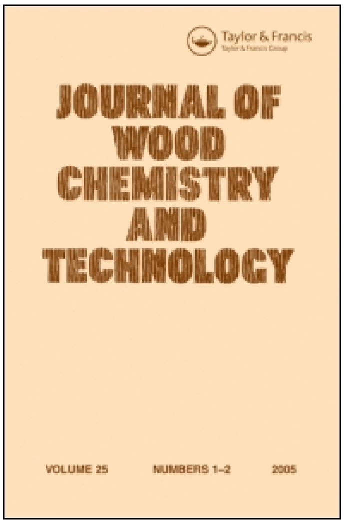

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Quantitative ^{13}C NMR Characterization of Milled Wood Lignins Isolated by Different Milling Techniques

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Abstract: Milled wood lignins (MWL) prepared from finely milled wood flour produced by different milling techniques were compared by quantitative ^{13}C NMR. Wood meal produced in a Wiley mill was milled for either six weeks in a porcelain rotary mill with porcelain balls, or by two variations of our standard technique. Specifically the Wiley wood meal was milled for one week in the rotary mill followed by 48 h of vibratory ball-milling with steel balls either in toluene or under a N_2 atmosphere. Results showed that the vibratory-milled samples were similar in structure with the exception that the preparation milled under N_2 had higher aliphatic and phenolic hydroxyl contents. The rotary-milled sample on the other hand had a much lower β-O-4′ and hydroxyl content along with a higher degree of condensation and oxidized side chain structures.

Keywords: Lignin, characterization, quantitative ^{13}C NMR, milled wood lignin (MWL), vibratory ball-milling, rotary ball-milling

This article is dedicated to the memory of Professor Josef S. Gratzl.

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INTRODUCTION

Ball-milling according to Bjorkman's method is utilized almost exclusively to isolate milled wood lignin (MWL).[1] The purpose of this effort is to reduce the wood to a particle size small enough to extract the lignin. As a result, varying methods of milling may be employed to achieve the end result. Bjorkman concluded that vibratory ball-milling by itself was sufficient to extract lignin from wood, but that the yield was improved by a two-step process utilizing rotary ball-milling as the first step.[1] Additionally, Bjorkman determined that vibratory ball-milling in a non-swelling solvent such as toluene will facilitate handling by reducing the tendency of the milled wood to cake on the steel balls.[1] Toluene is also known to be an efficient radical scavenger and may reduce side reactions occurring during the milling process.[2]

The use of rotary ball-milling exclusively has been shown to be sufficient to reduce the particle size of wood if milling is extended to a period of weeks.[3,4] This may be beneficial from the standpoint that milled wood can be isolated in larger portions with less handling and metal contamination from the steel balls can be avoided.

Previously we reported that vibratory ball-milling under a N_2 atmosphere may result in condensation reactions based on decreased monomer yields from a modified DFRC method.[5] In addition, our results showed that rotary ball-milling for a period of six weeks produced isolatable MWL yields comparable to the standard Bjorkman method in toluene with similar chemical structure based on modified DFRC results. The drawback of using any degradative method exclusively for structural analysis is that conclusions are drawn only from partial data obtained from the release of monomers and dimers from the lignin polymer. A case in point is our attempts to verify the modified DFRC data by using a modified thioacidolysis technique that acts by a similar mechanism.[6] The data using this method gave us conflicting results and upon further examination we determined that the modified DFRC was inefficient at cleaving β-aryl ether bonds.

As a result, we have begun to perform quantitative ^{13}C NMR as a routine method to examine the entire structure of soluble lignins.[7] The advantage of using NMR techniques is that one is able to study the entire soluble lignin structure intact without degradation. In this article we report on the structure of MWL isolated from milling processes involving the standard Bjorkman method with vibratory ball-milling in toluene and under a N_2 atmosphere, as well as rotary ball-milling in a porcelain ball mill with porcelain balls for six weeks. The effects of the different types of milling will be compared.

EXPERIMENTAL

Materials

DMSO-d_6 was purchased from Cambridge Isotope Laboratories in sealed ampules of 0.25 mL volumes. 1,4-Dioxane was purchased from Fisher

Scientific and distilled over $NaBH_4$ prior to being used. All other chemicals were purchased from either Fisher Scientific or Aldrich Chemicals and used as received.

All MWL samples were produced from Loblolly pine (*Pinus taeda*). Sapwood was ground to pass a 20-mesh screen in a Wiley mill and Soxhlet-extracted with 1:2 (v/v) ethanol:benzene for 24 h, followed by ethanol for 24 h. Milling of the Wiley wood meal was achieved by three different techniques to produce three separate MWLs. The standard method involves 1 week of rotary ball-milling with glass balls followed by 48 h of vibratory ball-milling in toluene. To assess the effects of milling on the lignin structure, a MWL of comparable isolatable yield was produced by rotary ball-milling with porcelain balls for a period of six weeks. In this case, no vibratory ball-milling was employed. In order to determine whether milling without toluene results in condensation reactions, we performed the standard milling procedure under a N_2 atmosphere (Figure 1).

Using our standard milling procedure, Wiley wood meal (100 g) was milled for one week in a rotary ball mill with glass balls. Ten gram batches of the rotary-milled wood (Figure 1) were then placed in two vibratory ball mills with steel balls and milling was performed for 48 h. The vibratory ball-milling was performed either in toluene or under a N_2 atmosphere and the samples are referred to as Vibratory in Toluene Wood and Vibratory (Dry) Wood, respectively.

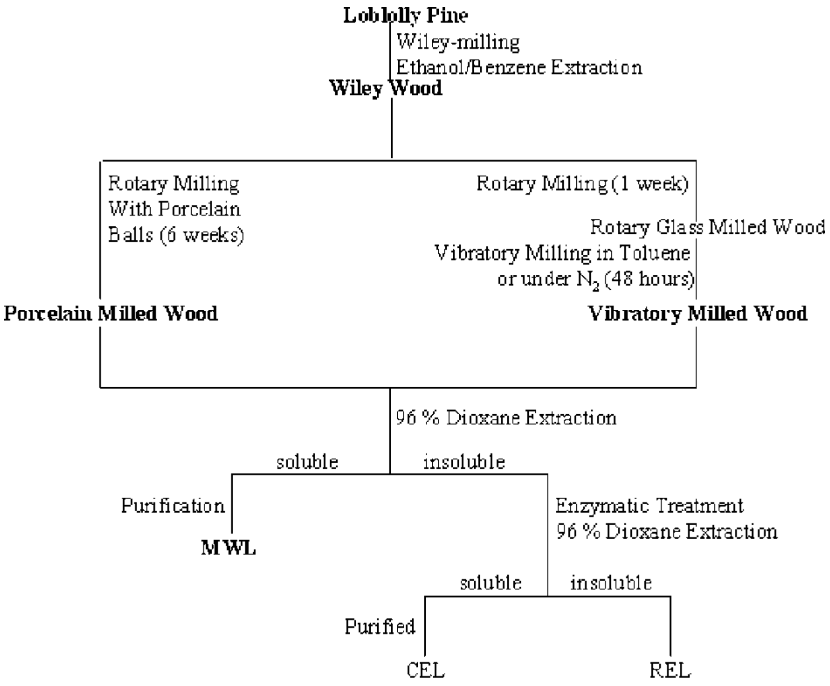

Figure 1. Procedures for the different milling techniques and the MWL isolation.

Alternatively, Wiley wood meal (100 g) was milled in a 1-gallon porcelain jar with porcelain balls under a N_2 atmosphere for six weeks to achieve a particle size small enough to extract MWL.[7] An advantage of this technique is that all of the Wiley wood meal can be milled in one batch, unlike milling in a vibratory ball mill. This sample is referred to as Rotary Porcelain 6 Week Wood.

The MWL preparations were produced according to the method of Bjorkman[1] (Figure 1). Twenty-five grams of either the Rotary Porcelain 6 Week Wood, Vibratory in Toluene Wood, or the Vibratory (Dry) Wood were weighed into a 500 mL centrifuge bottle, 250 mL of 1,4-dioxane/water (96:4, v/v) solution were added, and the solution was stirred at room temperature for 24 h under a N_2 atmosphere. The solution was centrifuged and the supernatant was collected. The remaining solid material was once again dispersed in the 96% dioxane solution and the earlier procedure repeated. The final solid material was washed twice with deionized water and freeze-dried.

The solvent from the supernatant was evaporated under reduced pressure to a volume of 100 mL. The concentrated dioxane solution was then added drop-wise to deionized water in a freeze-dry flask with stirring to induce precipitation. The samples were freeze-dried and the resulting crude MWL preparations were stored in a desiccator under vacuum over P_2O_5.

The crude MWL was dissolved in 20 mL of 90 % acetic acid (9:1, v/v) and precipitated into deionized water (400 mL). The precipitated lignin was filtered, dissolved in 10 mL of 1,2-dichloroethane/ethanol (2:1, v/v), precipitated into 200 mL of ether and washed with petroleum ether. The final lignin materials were air-dried, ground to further facilitate evaporation of ether, placed in a vacuum oven at 40°C and 30 in. Hg overnight, and finally stored in a desiccator under vacuum over P_2O_5. The samples were labeled Rotary Porcelain 6 Week MWL for the rotary ball-milled sample, Vibratory in Toluene MWL for the sample that was vibratory ball-milled in toluene, and Vibratory (Dry) MWL for the sample that was vibratory ball-milled sample under a N_2 atmosphere. MWL yields were reported previously.[5]

Acetylation of the MWL preparations was carried out according to published procedures.[8] No purification of the acetylated preparation was used to avoid material loss.

1D Quantitative ^{13}C NMR Spectroscopy

The NMR spectra were recorded in DMSO-d_6 on a Bruker AVANCE 500 MHz spectrometer (1996) at 300 K using an Oxford narrow bore magnet (1989). The methyl peak at 39.5 ppm was used as an internal reference. The operational frequency for the ^{13}C nucleus was 125.032 MHz and a ^{13}C GE probe was used. Samples were accurately weighed to 70 mg and dissolved in 0.25 mL of DMSO-d_6 and placed in a Shigemi microtube;

a 90° pulse width, a 1.4 s acquisition time and 1.7 s relaxation delay were used.[7] Chromium (III) acetylacetonate (10 μL of a 0.25 mg/mL solution) was added to the lignin solution to provide complete relaxation of all nuclei.[9,10] As has been shown in our previous work,[11] these experimental conditions do not affect the quality of the spectra as compared to the common conditions for quantitative ^{13}C NMR of lignin without relaxant addition, but allow a 4-fold decrease in the experimental time. A total of 20,000 scans were collected.

RESULTS AND DISCUSSION

Quantitative ^{13}C NMR

The frequencies of interunit linkages in each of the milled wood lignin preparations were calculated from the nonacetylated and acetylated spectra as outlined previously.[7] The nonacetylated spectrum from the Vibratory in Toluene MWL preparation is shown in Figure 2 with the oxygenated aliphatic region of the acetylated spectrum shown in the inset. All peak identifications are given in Table 1. All integrations were performed by setting the aromatic region from 160–103 ppm equal to 6.12 as discussed previously and therefore all moieties are based on equivalences per aromatic ring.[7,12,13]

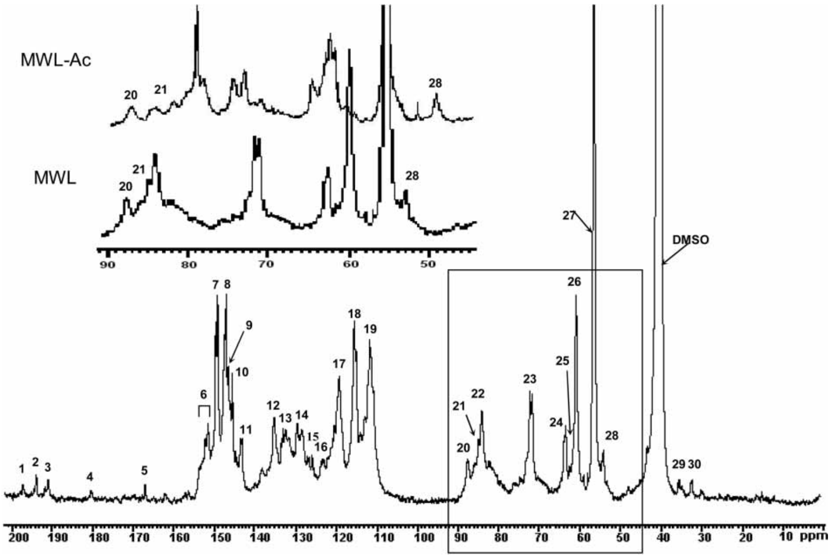

Figure 2. Quantitative ^{13}C NMR spectra of MWL. Included is an expansion of the oxygenated aliphatic region of the MWL and an inset of the acetylated MWL (MWL-Ac).

Table 1. Chemical shift assignments for the nonacetylated spectrum of the quantitative ^{13}C NMR experiment

Peak number	δ (ppm)	Assignment	Peak number	δ (ppm)	Assignment
1	198	C=O in (**IV**)	**16**	124.5–122	C_1 and C_6 in α-C=O units
2	194	C=O in (**III**)	**17**	122–117	C_6 in G units
3	191	C=O in (**I**)	**18**	117–113	C_5 in G units
4	181	C=O in (**XIV**)	**19**	113–108	C_2 in G units
5	168	C=O in −**OAc**	**20**	87	C_α in (**V**)
6	155–151	$C_3/C_{3'}$ in etherified (**V**) C_α in (**III**) C_5 in ring B of (**XI**)	**21**	86.5–85	C_α in (**VIII**) C_α and C_β in (**IX**)
7	151–148.5	C_3 in etherified G units	**22**	85–82	$C_\beta(e,t)$ in (**X**)
8	148.5–146.8	C_4 in etherified G units C_3 in nonetherified (**X**)	**23**	74–71	$C_\alpha(e,t)$ in (**X**) C_γ in (**VIII**)
9	146.2	C_4 in nonetherified G units C_4 in ring B of (**V**)	**24**	64–62	C_γ in (**III**), (**V**), and (**XII**)
10	145.2	$C_4/C_{4'}$ in etherified (**VI**)	**25**	61.5	C_γ in (**VII**)
11	143.5	C_3 in ring B of (**V**) $C_4/C_{4'}$ in nonetherified (**VI**)	**26**	61–59	C_γ in (**IX**) and (**X**)
12	137–134	C_1 in etherified G units	**27**	56	−**OCH_3**
13	134–130	C_1 in nonetherified G units $C_5/C_{5'}$ in etherified (**VI**)	**28**	54–52	C_β in (**V**), (**VIII**), and (**XII**)
14	130–127	C_β in (**II**) C_α and C_β in (**VII**)	**29**	34.5	C_α in (**XIII**)
15	127–124.5	$C_5/C_{5'}$ in nonetherified (**VI**)	**30**	31.5	C_β in (**XIII**)

The spectrum can be subdivided into regions of interest in order to obtain information about the overall structure of the lignin polymer. The results are shown in Table 2 and the methoxyl content can be used as a type of calibration for each of the spectra. In other words, if the integration of the methoxyl peak is accurate, then the phasing and baseline of the spectrum are likely adequate. The methoxyl content for the Vibratory in Toluene MWL and the Rotary Porcelain 6 Week MWL are 0.96 and 0.97 which are typical values for guaiacyl-based lignins such as those found in conifers. The Vibratory (Dry)

Table 2. Value of integrations of the spectral regions of the nonacetylated quantitative ^{13}C NMR spectra

		Number of moieties per aromatic ring		
Spectral region	Chemical shift range (ppm)	Vibratory MWL	Vibratory (Dry) MWL	Rotary Porcelain 6 week MWL
Methoxyl content	57–54	0.96	1.00	0.97
Aromatic methine carbons	125–103	2.57	2.56	2.46
Aromatic carbon-carbon structures	141–125	1.53	1.48	1.54
Oxygenated aromatic carbons	160–141	2.02	2.07	2.12
Carbon from carbonyl type structures	195–190	0.12	0.09	0.14
Carbon from carboxyl type structures	176–163	Negl.	Negl.	0.05
Degree of condensation[a]	125–103	0.43	0.44	0.54
Aliphatic hydroxyl content	171–168.5	1.02	1.26	0.75
Phenolic hydroxyl content	168.5–166	0.17	0.29	0.30

[a] Calculated from $3.00 - I_{125-103}$.

MWL is slightly high at 1.00, however the value is within the 5% error associated with this technique.[14]

Carboxyl Structures

The spectrum of the nonacetylated Rotary Porcelain 6 Week MWL shows several small signals in the region representing –COO type structures. Meanwhile, this region in the spectra of the nonacetylated Vibratory in Toluene MWL and Vibratory (Dry) MWL preparations is basically devoid of signals. The total integration from 176–163 ppm yields a value of 0.05 per aromatic ring for the Rotary Porcelain 6 Week MWL sample (Table 2). The downfield signals (176–168 ppm) are likely due to unconjugated aliphatic carboxyl groups, whereas the upfield signals (168–163 ppm) are possibly conjugated carboxyl structures.[15] The conjugated carboxyl structures are present at levels of 0.04 per aromatic ring (Table 3) and are likely of the benzoic acid type, represented by structure XV in Figure 3.

Carbonyl Structures

Table 2 indicates that the Vibratory (Dry) MWL has a slightly lower degree of oxidation than the Vibratory in Toluene MWL as determined by integration of

Table 3. Estimation of interunit linkages in vibratory in Toluene MWL, vibratory (Dry) MWL, and rotary porcelain 6 week MWL via quantitative ^{13}C NMR

		Number of moieties per aromatic ring		
Spectral region	Chemical shift range (ppm)	Vibratory MWL	Vibratory (Dry) MWL	Rotary Porcelain 6 week MWL
Ar-CHO (I)	191	0.04	0.02	0.05
Ar-CH=CH-CHO (II) + α-C=O in β-O-4 (III)	194	0.04	0.04	0.04
Ar-CO-CH_2-CH_2OH (IV)	198	0.03	0.02	0.05
β-5′ (V)	Ac(90–86)[a]	0.09	0.07	0.09
5-5′ (VI)[b]	145–141[b]	0.32	0.34	0.36–0.38
Ar-CH=CH-CH_2OH (VII)	62	0.04	0.03	0.02
β-O-4′ (VIII)	61.5–57.5[c]	0.41	0.42	0.32
β-β′ (IX)	54.3–52.0[d]	0.02	0.02	0.02
Dibenzodioxocin (X)	Ac(86–83)[e]	0.08	0.06	0.03–0.04
β-1′ (XII)	Ac(51–48)[f]	<0.01	0.01	Negl.
Ar-CH_2-CH_2-CH_2OH (XIII)	31	0.03	0.03	0.02
Spirodienone (XIV)	181	0.01	<0.01	0.01

[a] β-5′ (V) was directly integrated from 90–86 ppm in the acetylated ^{13}C spectrum. (inset, Figure 2).

[b] 5-5′ was determined by integration of 145–141 ppm of the nonacetylated spectrum minus the value of (V). As the 5-5′ moieties are symmetric, the number of 5-5′ moieties is half of the aromatic units involved.

[c] β-O-4′ (VIII) calculated from region of 61.5–57.5 in the nonacetylated spectrum minus the values for (III), (IV), (X), (XII), and (XIII).

[d] β-β′ (IX) calculated from integration of the region 54.3–52 in the nonacetylated spectrum minus the value of (V).

[e] Dibenzodioxocin was calculated by integration of 86–83 ppm in the acetylated ^{13}C spectrum minus the value of (IX).

[f] β-1′ (XII) was calculated by integration of 51–48 ppm of the acetylated ^{13}C spectrum minus the value of (V).

the carbonyl region from 195–190 ppm. The resulting values are 0.12 and 0.09 per aromatic ring for the Vibratory in Toluene MWL and Vibratory (Dry) MWL, respectively. The Rotary Porcelain 6 Week MWL sample on the other hand is slightly higher with a value of 0.14 per aromatic ring after being milled over a period of 6 weeks.

Table 3 indicates that the Vibratory (Dry) MWL contains a slightly lower amount of vanillin (I), 0.02 per aromatic ring, compared to the other two preparations (0.04 per aromatic ring). The presence of the 3-hydroxy-1-(4-hydroxy-3-methoxy-phenyl) propan-1-one (IV)[16] structure is higher in the Rotary Porcelain 6 Week MWL compared to the other 2 materials, 0.05

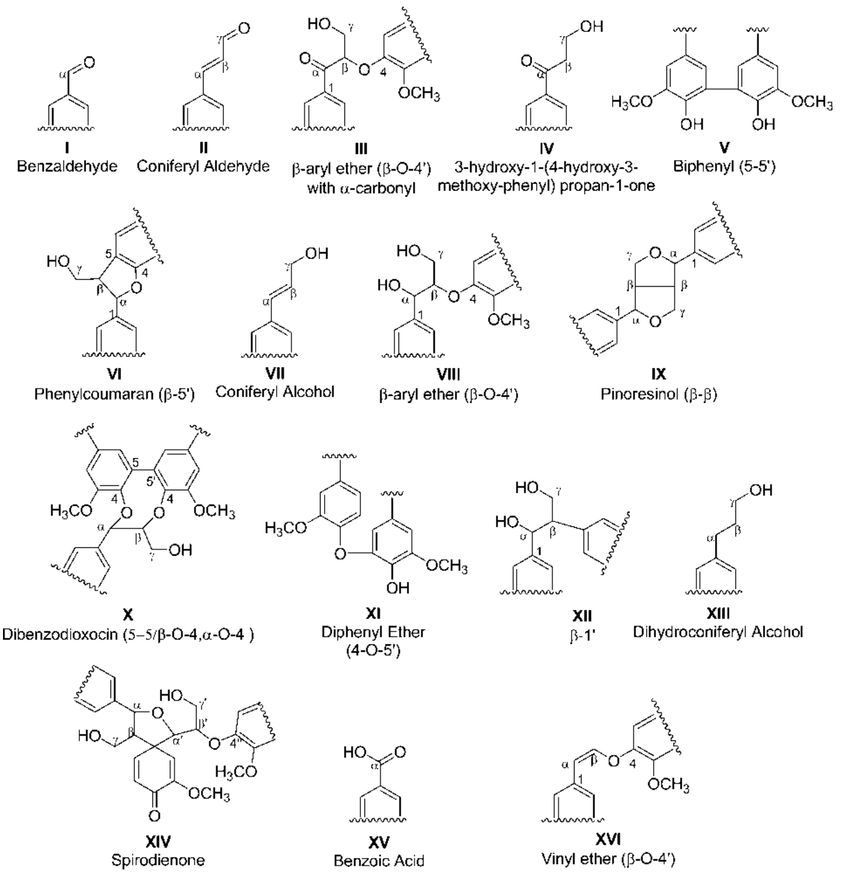

Figure 3. Commonly found interunit linkages in MWL preparations.

versus 0.03 and 0.02 (Vibratory in Toluene MWL and Vibratory (Dry) MWL) moieties per aromatic ring. The integration of the peak at 195 ppm indicates that there is no difference in the combined amount of coniferaldehyde (II) and α-carbonyl structures (III) between the Vibratory in Toluene MWL and Vibratory (Dry) MWL samples (0.02 per aromatic ring), but that the Rotary Porcelain 6 Week MWL contains a larger amount of these units, 0.05 per aromatic ring. All structural moieties discussed are shown in Figure 3 and are consistent with the labelling in a previous article.[7]

Zhang and Gellerstedt has suggested that the spirodienone (XIV) structure, based on two-dimensional HMQC ^{1}H-^{13}C NMR and the C_4 carbonyl signal at 181 ppm in the ^{13}C NMR spectrum,[17] may be a major contributor of β-1$'$ moieties in native spruce lignin. Our samples have not indicated it to be anything more than a minor contributor to the lignin structure. All 3 MWLs contain this moiety at no more than 0.01 per aromatic ring based on integration of the peak at 181 ppm.

The increased levels of oxidized carbon structures in the Rotary Porc 6 Week MWL preparation indicate that oxidation is likely occurring during milling. The comparison of this preparation to those milled in the vibratory mill provides evidence that oxidized side chain structures are likely produced by milling and not incorporated during biosynthesis.

Aliphatic and Phenolic Hydroxyl Content

Table 2 lists the values for the aliphatic (171–168.5 ppm) and phenolic (168.5–167 ppm) hydroxyl groups as determined for each MWL. The data for the Rotary Porcelain 6 Week MWL supports the theory of side chain oxidation as the aliphatic hydroxyl content (0.75 per aromatic ring) is much lower than typically reported, whereas the phenolic hydroxyl content is similar to literature values.[14,18]

Comparison of the vibratory-milled samples shows an unexplainable discrepancy. Although both are comparable to literature values,[14] the Vibratory (Dry) MWL is much higher in both aliphatic and phenolic hydroxyl groups, 1.26 *versus* 1.02 and 0.30 *versus* 0.17 per aromatic ring, respectively. A higher value for the phenolic hydroxyl content is consistent with more depolymerization of the lignin polymer. A higher isolatable MWL yield was also achieved for this sample with equivalent milling time. More extensive polymer degradation may be expected as the toluene is not present to stabilize radicals or absorb energy during milling. A decrease in the aliphatic hydroxyl content is consistent with degradation of the side chain; however, there is little difference in oxidized moieties between the two preparations.

β-O-4′ Content

Prolonged milling in the rotary ball mill leads to a decrease in the β-O-4′ (VIII) content compared to the vibratory ball-milled preparations as determined by integration of the γ-carbon from 61.5–57.5 ppm. The value obtained, 0.33 per aromatic ring, is much lower than those obtained for the Vibratory in Toluene MWL and Vibratory (Dry) MWL, which were 0.42 and 0.41, respectively.

It is possible that the Rotary Porcelain 6 Week MWL has a β-O-4′ content similar to the vibratory-milled samples except that side chain modification has resulted in cleavage of the γ-carbon. This would result in an under calculation of the β-aryl ether content and shows a disadvantage to evaluation of lignin structure by quantitative ^{13}C NMR. This theory is also supported by the discrepancy in aliphatic hydroxyls discussed in the previous section. It has been reported that there is sufficient energy present in milling to cause C–C bond cleavage.[19] Loss of the γ-carbon may result in the formation of vinyl ether (XVI) structures.

Aromatic Region and the Degree of Condensation

The aromatic region of the spectrum can be divided into the protonated aromatic (125–103 ppm), the condensed aromatic (141–125 ppm), and the oxygenated aromatic (160–141 ppm) regions. In theory, the oxygenated aromatic region should contain the C_3 and C_4 carbons on the aromatic ring along with any carbons deriving from diphenyl ether structures (XI), expected to be minor components in MWL. The condensed aromatic region should consist of C_1 carbons plus any ring carbons involved in cross-links such as in the 5-5′ (V) or β-5′ (VI) substructures. Finally the protonated aromatic region will consist of the C_2, C_6, and any uncondensed C_5 carbons (Table 2).

As discussed previously, the oxygenated aromatic and condensed aromatic regions have some degree of overlap, making calculation of the degree of condensation less precise.[7] The integrations of these regions for all three spectra are presented in Table 2. The protonated aromatic region does not suffer from overlap and as a result the degree of condensation can be easily estimated. Three minus the value of integration for this region will yield an estimate of the degree of condensation based on the assumption that a completely uncondensed lignin will have protons at the C_2, C_5, and C_6 positions. The vibratory-milled samples show similar integrations for this region, with values of 2.57 and 2.56, respectively, for the Vibratory in Toluene MWL and Vibratory (Dry) MWL samples, yielding degrees of condensation of 0.43 (3.00–2.57) and 0.44 (3.00–2.56). The porcelain-milled wood, however, yielded an MWL with a degree of condensation of 0.54 indicating that condensation reactions are occurring during the milling process.

Contributing to the degree of condensation are the 5-5′ and β-5′ units. The β-5′ content was determined to be 0.07–0.09 per aromatic ring as determined by integration of the 90–86 ppm region of the acetylated MWL spectrum. The number of C-5 carbons contributing to the 5-5′ structures was determined by subtracting the β-5′ values from the integral of the 145–114 ppm region of the nonacetylated spectrum. In the case of these samples, 0.32–0.36 aromatic carbons per 5-5′ unit were calculated for all samples. As the structure of 5-5′ moieties is symmetric, the contribution of the aromatic carbon integral is half per 5-5′ moiety. Thus, the number of 5-5′ moieties is approximately 0.16–0.18 units per aromatic ring.

As mentioned in a previous article, the calculation of 5-5′ units is not precise.[7] However, the values for the 5-5′ and β-5′ carbons (0.41) agree fairly well with the degree of condensation for the vibratory-milled samples (0.43 and 0.44); although there is a discrepancy of 0.09 carbons per aromatic ring for the Rotary Porcelain 6 Week MWL. Although it cannot be substantiated, condensation reactions other than the formation of 5-5′ substructures may be occurring. In addition, there may be other side reactions occurring that will cause shifts in side chain carbons resulting in misleading calculations. This is a potential drawback to the quantitative ^{13}C NMR analysis of modified lignin samples. A possible example is the appearance of a fairly broad signal at 141–140 ppm not present in the other NMR spectra. According to literature values, this signal may coincide with the β-carbon from vinyl ether type structures (XVI), possibly corroborating the theory of γ-carbon elimination mentioned previously.[15] Further support for structural changes occurring is the accumulation of oxidized material in the insoluble lignin portion as determined by solid-state ^{13}C NMR spectroscopy.[20]

Aliphatic Side Chain Region

Other interunit linkages known to be present based on dehydrogenative polymerization show convergence between the lignin preparations and the literature values.[21,22] Specifically, integration for β-5′ (V) substructure in the acetylated spectra results in values 0.09 units per aromatic ring each for Vibratory in Toluene MWL and Rotary Porcelain 6 Week MWL and 0.07 for Vibratory (Dry) MWL. The β-β' substructure (IX) is also present in typically reported values, 0.02 per aromatic ring, for each sample (Table 3).

Dibenzodioxocin (X) substructures can be calculated by integration of peaks from 86–83 ppm in the acetylated lignin spectra. Values 0.08 and 0.06 per aromatic ring were obtained for the Vibratory in Toluene MWL and Vibratory (Dry) MWL, respectively, whereas the Rotary Porcelain 6 Week MWL sample was only 0.03. Dibenzodioxocin is a specific type of β-aryl ether substructure and their decrease in the rotary-milled sample is consistent with the β-O-4′ content discussed earlier. It is possible that a decrease in dibenzodioxocin in the Rotary Porcelain 6 Week MWL could result from a shift of the side chain carbon resonances due to cleavage of the γ-carbon or ring-opening depolymerization.

Coniferyl alcohol (VII) is thought to be incorporated as an end group in the lignin polymer by etherification at the 4′-position. The values obtained by integration at 62 ppm may be slightly overestimated due to overlap of the γ-carbon of the β-O-4′ subunit. In any case, the values obtained per aromatic ring are 0.04 and 0.03 for the Vibratory in Toluene MWL and Vibratory (Dry) MWL, respectively, and 0.02 for the Rotary Porcelain 6 Week MWL (Table 3). The decrease in the Rotary Porcelain 6 Week MWL may be attributable to cleavage of these end groups from the lignin polymer and their subsequent loss by solubilization during the purification steps.

Finally, the contribution of minor structural moieties such as β-1′ (XII), and dihydroconiferyl alcohol (XIII) should be mentioned. Dihydroconiferyl alcohol was the most prevalent of these substructures, with amounts of 0.03 per aromatic ring found in the vibratory-milled samples, and 0.02 for the rotary-milled sample. Again the slight decrease may be attributable to side chain cleavage or the loss of end groups. The β-1′ substructures were found to be relatively minor with the maximum level detected in any case was 0.01 per aromatic ring (Table 3).

CONCLUSIONS

Rotary-milling for a prolonged period to produce MWL has been shown to result in more structural changes than milling for a short period of time in vibratory milling. Oxidation was apparent with an increase in carbonyl content as well as the appearance of acidic moieties absent in the other preparations. In addition the β-aryl ether content was lower in the Rotary Porcelain 6 Week MWL than in the other preparations indicating depolymerization was occurring. The appearance of possible vinyl ether structures may contribute to this decrease of β-aryl ether content as estimated from the oxygenated aliphatic side chain region. The degree of condensation was higher in this sample indicating crosslinking reactions may occur during the milling process.

Two vibratory-milled MWL preparations were compared to determine whether previous data indicating that extensive condensation occurs during milling under a N_2 atmosphere. Results showed that the MWL produced by milling under N_2 or in toluene were very similar with the only structural differences being in the aliphatic and hydroxyl content. Total yield of MWL was higher for the sample milled under N_2 than that milled in toluene. Therefore, it can be concluded that the use of the standard Bjorkman method either under N_2 or in toluene is acceptable to produce MWL, although the former may be preferable based on total yield.

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