RAPID COMMUNICATION

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Quantitative evaluation of milling effects on lignin structure during the isolation process of milled wood lignin

Results and discussion

Milled wood lignin (MWL) is the best lignin sample among many isolated lignins that can be used to study the chemical structure and reactivity of native lignin. It is well recognized that MWL represents only a part of native lignin in the wood cell wall.[1,2] It also has been demonstrated that lignin could undergo structural change during the MWL isolation process, especially the milling process.[3,4] However, there have been no quantitative relationships found between the structural change of lignin and the degree of milling.

In the present article, we describe our efforts to quantify the effect of milling on the structural changes of lignin. Coarse wood meal of sweetgum (*Liquidambar styraciflua* L.) was further milled for 150–1200 min with the use of a planetary ball mill under six different conditions. Lignin analysis was conducted on the whole milled wood but not on the isolated lignin (MWL) to characterize the structural changes having occurred to whole lignin. As an analytical method, ozonation analysis was employed. By this method, both the *erythro/threo* ratio and the content of *β-O*-4 structure in lignin can be evaluated by determining two ozonation products, erythronic and threonic acids. Ratio of erythronic and threonic acids (E/T ratio) gives the *erythro/threo* ratio of *β-O*-4 structure while the total yield of

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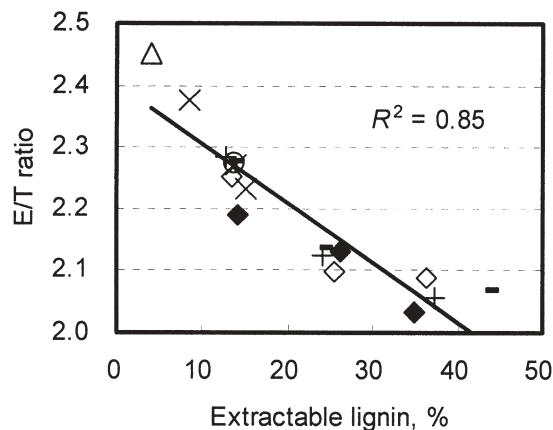
erythronic and threonic acids (E + T) gives information about the content of this structure.

If structural changes of lignin caused by milling proceed via a radical process,[5–9] radicals thus produced might initiate further degradation. For example, if the initial reaction caused by mechanical effects was the homolytic cleavage of the *β-O*-4 structure, resultant radicals at the side chain part would combine with molecular oxygen under aerobic conditions and create new reactive species, which might further degrade lignin. The presence of some radical scavenging reagents, such as a reducing agent, would prevent this further degradation even though the initial reaction caused by mechanical effects cannot be prevented. If produced, phenoxy radicals would undergo a dimerization reaction, which could also be prevented by a reducing agent. On the other hand, milling temperature might affect the initial cleavage reaction itself. Ikeda et al.[3] reported that lignin underwent more structural changes in the absence of toluene than in its presence during the milling by vibratory milling apparatus and suggested the occurrence of condensation reactions during milling process. Based on these considerations, milling was conducted under several different conditions: aerobic, anaerobic, and reductive conditions.

There was, however, one difficulty in comparing the results obtained for different milling conditions. This was the lack of a proper measure to evaluate the milling effect caused by different milling conditions. For example, it is not adequate to compare structural changes caused by 5 h of milling in the air and 5 h of milling in toluene because the milling efficiency of the former is obviously much greater than that of the latter. Thus, milling time cannot be a measure to compare the results obtained by different milling conditions. This problem was overcome by employing the amount of extractable lignin from milled wood as a measure of the milling effect. The amount of lignin extracted with 96% aqueous dioxane was quantified by measuring the ultraviolet (UV) absorbance of the extracts at 280 nm.

In Figs. 1 and 2, analytical results of lignin in milled woods obtained under different milling conditions are compared based on the amount of extractable lignin from those milled woods. The changes in E/T ratio of *β-O*-4 structure

Fig. 1. Change of *erythro/threo* ratio (*E/T ratio*) of *β-O-4* structure of lignin in milled woods with the progress of milling under different milling conditions. *Triangles*, original wood; *open diamonds*, milling in air at room temperature (150, 300, 450 min); *crosses*, milling in toluene at room temperature (300, 750, 1200 min); *circles*, milling in toluene with tetralin at room temperature (750 min); *dashes*, milling in argon at room temperature (150, 300, 450 min); *plus signs*, milling in hydrogen at room temperature (150, 300, 450 min); *filled diamonds*, milling in air at cold temperature (150, 300, 450 min)
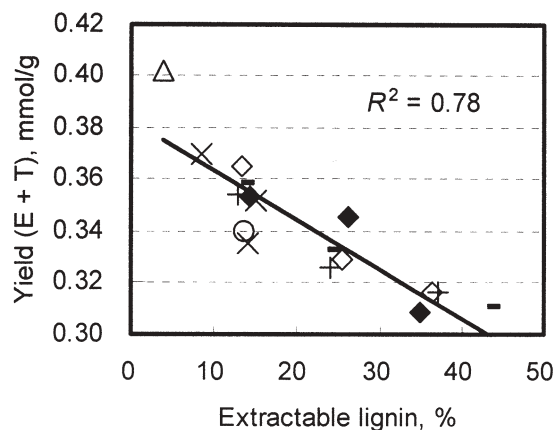

Fig. 2. Decrease in the content of *β-O-4* structure of lignin in milled woods with the progress of milling under different milling conditions. Yield from 1 g of wood meal. Lignin content of original wood was 25.6% as Klason lignin

are shown in Fig. 1. The total yields of erythronic and threonic acids (E + T) from milled woods, which are used as the measure of the content of *β-O-4* structure, are shown in Fig. 2. All the data obtained by 150–1200 min of milling under six different conditions are given in these figures. Interestingly, all the data gave one line even though rather wide variation was observed for the content of *β-O-4* structure. As to the degradation of *β-O-4* structure, E/T ratio decreased significantly from the original value (2.45), demonstrating that preferential *erythro* degradation proceeded under all milling conditions. It seemed that there was no significant difference among the different milling conditions, which suggests that changing milling conditions cannot prevent degradation of lignin during milling. From the data in Fig. 2, the degradation of *β-O-4* structure during

milling is expressed as a simple linear function of the amount of extractable lignin, which was employed as the measure of milling effect. From this relationship, the degradation of the *β-O-4* structure is estimated to be about 25% when milling is continued until 40% of lignin in wood becomes extractable. Here, it should be noted that the yield of MWL, especially that of a purified sample, from wood meal should be smaller than the amount of extractable lignin determined in this study.

There is a possibility that either hydrogen or tetralin did not function as reducing agent of radicals, and thus, there is still room to further investigate the use of reducing agents to prevent degradation of lignin during milling. However, it should be emphasized that aerobic and anaerobic conditions resulted in the same degree of degradation. These data suggest that structural change of lignin is fatal and cannot be prevented. In order to prepare lignin from wood that is structurally less modified, the use of some other method such as enzymatic treatment in combination with a low level of milling must be considered, as was tried by Chang et al.[4]

Experiments

Milling of wood meal. Sweetgum (*Liquidambar styraciflua* L.) wood was ground in a Wiley mill to pass through a 20-mesh screen, and was extracted with ethanol/benzene (1:2, v/v). Milling was conducted by the use of planetary ball mill (Fritsch, Idar-Oberstein, Germany). Wood meal (2 g) was put in a 45-ml bowl with 18 balls (1 cm diameter, ZrO_2). When milling was conducted under gaseous atmosphere, each gas (air, argon, or hydrogen) was purged in the bowl sealed in a plastic bag. For milling in toluene or tetralin, 25 ml of toluene or 20 ml of toluene with 5 ml tetralin, respectively, was put in the bowl together with wood meal (2 g) and 18 balls. Milling frequency was 600 rpm. When milling was conducted at room temperature, in order to prevent overheating, a 10-min pause was used every 30 min. In this way, the temperature of the bowl was maintained below 55°C. When milling was conducted in a cold room, each 30 min of milling was followed by a 30-min pause. In this case, the temperature was kept below −5°C.

Measurement of extracted lignin. Wood meal (20 mg) prepared by each milling condition was put in a 20-ml glass tube together with 10 ml of 96% aqueous dioxane. The glass tube was kept at room temperature with agitation for 48 h. Five milliliters of the extract was reduced with 1 mg of sodium borohydride in 1 ml of 0.05 M NaOH solution for 24 h and was poured into 4 ml of glacial acetic acid. The UV absorbance at 280 nm was measured and was used to calculate the amount of lignin with the use of 13 as the gram absorptivity.

Ozonation analysis. Ozonation was performed according to the procedure reported by Akiyama et al.[10]

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