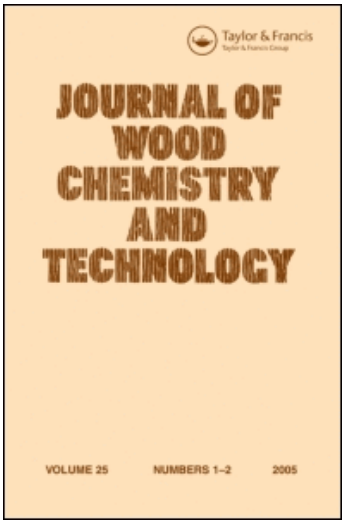

Journal of Wood Chemistry and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597282

PULPING AND BLEACHING OF CAD-DEFICIENT WOOD

D. R. Dimmel[a]; J. J. MacKay[a]; E. M. Althen[a]; C. Parks[a]; R. R. Sederoff[b]
[a] Institute of Paper Science and Technology, Atlanta, Georgia [b] North Carolina State University,

Online publication date: 28 February 2001

To cite this Article Dimmel, D. R. , MacKay, J. J. , Althen, E. M. , Parks, C. and Sederoff, R. R.(2001) 'PULPING AND BLEACHING OF CAD-DEFICIENT WOOD', Journal of Wood Chemistry and Technology, 21: 1, 1 — 17
To link to this Article: DOI: 10.1081/WCT-100102651
URL: http://dx.doi.org/10.1081/WCT-100102651

JOURNAL OF WOOD CHEMISTRY AND TECHNOLOGY, 21(1), 1–17 (2001)

PULPING AND BLEACHING OF CAD-DEFICIENT WOOD

D. R. Dimmel,[1] J. J. MacKay,[1] E. M. Althen,[1] C. Parks,[1] and R. R. Sederoff[2]

[1] Institute of Paper Science and Technology, 500 10th Street NW, Atlanta, Georgia 30318
[2] North Carolina State University

ABSTRACT

Mutant loblolly pine trees that are deficient in the enzyme cinnamyl alcohol dehydrogenase (CAD) have been obtained through directed breeding. The lignin in the wood of CAD-deficient trees has a different pool of precursors, resulting in high levels of pulping-resistant C-5 linkages. Wood from a 12-year-old CAD-deficient tree has been pulped under soda and kraft conditions in microdigestors. In comparison to a normal 12-year-old loblolly pine, the CAD-deficient wood was much more easily delignified. In addition, the pulp from CAD-deficient wood was as easy to bleach as a control pulp. The high reactivity of CAD-deficient wood may be related to the lignin size and phenolic content. The molecular weight of an isolated milled wood lignin from CAD-deficient pine was ~35% less than that from a normal pine tree.

INTRODUCTION

Wood that contains less lignin and/or more reactive lignins could be an attractive raw material for pulp production. Manipulation of lignin in

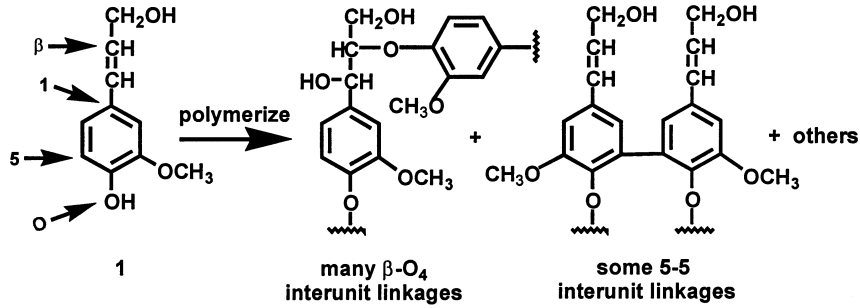

Figure 1. Polymerization of normal lignin monomer building blocks.

trees has been achieved in poplar by generic transformation (introduction of foreign genes); potential pulping benefits have been shown.[1,2] Genetic transformation of commercial softwoods is not yet possible on a routine basis; however, cross-breeding offers an alternative way to achieve a similar result. This report outlines the reactivity of an unusual wood that has been obtained through cross-breeding.

Softwood lignins are derived mainly from coniferyl alcohol (**1**).[3,4] Oxidation of coniferyl alcohol to a radical, followed by coupling one radical form with another, leads to the lignin polymer network. The preferred coupling involves union of an O_4-radical with a C_β-radical; approximately 50% of the interunit linkages in softwood lignin are of this type (Figure 1).[5] Several other linkages are also present in varying amounts, including C_5-C_5 (Figure 1), C_1-C_β, C_β-C_β, C_5-O_4, etc.[5]

Coniferyl alcohol is formed in the plant by reduction of coniferaldehyde (**2**), a step that requires the enzyme cinnamyl alcohol dehydrogenase (CAD).[6,7] CAD-deficient pines have been obtained through crosses of trees that have a mutant gene, the *cad-nl* allele, found in breeding stocks of loblolly pine. Homozygous *cad-nl* trees are obtained from well-defined crosses and are almost *totally deficient* in CAD activity.[8,9] Lignins from CAD-deficient trees are built up from unusual monomers. Analysis of milled wood lignins by NMR techniques[9] and pyrolysis GC/Ms[10] indicates that it contains elevated levels of coniferaldehyde (**2**); *vanillin* (**3**), and dihydroconiferyl alcohol (**4**) and, is low in coniferyl alcohol (**1**) (Figure 2). Relative to normal pinewood, lignin in these CAD-deficient trees contains fewer C_β-O_4 linkages and a high number of C_5-linkages.[11]

Coniferyl alcohol (**1**), the building block of normal lignin, has four available radical reaction sites (O_4, C_5, C_1, and C_β). Linkages involving C_β are not possible with CAD-deficient lignin precursors **3** and **4**, and probably are low in frequency with precursor **2**, as Kim *et al.* have recently reported.[12]

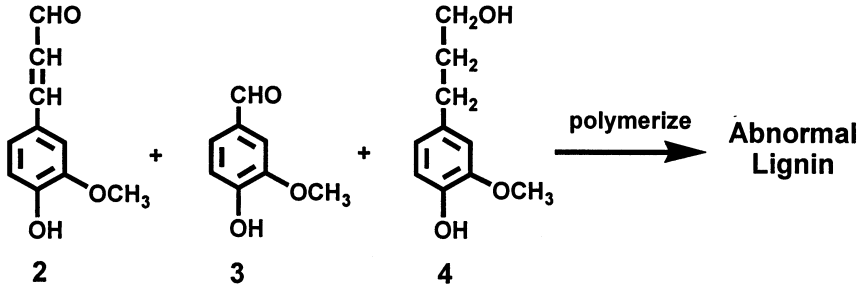

Figure 2. The building blocks for lignin production in CAD-deficient trees.

Since C_β-linkages bio-synthetically produce quinone methide intermediates that lead to C_α-linkages with other lignin units and with carbohydrates, the latter should also be low in CAD-deficient lignin. Linkages at C_1 may occur since viable mechanisms exist for loss of the **2-4** precursors' side-chains following radical coupling at the C_1-sites.

Except for coniferaldehyde (**2**), the unusual CAD-deficient precursors only have two reactive radical sites (O_4 and C_5). Lapierre has shown that O_4-C_5 linkages are more abundant in CAD-deficient wood.[11] Bonding at these sites would result in no cross-linking and potentially few residual phenol groups. However, the decrease in C_β-O_4 bonding should give more free phenol groups. CAD-deficient lignin is known to have a higher amount of phenolic groups than control wood.[11] Because of the lack of active C_β-sites in the precursors, the lignin in totally CAD-deficient pines will likely be less cross-linked (to lignin and carbohydrate chains), be inhibited in polymer growth, and have a lower molecular weight. These factors, and the higher phenolic content, would facilitate dissolution of lignin in alkali. In contrast, its high abundance of pulping-resistant C5-linkages will hurt dissolution. Our studies are directed at establishing the pulping and bleaching reactivity of CAD-deficient wood.

RESULTS AND DISCUSSION

CAD-Deficient Trees

The wood used in this study came from two 12-year-old lolblolly pine trees, one tree with normal wood (control) and one CAD-deficient tree (*cad-nl* homozygous). The trees were grown on the same site and were genetically related because they were derived from the same parents. CAD-deficient trees have, so far, only been found in inbred families that are

characterized by low survival and growth rates. CAD-deficient trees are often less straight than normal trees. It is unclear if the aberrant growth is solely related to the CAD-deficiency or, at least in part, to the inbred nature these trees. In the family identified for this study, a single CAD-deficient tree was found: it was a leaning and slow growing tree, approximately 2.5 m tall and 6–7cm in diameter at breast height. A normal (control) tree was selected from the same family: it was approximately 8 m tall and 20–22 cm in diameter. Their lignin contents were 29.5% (control tree) and 28.5% (CAD-deficient tree).

The CAD-deficient tree contained relatively high amounts of compression wood lignin (~10%).[9,11] The two trees were very similar in cellulose and hemi-cellulose contents.[13] Fibers lengths, determined after pulping, ranged from 0.68 to 0.73 mm (length weighted averages) in the CAD-deficient tree and from 1.36 to 1.63 mm in the control, clearly indicating a predominance of juvenile wood. Air dried chips were prepared from the entire bole of the trees, from the stump up to a diameter of approximately 2 cm, which were debarked manually before chipping.

Pulping of CAD-Deficient Wood

Our previous studies showed that 30% of the lignin was removed from CAD-deficient wood by mild alkaline treatment at room temperature, compared to 10% for the wild type.[14] Here we present an extensive study of soda and kraft pulping of normal and totally CAD-deficient wood.

One of the first challenges that we had to address was conducting a large number of cooks with a supply of 400 grams of dry CAD-deficient chips. Initial studies were conducted to show that small-scale (0.5 g) cooks in 4-mL pressure vessels and mini-kappa number determinations gave results similar to 1-kg cooks and regular kappa determinations for normal loblolly pine. The dried chips were rewet and reduced in size prior to cooking. In some cases, the number of days required for the chips to sink in water under vacuum varied considerably; however, control experiments indicated that the number of days had no impact on subsequent pulping response.

The reactor size dictated that a high (7:1) liquor-to-wood ratio be used. If a standard 4:1 liquor-to-wood ratio was used, the swelled chips in the small bombs were basically void of bulk liquor. For many of the cooks, the chemicals charged into the reactor represent the absolute amounts of NaOH and NaSH that would have been present in a 4:1 cook. Performing 7:1 liquor-to-wood ratio cooks this way meant that NaOH and NaSH *concentrations* were less than normally employed in a typical 4:1 cook.

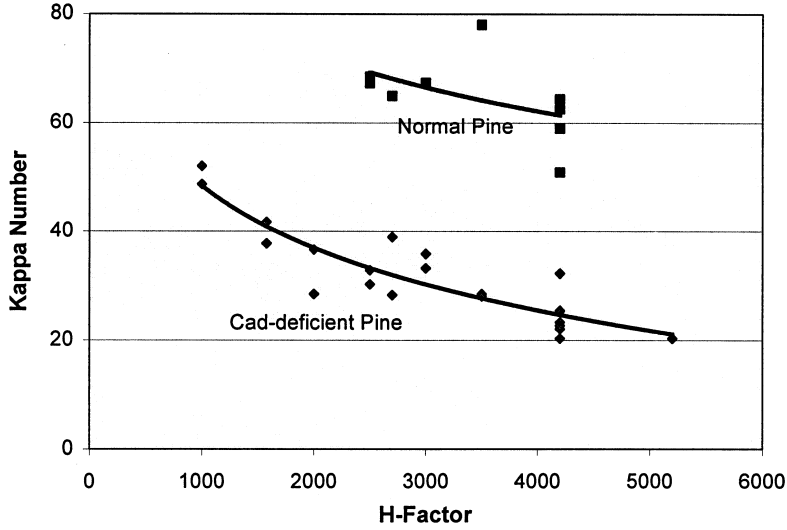

Figure 3. Relationship between kappa number and H-factor for soda cooks done with 18% active alkali and a liquor-to-wood ratio of 7:1.

Therefore, some cooks were also done at standard concentrations, but with higher absolute amounts of chemicals.

Soda pulping of CAD-deficient and normal wood with 18% active alkali at several different H-factors showed that CAD-deficient pines are much more easily pulped (Figure 3). The relatively low response of the normal wood to changes in the H-factor is probably related to the lower concentration of NaOH available in the 7:1 liquor-to-wood ratio cooks. Delignification was only slightly decreased when the charge of active alkali was lowered to 16.5 and 15% for CAD-deficient cooks (done at 1575 H-factor).

For kraft pulping, the CAD-deficient wood again delignified more easily than normal wood when the NaOH and NaSH concentrations were moderate; at higher concentrations, the degree of delignification was similar (Figure 4). Delignification occurs in three stages: initial, bulk, and residual. The results suggest that, with CAD-deficient wood, the initial and bulk phase reaction rates are quite fast, but the residual phase rate is slow, similar to normal wood. For harsh cooks, each wood delignifies down to roughly the same level of residual lignin.

A large number of high-chemical kraft cooks were conducted (Figure 5). Two trends were observed for the CAD-deficient wood, depending upon which set of chips was used. The upper trend line, obtained from the same

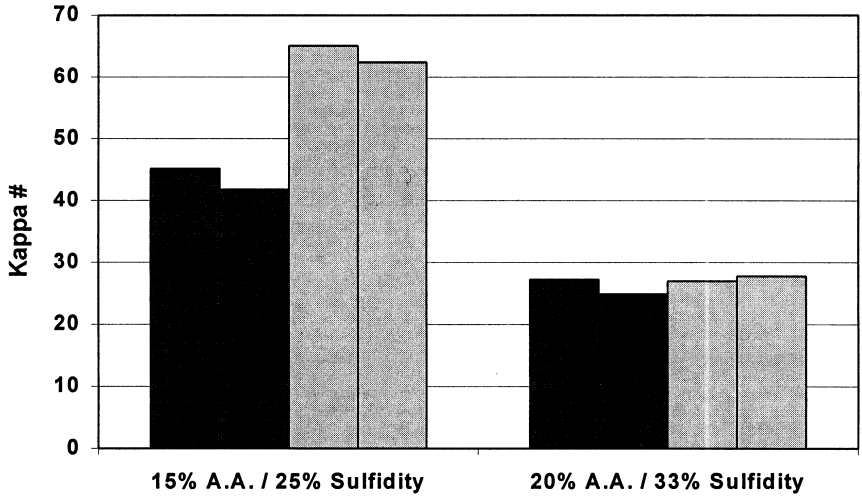

Figure 4. Relationship between kappa number and chemical charge for duplicate CAD-deficient (dark-colored bars) and normal (light-colored bars) loblolly pine pulping (7:1 liquor-to-wood ratio, 2000 H-factor).

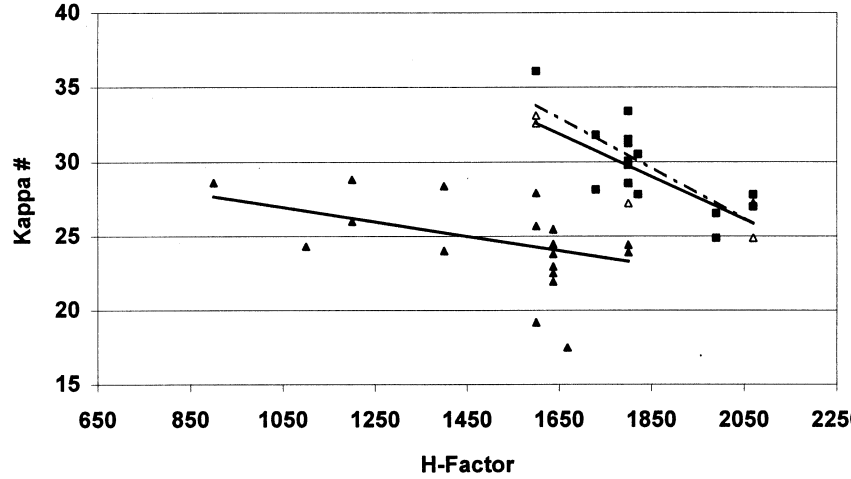

Figure 5. Relationship between kappa number and H-factor for kraft cooks (20% active skills, 33% sulfidity and a liquor-to-wood ratio of 7:1) for CAD-deficient wood (▲, △, —) and control wood (■, ----).

Table 1. Kappa and Yield Data for Cooks of CAD-Deficient and Control Woods.

Sample	Kappa Numbers	Kappa Average	Pulp Yield (%)	Ave. Pulp Yield (%)	Ave. Lignin-Free Yield (%)
CAD-Deficient	20.3, 23.2, 22.7	22.1	32.6, 32.6, –	32.6	29.3
Control	59.0, 64.4, 62.6	62.0	46.6, 44.7, 49.8	47.0	37.7

CAD-deficient chips as in the soda cooks, indicates that CAD-deficient wood is only slightly easier to pulp than control wood. The bottom trend line represents data obtained from CAD-deficient chips that were rewet for different length of time.The new chips were considerably more reactive than the first batch, further verifying the special nature of CAD-deficient wood. The reasons for the pulping differences between the first and subsequent CAD-deficient chip batches are not clear; we suspect that the first batch contained one or more uncharacteristic chips. Different batches of normal chips behaved identically.

The pulp yields of CAD-deficient and control wood were compared using identical chemical charges and H-factors for soda, kraft, and soda/0.1% AQ cooks. The data for triplicate soda cooks are given in Table 1. A consistent trend was observed toward lower lignin-free yield with the CAD-deficient wood: 8.5% lower, on average, for soda cooks, 15% lower for kraft cooks, and 20% lower for soda/AQ cooks. The yield data were from very small cooks, where chip differences magnify errors; even so, the trend is obvious.

Nature of the Dissolved and Residual Lignin

The comparison of kappa numbers from the pine cooks could be a problem if the lignins from CAD-deficient and control wood had different reactivities toward $KMnO_4$. Decreased consumption of $KMnO_4$ might be expected due to the increased amounts of aldehyde in the lignin of the CAD-deficient wood. However, this concern is not supported by $KMnO_4$ consumption[15] experiments, which show no significant differences between milled wood lignins[14] isolated from CAD-deficient and control trees (Table 2).

Pulping liquors from identical (chemical charge and H-factor) cooks were acidified to precipitate the dissolved lignin. Fifty percent more lignin was collected from the CAD-deficient liquor. This was more than expected

Table 2. Potassium Permanganate Consumption Data for MWL Lignins.

Sample	Amount (mg)	$KMnO_4$ consumed (mL)	% $KMnO_4$ consumed	Equivalent (mL $KMnO_4$/mg)
CAD-deficient MWL	5.72	3.40	6.8	0.594
	17.16	10.20	20.4	0.594
	34.32	20.78	41.6	0.605
	45.76	27.60	55.2	0.603
Control MWL	5.78	3.00	6.0	0.519
	17.33	10.15	20.3	0.585
	34.65	20.67	41.3	0.596
	46.20	26.79	53.6	0.579

based on material balances, meaning that recovery of lignin was greater in the CAD-deficient case (92% vs. 67% for the control). This result suggests that the control wood produced smaller (less easily recovered) lignin fragments. The precipitated lignins from CAD-deficient and control pine cooking liquors were acetylated and analyzed by gel phase chromatography; they displayed nearly the same molecular weights, regardless of the cooking time. However, the isolated acetylated lignins displayed variable solubility in THF, the solvent for the molecular weight studies, raising doubts as to whether the molecular weights were representative.

Bleachability

To learn more about the relationship between lignin structure and reactivity, we compared the bleachability of CAD-deficient and normal pulps that were produced in a similar manner and had identical 29-kappa numbers. Both pulps were produced using 7:1 liquor-to-wood ratio, 20% active alkali, and 33% sulfidity; however, the CAD-deficient wood was cooked at *less than half* the H-factor (790 vs. 1800) of the control wood.

Unbleached CAD-deficient and normal pulps (2 g each) were treated with an identical $D_oE_1D_1E_2D_2$ bleach sequence and the brightness values determined. Bleaching on such low scale presents several challenges; great care was taken to try to mimic the exact conditions, such as exiting pH values, used in typical pulp bleaching. Roughly 3 cm-diameter pads of bleached pulp were prepared for brightness determinations (see the Experimental Section for exact details). The brightness readings for the

Table 3. Density Values (g/cm^3, Average of 3–5 Measurements) Determined by Two Methods for CAD-Deficient and Control Unbleached and Bleached Pulps.

Pulp Type	Soft Platen Density	Hard Platen Density
CAD-Unbleached	0.630	0.579
Control Unbleached	0.568	0.533
CAD-Bleached	0.999	1.023
Control Bleached	0.987	0.935

micro-pads were very close to the normal TAPPI pad readings for a sample pulp when the basis weights were above 200 g/m^2.

There was no difference in the D_1-brightness values for the CAD-deficient and normal pulps; both were 65.3 ± 0.1. The measured brightness values after the D_2 stage were problematic because the CAD-deficient pulp pad had a relatively low opacity, which allowed light to penetrate through the pad and reflect off the black background, giving the appearance that the pulp itself was darker. Against a white background, the CAD-deficient pad *appeared as bright as* (if not more than) the control pulp pad.

Some physical measurements were performed on the fully bleached and unbleached CAD-deficient and normal pulps. The data in Table 3 indicate that the CAD-deficient pulps were denser than control pulps, but the differences were not large. However, the small pad size and warped physical state of the fully bleached CAD-deficient pad may have influenced the observed values. Therefore, we also examined the pulps using scanning electron microscopy (SEM). The SEM cross-sectional pictures (Figure 6) indicate that the fully bleached CAD-deficient pulp was denser than the fully bleached control pulp. This same density difference was observed in the cross-sectional SEM pictures of the unbleached pulps. The SEM surface views also revealed that the CAD-deficient fibers were flatter and more thin-walled than the control pulps, both in the bleached and unbleached cases. These characteristics are common for early-wood fibers. The data suggest that there are important structural and/or carbohydrate compositional differences between these pulps that warrant further study.

In summary, the bleachability of CAD-deficient pulps appears to be about the same as a normal pulp. The pad formation is very different in the CAD-deficient case; this leads to a more dense, low opacity sheet that gives a false brightness value. We are uncertain what causes this unusual sheet formation property; however, we speculate that it may be related to altered deposition of cell wall components in response to, and in compensation for, the altered lignin structure.

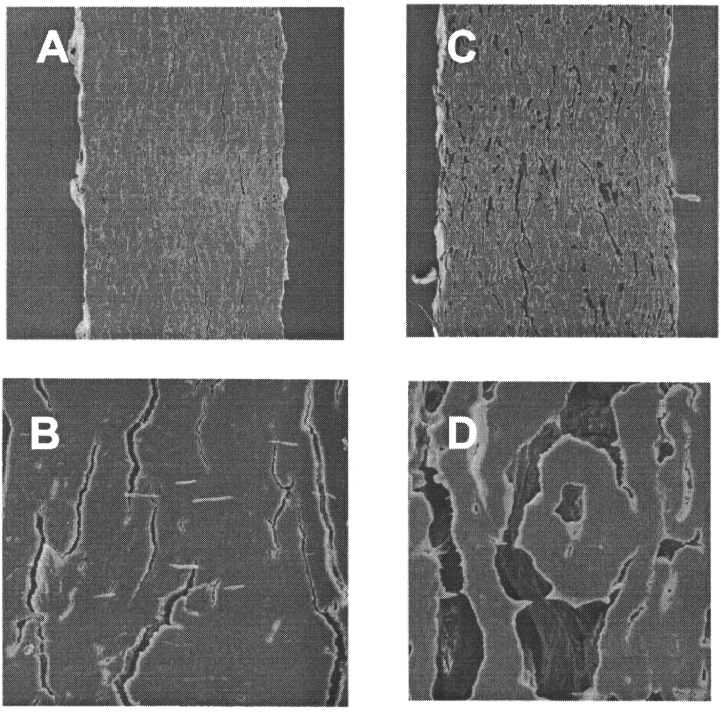

Figure 6. Scanning electron micrographs of fully bleached pulps: CAD-deficient at 150 magnification (A) and 1500x (B), normal at 150x (C), and at 1500x (D).

Lignin Molecular Weight Characterization

The molecular weight of milled wood lignin obtained from CAD-deficient wood was analyzed in-house and by an outside lab and was found to be ~2/3 that of normal pine (Figure 7).

CONCLUSIONS

With a higher amount of 5-5 lignin linkages, CAD-deficient wood should be more difficult to delignify than normal wood. But just the opposite is found. Totally CAD-deficient loblolly wood is much more easily delignified in a pulping stage than normal pine. The relatively low molecular weight of the totally CAD-deficient lignin is likely the principal reason for the easy lignin removal; it takes fewer cleavages to get a water-soluble piece.

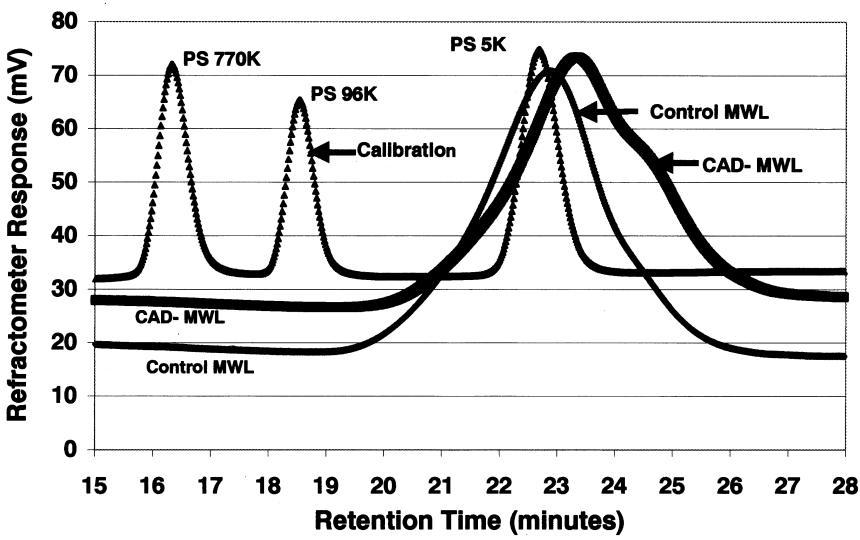

Figure 7. Comparison of the molecular distributions of CAD-deficient and normal milled wood lignins with polystyrene (PS) standards.

Another factor could be that the lignin is less cross-linked. Interestingly, even though there are probably a higher number of C_5-linkages in the lignin present in the unbleached CAD-deficient pulp, the bleachability of this pulp was similar to that of normal wood.

The significant variability of lignin structure in plant species is well-established;[16,17] however, it appears that lignin composition and structure may be manipulated beyond what was previously anticipated.[9,11] We are only beginning to evaluate the impact of these changes in subunit composition and linkages; however, they point to promising opportunities to manipulate the reactivity wood in pulping and bleaching.

EXPERIMENTAL

Chip Preparation

The air-dried CAD-deficient and normal pine chips were rewetted by placing the chips in a desiccator containing sufficient water to cover the chips once they sank. The chips sank after ~2 days of applied 30mm vacuum. The wet chips were reduced in size using a Wiley mill, screen size 1, and then conditioned in sealed bags for at least 24 hours. The consisten-

cies of the chips were determined, in duplicate, by weighing the chips after oven drying at 105°C in a tarred pan for 2+ hours.

When the first batch of CAD-deficient chips ran out, more chips were rewetted for continued studies. The rewetting procedure in this case took longer than normal, 5 vs. 2 days. The chips used from this rewetted batch had a much lower than expected kappa number after pulping. However, new batches of CAD-deficient that were rewet for different lengths of time and then pulped gave similar kappa numbers; the same was observed for control chips. The rewetting procedure did not extract much lignin, based upon their A_{280nm} of the residual rewetting liquors. The UV absorbance measurement involved evaporating the rewetting liquors to ~20 mL and then diluted to 50.00 mL with water. A 1.00 mL-aliquot of the 50-mL sample was then diluted to 10.00 mL with 4 mL of water and 5 mL of *p*-dioxane. The UV spectra (200–500 nm) of 50% aq. dioxane solutions of CAD-deficient and control pine rewetting liquors were taken using a Shimadzu UV160-U spectrophotometer. The amount of lignin in each sample was calculated from its $A_{280\ nm}$ and a calibration curve constructed with Repap lignin in 50% aq. dioxane. The lignin extracted by rewetting represented 0.25% of CAD-deficient wood (38.5 mg of lignin from 14.4 g of wood) and 0.05% of the control pine (6.5 mg from 14.2 g). These lignin %'s correspond to 1.6 and 0.3 kappa units, respectively. Obviously, very little material was extracted in both cases, and the difference between the CAD-deficient and normal wood was insufficient to explain the differences in delignification.

Micro-Pulping Reactions

Cooking with 4.5 mL pressure vessels (bombs), utilizing a Techne SBL-1 fluidized sand bath and TC-AD temperature controller, has previously been described for model studies.[18] The bomb was filled with 0.5000 g of o. d. chips and the pulping liquor. A 7:1 liquor-to-wood ratio was used to adequately wet the chips and to provide enough residual black liquor for post-run lignin analyses. The active alkali and sulfidity levels in the liquor were determined by the ABC titration method.[19]

The cook temperature profile consisted of a 100–170°C temperature rise over a 60-min time period and 170°C for the time needed to achieve the desired H-factor. At the conclusion of the run, the bomb was removed from the hot sand bath, cooled with ice water, opened, and emptied (with rinsing) into a Waring blender and chips disintegrated. Because the product yield was typically ~200 mg, there was no attempt to separate shives that were typically present in higher kappa number pulps. Consequently, this introduced possible errors in the kappa numbers of such pulps. The kappa of

each sample was performed according to TAPPI UM-246,[20] with two variations: the sample volume was diluted by two in order to allow good sample disintegration in the blender, and the sample was oven-dried, rather than used wet.

Lignin Isolation from Pulping Liquor

After disintegration, the pulp from a micro-pulping run was washed with ~500 mL water. This liquor volume was evaporated to ~200 mL and ~2 mL of 0.025 M diethylenetriamine pentaacetic acid (DTPA) was added to chelate any metals. After stirring for 1 hr, the liquor was acidified to pH 2 with lN HCl. This caused the lignin to precipitate. Freezing, thawing, and centrifuging the sample facilitated further precipitation. The supernatant was decanted and put through the acidification, freezing, thawing, and centrifugation cycle two more times. The lignin samples were combined, freeze dried, and purity determined from their $A_{280\ nm}$ compared to a calibration chart of Repap lignin concentration vs. absorbance. Considerably more lignin was isolated from CAD-deficient liquors than from control liquors; for example, 126 mg of lignin was isolated from the black liquor of a CAD-4200 H-factor soda cook, while 84 mg was isolated from a control run, employing the same conditions and amount of starting o.d. wood. Based on the amount of lignin known to be in the wood, the pulp yield, and pulp kappa number, we calculate that 137 mg of lignin should be in the liquor in the CAD-deficient case and 122 mg in the control case. Roughly 92% of the CAD-deficient dissolved lignin was recovered, but only 67% of the control lignin.

Micro-Brightness Determination

A set of micro-brightness pads of varying basis weights was prepared from a standard bleached pulp. A ~1% consistency slurry of pulp was acidified with ~ 0.5 N H_2SO_4 to a pH of 4.8-5, divided in half, and two pads were prepared by pouring the slurries into a pad-forming stand tube.[21] After drainage and aspiration for 1-2 minutes, the pad was removed from the centrifuge tube and pressed under 50 psi to force excess water from the sample. The pad was then conditioned in a constant humidity room overnight and read for directional brightness using standard TAPPI method 452.[22] The brightness readings for the micro-pads were very close to the normal TAPPI pad readings for the same pulp when the basis weights were $> 200\ g/m^2$.

Table 4. Bleaching Conditions; All Stages were done at 10% Consistency and 70°C.

Stage	Pulp o.d. wt. (g)	ClO_2 Level[a]	% NaOH	Time (min)
D_0	2.00	0.25 KF	------	45
E_1	2.00	------	[b]	60
D_1	1.50	1.00%	0.5%[c]	90
E_2	1.00	------	0.5%	60
D_2	0.50	0.40%	------	180

[a] For D_0 stage, $\%ClO_2$ = [Kappa Factor (KF) x kappa #]/2.63
[b] %NaOH = KF x kappa # of brownstock x 0.55
[c] %NaOH in D_1 stage determined to provide an appropriate pH_{out}

Bleaching Procedures

Bleaching was done in a tarred Kapak pouch, using a syringe to deliver water, ClO_2 solution, and NaOH volumes that were required. The ClO_2 concentration was determined by sodium thiosulfate titration. After addition of the required reagents, the pouch was heat-sealed, massaged to achieve good mixing, placed in a 70°C constant temperature bath, and at intervals, removed from the bath and further massaged. At the conclusion of the reaction time, the pouch was removed from the water bath and cut open, and the pH of the reaction mixture recorded. The pulp was then filtered and washed with copious amounts of water.

SEM Pictures

The pulp samples were embedded in a resin, etched to remove the top surface of the resin and then polished with grinding paper to expose the pulp. White streaks of rubbery-like material formed on the surface of the resin-embedded, fully bleached CAD-sample, but not the other samples. The streaks can be seen on close examination of the SEM cross-sectional pictures (Figure 6B).

Lignin Molecular Weight Determination

Isolated black liquor lignins of several of the CAD-deficient and control pine cooks, as well as milled wood lignins prepared at NC State

University,[23] were examined by gel phase chromatography (GPC). The lignins were acetylated prior to analysis.[23] Immediately before injection into the liquid chromatograph (LC), the sample was diluted to 25 mL with LC grade THF and then filtered through a 0.2μm pore Whatman syringe filter. The GPC analysis was performed on a Hewlett-Packard 1090 Liquid Chromatograph using a photodiode array UV detector, Waters Stryagel 7.8 × 300 mm HR6, HR4, and HR3 columns, and LC grade THF as the eluent at 1 mL/min. The collected data was compared to a calibration plot of log MW vs. time for polystyrene standards.

The GPC analyses of black liquor-isolated lignins were problematic. Select samples were submitted to Prof. Richard Helm's lab at Virginia Tech for molecular weight determinations. The VA Tech lab used polystyrene standards and an RI (concentration) detector in conjunction with a viscosity (universal calibration) detector. They had difficulties dissolving the acetylated lignin samples in THF, the mobile phase used for their GPC analysis. We did not experience noticeable solubility problems, but the GPC baseline varied from sample to sample. This could be related to non-specific interactions between the sample and the column. However, we assumed that it was a simple baseline drift and applied a correction to provide a Gaussian-shaped curve between two time periods for which the majority of the sample eluted and represented weight average molecular weight (M_w) range of 162-100,000. Using this integration method, we observed roughly the same M_w of 19-21,000 for every black liquor-isolated lignin examined, regardless of the duration of the cook, the cook type, or wood sample.

The MLW lignin samples were much easier to deal with; no baseline corrections were required. Our results are shown in Table 5. The same samples were also analyzed at Virginia Tech lab that used polystyrene standards and an RI (concentration) detector in conjunction with a viscosity (universal calibration) detector. The data is shown in Table 5, as well as graphically in Figure 7. While our numbers are not in good agreement with one another, we observed that the M_w and number average molecular

Table 5. Milled Wood Lignin Molecular Weight Determinations by IPST and VA Tech.

Lignin Type	IPST M_w	VA Tech M_w	IPST M_n	VA Tech M_n
Control MWL	15,304	11,000	6,849	5,690
CAD-MWL	10,130	7,060	4,576	3,310

weights, M_n, of the CAD-deficient MWLs were roughly 60-67% that of the control pine.

ACKNOWLEDGEMENTS

We are grateful to Dr. Jiebing Li for performing the $KMnO_4$ consumption tests and to Dr. Hiroki Nanko and Ms. Georgeta Maghiari for providing the STEM results. Support for this work came from a research grant from the US department of Agriculture, grant number 97-35103-4796.

REFERENCES

1. M. Baucher, B. Chabbert, G. Pilate, J. van Doorsselaere, M.-T. Tollier, M. Petit-Conil, D. Cornu, B. Monties, M. van Montagu, D. Inzé, L. Jouanin, and W. Boerjan, *Plant Physiol.*, *112*, 1479 (1996).
2. C. Lapierre, B. Pollet, M. Petit-Conil, G. Toval, J. Romero, G. Pilate, J.-C. Laple, W. Boerjan, V. Ferret, V. De Nadai, and L. Jouanin, *Plant Physiol.*, *119*, 152 (1999).
3. B. Monties, in *Methods in Plant Biochemistry*, P. M. Dey, and J. B. Harborne (eds.), Academic Press, London, Vol. 1, pp. 113–158 (1989).
4. E. Adler, *Wood Sci. Technol.*, *11*, 169 (1977).
5. E. Sjostram, *Wood Chemistry Fundamentals and Applications*, 2nd Edition, Academic Press, San Diego, CA, 1993, Chapter 4.
6. J. J. MacKay, W. Liu, R. W. Whetten, R. R. Sederoff, and D. M. O'Malley, *Molecular and General Genetics*, *247*, 537 (1995).
7. D. O'Malley, S. Porter, and R. R. Sederoff, *Plant Physiol.*, *98*, 1364 (1992).
8. J. L. MacKay, D. M. O'Malley, T. Presnell, F. L. Booker, M. M. Campbell, R. W. Whetten, and R. R. Sederoff, *Proc. Natl. Acad. Sci. USA*, *94*, 8255 (1997).
9. J. Ralph, J. J. MacKay, R. D. Hatfield, D. M. O'Malley, R. W. Whetten, and R. R. Sederoff, *Science*, *277*, 235 (1997).
10. J. MacKay, D. R. Dimmel, and J. J. Boon, J. Wood Chem. *Technol.*, *21*, 19 (2001).
11. C. Lapierre, B. Pollet, J. MacKay, and R. Sederoff, *Journal of Agricultural and Food Chemistry*, *48*, 2326 (2000).
12. H. Kim, J. Ralph, N. Yahiaoui, M. Pean, and A-M. Boudet, *Org. Letters*, *2*, 2197 (2000).
13. R. D Hatfield, Unpublished communication.

14. J. J. MacKay, T. Presnell, H. Jameel, H. Taneda, D. M. O'Malley, and R. R. Sederoff, *Holzforschung*, *53*, 403 (1999).
15. J. Li, and G. Gellerstedt, *Proceed. Intern. Symp. Wood and Pulping Chem.*, Montreal, Canada, June 7-12, 1997, p. Gl-l.
16. R. W. Whetten, and R. R. Sederoff, *Plant Cell*, *7*, 1001 (1995); R. W. Whetten, J. J. MacKay, and R. R. Sederoff, *Ann. Rev. Plant Physiol. & Plant Mol. Biol.*, *49*, 585 (1998).
17. A. M. Boudet, C. Lapierre, and J. Grima-Pettenati, *New Phytol.*, *129*, 203 (1995).
18. B. N. Brogdon, and D. R. Dimmel, *J. Wood Chem. Technol.*, *16*(3), 261 (1996).
19. C. E. Libby (ed.), *Pulp and Paper Science and Technology*, Vol. 1, McGraw-Hill, New York, NY, 1962, p. 229.
20. Method UM-246, TAPPI Test Methods, TAPPI PRESS, Atlanta, GA, 1992.
21. E. F. Thode, J. G. Bergomi, Jr., and R. E. Unson, *Tappi*, *43*(5), 505 (1960).
22. Method 452, TAPPI Test Methods, TAPPI PRESS, Atlanta, GA, 1992.
23. G. Gellerstedt, in *Lignin Chemistry*, Springer Series in Wood Science, S. Y. Lin, and C. W. Dence, (eds.), Springer-Verlag, Berlin, 1992, p. 491.