$, was not affected by the milling, but the yield of the nitrobenzene oxidation products, $S + V$, decreased with the progress of the milling. The yield decreases were 2.6 and 6.5% after the 4 and 10 h millings, respectively. When milling conditions, apparatus, intensities, and so on are different, milling time cannot be utilized as a criterion of milling degree to compare milling effect on lignin structure. The yield of extractable lignin from milled wood can be used as a general criterion of milling degree. Fujimoto et al. (7) and Hu et al. (9) showed that when the yields of extractable lignin from milled woods are the same, the structural changes of lignin caused by the milling are similar regardless of the difference in milling conditions and apparatus. Figure 3 shows the $S/(S + V)$ and $S + V$ values as functions of the yield of extractable lignin. The $S + V$ value decreased with the increase in the extractable lignin yield from the milled wood. However, the changes of both values of $S/(S + V)$ and $S + V$ were not significant when the milling time was 2 h or, in other words, when the yield of extractable lignin from the milled wood reached 23%. On the basis of these results, it can be stated that although the structure of the aromatic part of lignin

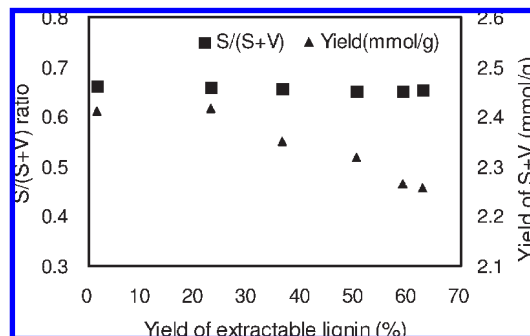


Figure 3. Dependence of the syringyl ratio $S/(S + V)$ and yield of alkaline nitrobenzene oxidation products ($S + V$) on the yield of extractable lignin in beech milled wood prepared by different milling time. [$S/(S + V) = (\text{syringaldehyde} + \text{syringic acid})/(\text{vanillin} + \text{vanillic acid} + \text{syringaldehyde} + \text{syringic acid})$.]

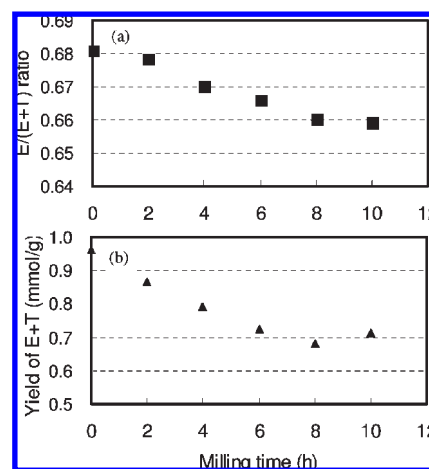


Figure 4. Changes of the erythro ratio $E/(E + T)$ and yield of ozonation products ($E + T$) from the beech milled wood prepared at different milling times: (a) erythro ratio $E/(E + T)$ versus milling time; (b) yield of $E + T$ versus milling time. [$E/(E + T) = \text{erythronic acid}/(\text{erythronic acid} + \text{threonic acid})$.]

changes depending on the milling time, the degree of the change is negligible after 2 h of milling.

Effect of Milling on β -O-4 Structure in Lignin. The effect of milling on the β -O-4 structure in lignin was evaluated by applying the ozonation method. The yield ratio and total yield of two major ozonation products from the β -O-4 structure, erythronic acid (E) and threonic acid (T), provide important information on β -O-4 structure (10, 17). Furthermore, the erythro and threo forms of this structure are known to exhibit different chemical reactivities in some chemical reactions. If this is also the case during milling, even a slight change in the erythro ratio, $E/(E + T)$, should be a good indication for the occurrence of some chemical modifications of β -O-4 structure during milling. From the results shown in Figure 4, it was found that both $E/(E + T)$ and the total yield of the ozonation products, $E + T$, decrease depending on the milling time. The $E + T$ value remarkably decreased from 0.96 to 0.79 mmol/g (17.7% decrease) with 4 h of milling. This value reached that which corresponded to the loss of about 26% of β -O-4 linkage after a milling time of 10 h. The decreases of both $E/(E + T)$ (0.35% decrease) and $E + T$ (9.8% decrease) were much more moderate after the 2 h milling than after the longer milling time. The same results are expressed as a function of the extractable lignin yield (Figure 5). This confirms that the β -O-4 structure is degraded during the milling process and that the erythro isomer is

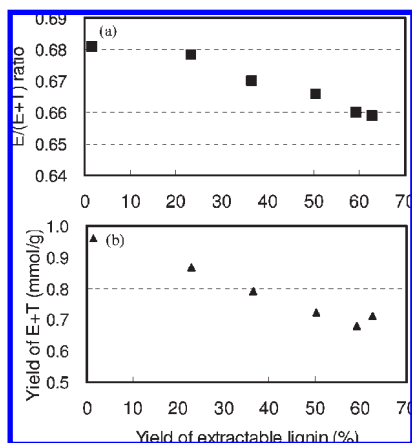


Figure 5. Dependence of the erythro ratio $[E/(E + T)]$ and yield of ozonation products on the yield of extractable lignin in beech milled wood prepared at different milling times: (a) erythro ratio $[E/(E + T)]$ versus yield of extractable lignin; (b) yield of E + T versus yield of extractable lignin. $[E/(E + T) = \text{erythronic acid}/(\text{erythronic acid} + \text{threonic acid})]$.

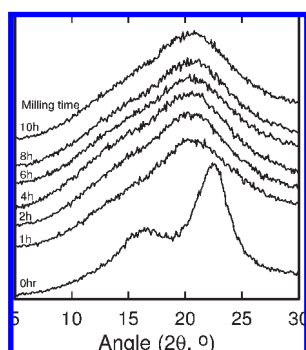


Figure 6. X-ray diffraction patterns of Wiley wood and milled wood prepared at different milling times from beech wood. (The individual diffractograms were shifted on the y-scale for the total display. The Y-axis represents intensity.)

preferentially degraded, which coincides well with the results of Fujimoto et al. (7) and Hu et al. (9). The results obtained by the nitrobenzene oxidation (Figures 2 and 3) and ozonation method (Figures 4 and 5) indicate that the structural changes of lignin cannot be avoided during the milling. The degree of lignin degradation increases with the progress of milling. It is quite important for a solvent system to dissolve milled wood prepared by short milling time. As shown above, the structural change of lignin was not so significant when the milling time was 2 h, which is a significant advantage of the LiCl/DMSO system.

Effect of Milling on X-ray Diffraction Pattern of Milled Wood.

X-ray diffraction patterns of the beech Wiley and milled woods are shown in Figure 6. The crystal region of cellulose disappeared when the milling time was as short as 1 h, indicating that the milling process affects the structure of not only lignin but also cellulose. The destruction of the crystal region should be one of the decisive factors for the dissolution process of the milled woods in the LiCl/DMSO solvent system.

Conclusions. The novel solvent system developed in this paper, LiCl/DMSO, completely dissolved milled wood prepared by as short as 2 h of milling using a planetary ball mill. The nitrobenzene oxidation and ozonation analyses indicated that the structural change of lignin caused by the 2 h of milling is not significant. In contrast, the destruction of the cellulose crystalline region in the

milled wood was serious even after 1 h of milling. Compared with already reported solvent systems, the LiCl/DMSO system, developed in this paper, has advantages of high solubility and low degradation of lignin and is expected to be applied widely to the analysis of the entire lignin fraction in wood cell wall.

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