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Organosolv Ethanol Lignin from Hybrid Poplar as a Radical Scavenger: Relationship between Lignin Structure, Extraction Conditions, and Antioxidant Activity

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Twenty-one organosolv ethanol lignin samples were prepared from hybrid poplar (*Populus nigra* × *P. maximowiczii*) under varied conditions with an experimental matrix designed using response surface methodology (RSM). The lignin preparations were evaluated as potential antioxidants. Results indicated that the lignins with more phenolic hydroxyl groups, less aliphatic hydroxyl groups, low molecular weight, and narrow polydispersity showed high antioxidant activity. Processing conditions affected the functional groups and molecular weight of the extracted organosolv ethanol lignins, and consequently influenced the antioxidant activity of the lignins. In general, the lignins prepared at elevated temperature, longer reaction time, increased catalyst, and diluted ethanol showed high antioxidant activity. Regression models were developed to enable the quantitative prediction of lignin characteristics and antioxidant activity based on the processing conditions.

KEYWORDS: Antioxidant; biorefining; ethanol; free radical; organosolv ethanol lignin

INTRODUCTION

The utilization of lignocellulosic biomass for chemicals, fuels, and materials is now the subject of accelerating interest and awareness. Driven by increasing environmental concerns, high oil prices, and the instability/uncertainty of petroleum reserves, the production of bioethanol from lignocellulose is receiving increasing attention from governments, industries, and academics throughout North America and Europe (*1*−*3*). However, the current lignocellulose-to-ethanol conversion processes are not yet economically feasible, requiring expensive pretreatment steps and producing only low-value coproducts. A promising strategy is to integrate ethanol production into a biorefinery scheme analogous to that of crude oil wherein a relatively low-value raw material is fractionated and refined to produce a wide range of value-added products (*4*). In the case of lignocellulose, the lignin, hemicellulose, and extractive components of the biomass need to be converted into high-value coproducts, offsetting the high costs of the pretreatments.

Among the various biorefining processes currently being assessed is an organosolv ethanol process. Initially developed for processing hardwoods into pulp for paper manufacturing (*5*−*7*), this process has been demonstrated as a promising pretreatment for the bioconversion of lignocellulose to bioethanol, chemicals, and materials (*8*). Results indicated that substrates pretreated using the organosolv ethanol process possess superior enzymatic digestibility over those pretreated with alternative processes, including the substrates with high residual lignin contents (*8*). Furthermore, this process produces a hemicellulose-derived sugar stream (*9*) and a particularly high-quality lignin fraction, which has potential use in several industrial applications (*10*−*12*).

In recent years, research into naturally occurring polyphenols has drawn increasing attention. Polyphenols have shown many favorable effects on human health. They can inhibit the oxidization of low-density proteins (*13, 14*), thereby decreasing the risks of heart disease (*15*). Polyphenols have anti-inflammatory activity and anti-carcinogenic properties (*16*−*18*) and are well-known to be effective antioxidants for food lipids (*19*). Lignin is a natural phenolic polymer. It is one of the most abundant natural polymers, composing up to one-third of the material found in plant cell walls. Lignin serves to affect water transport, protect trees against chemical and biological attack, and provide structural integrity. In addition to traditional application strategies (*20*−*22*), antioxidant is a potential application of lignin. It was reported that kraft lignin was as effective as vitamin E as an antioxidant of corn oil (*23*). Lignin functions as an effective radical scavenger to prevent autoxidation and depolymerization of cellulose in pulps and papers (*24*, *25*). Incorporation of lignin into synthetic polymer systems can stabilize the material against photo- and thermal oxidation (*26*−*28*). As a major component in dietary fiber, lignin can inhibit the activity of enzymes related to the generation of superoxide anion radicals and obstruct the growth and viability of cancer cells (*29*).

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Table 1. Extracting Conditions and Characterization of Lignin Preparations from Hybrid Poplar[a]

	lignin extracting conditions				characterization of lignin							
lignin no.	temp (°C)	time (min)	H_2SO_4 (% of wood)	ethanol concentration (% (v/v))	lignin yield (% of wood)	ArOH (mmol/g of lignin)	AlkOH (mmol/g of lignin)	MeO (mmol/g of lignin)	M_n	M_w	M_w/M_n	RSI
EL1	165	40	1.00	65	5.7	2.21	5.01	6.98	1515	3877	2.56	12.5
EL2	195	40	1.00	65	17.0	2.50	4.62	7.64	1377	4191	3.04	57.5
EL3	165	80	1.00	35	5.0	3.14	4.92	8.35	992	1962	1.98	58.2
EL4	195	80	1.00	35	4.4	4.63	3.29	8.04	783	1105	1.41	122.0
EL5	165	40	1.50	35	6.3	3.38	4.13	8.62	1056	1991	1.89	59.6
EL6	195	40	1.50	35	6.5	4.83	3.15	8.58	844	1195	1.42	66.7
EL7	165	80	1.50	65	17.7	2.94	3.78	8.45	1351	3596	2.66	26.3
EL8	195	80	1.50	65	20.9	4.38	2.73	8.11	1106	1888	1.71	68.5
EL9	155	60	1.25	50	7.1	2.31	5.14	7.91	1270	3840	3.02	26.3
EL10	205	60	1.25	50	15.8	4.24	3.01	7.86	994	1579	1.59	61.8
EL11	180	26	1.25	50	13.5	2.86	4.67	8.52	1167	2953	2.53	23.8
EL12	180	94	1.25	50	15.7	3.78	3.55	8.63	1087	1942	1.79	47.6
EL13	180	60	0.83	50	12.4	2.80	4.49	7.82	1230	3100	2.52	22.8
EL14	180	60	1.67	50	17.2	4.10	3.15	8.44	1123	1890	1.68	52.6
EL15	180	60	1.25	25	1.7	4.23	3.60	8.73	894	1381	1.54	50.0
EL16	180	60	1.25	75	17.3	2.63	4.08	8.25	1330	3991	3.00	21.8
EL17	180	60	1.25	50	15.6	3.39	3.81	8.49	1075	2089	1.94	37.1
EL18	180	60	1.25	50	14.8	3.50	3.85	8.61	1073	2020	1.88	38.5
EL19	180	60	1.25	50	15.2	3.51	3.83	8.63	1084	2065	1.90	37.6
EL20	180	60	1.25	50	16.0	3.48	4.00	8.77	1099	2105	1.92	35.7
EL21	180	60	1.25	50	16.2	3.50	3.80	8.64	1134	2250	1.98	33.8
VE												263.2
BHT												1.1

[a] EL = organosolv ethanol lignin; ArOH = phenolic hydroxyl groups; AlkOH = aliphatic hydroxyl groups; MeO = methoxyl groups; M_n = number-average molecular weight; M_w = weight-average molecular weight; RSI = radical scavenging index; VE = vitamin E; BHT = 3,5-di-*tert*-butyl-4-hydroxytoluene.

In the present work, we extracted and characterized 21 lignin preparations from hybrid poplar under varied conditions using an organosolv ethanol process. The organosolv ethanol lignins were assessed as antioxidant. The effect of processing parameters on the yield and structural features, i.e., functional groups, molecular weight, and polydispersity, of the resulting lignins was investigated. The relationship among the lignin features, extraction conditions, and antioxidant activity was discussed. The main objective of this study was to investigate the radical scavenging activity of the extracted lignins, identifying the most important structural features of the lignin for its antiradical efficiency. Mathematic models were also regressed from the results obtained in this study, allowing quantitative prediction of the structural characteristics and antioxidant activity of the resulting lignins.

MATERIALS AND METHODS

Materials. Hybrid poplar (*Populus nigra* × *P. maximowiczii*) wood chips, screened to pass a 1/4 in. (6.4 mm) round screen, were generously provided by the National Renewable Energy Laboratory (NREL) (Golden, CO). The wood chips were stored at −20 °C and used as received. The chemical composition of the poplar chips was reported previously (*30*). Anhydrous ethanol was purchased from Commercial Alcohol Inc. (Brampton, ON, Canada). Other chemicals were purchased from Sigma-Aldrich (Oakville, ON, Canada) and used as received.

Organosolv Ethanol Processing of Hybrid Poplar. The poplar chips were delignified using an organosolv ethanol process on a customer-designed, four-vessel (2 L each), rotating digester manufactured by Aurora Products Ltd. (Savona, BC, Canada). A 200 g (oven-dried weight) batch of chips was processed in each vessel. The flowchart of the process and experimental details were reported in a previous paper (*30*). The experimental matrix of 21 runs (**Table 1**) was designed using Response Surface Methodology (RSM) (*31*), including 8 factorial points (EL 1−8), 8 star points (EL 9−16), and 5 central points (EL 17−21). Extracting temperature, reaction time,catalyst (sulfuric acid) dosage, and aqueous ethanol concentration were varied within the ranges of 155−205 °C, 26−94 min, 0.83−1.67% on oven dry wood chips (w/w), and 25−75% (v/v), respectively, while the ratio of liquor-to-wood remained constant at 7 (v/w).

At the end of the delignification, the pulp was filtered using a nylon cloth and washed (3 × 300 mL) using warm (60 °C) aqueous ethanol with the same concentration of processing liquor. The filtrate and washings were combined and poured into a 3-fold volume of water (about 6 L) to precipitate lignin. The resulting lignin precipitate was filtered through Whatman no. 1 filter paper, washed thoroughly with water, and air-dried. The yield of crude lignin was calculated as the percentage of starting wood chips (oven-dried weight). Before being characterized, the crude lignin was purified by dissolving 3 g of the crude lignin in 15 mL of acetone containing molecular sieves and then filtering through a 0.45 μm syringe filter to remove insoluble impurities. The acetone was removed under reduced pressure, and the purified lignin was dried over P_2O_5 in a vacuum desiccator. The yields of the purified lignins were between 91 and 98%, depending on the extraction conditions of the lignins. In general, severer conditions (e.g., high temperature, more catalyst, and long reaction time) generated purer lignin (higher purification yield) due to less residual carbohydrate.

Acetylation of Lignin. Purified lignin (0.5 g) was dissolved in 6 mL of pyridine−acetic anhydride (1:1, v/v) and kept in the dark at room temperature for 72 h. The solution was added dropwise to 120 mL of ice-cold water containing 1 mL of concentrated HCl, under constant stirring. The precipitated lignin acetate was collected on a 10 μm nylon membrane filter, washed with water, and dried over P_2O_5 under vacuum.

Characterization of Lignin. Functional groups [phenolic hydroxyl (ArOH), aliphatic hydroxyl (AlkOH), and methoxyl (MeO) groups] of lignin were estimated using ^{1}H NMR (*32*). Lignin acetate (50 mg) and *p*-nitrobenzaldehyde (NBA, 5 mg as internal standard) were dissolved in 0.5 mL of $CDCl_3$. The ^{1}H NMR spectrum was recorded on a Bruker 300 UltraShield spectrometer. A total of 128 scans were collected. The contents of functional groups were calculated from integration ratios of the protons of functional groups to the protons of the internal standard according to eq 1

$$F(\text{mmol/g of lignin}) = \frac{\frac{I_F}{3} \times \frac{4}{I_{NBA}} \times \frac{W_{NBA}}{151} \times 1000}{W_L - \frac{I_{Ac}}{3} \times \frac{4}{I_{NBA}} \times \frac{W_{NBA}}{151} \times 42} \quad (1)$$

where F is the content of the functional groups (ArOH, AlkOH, and MeO), mmol/g of lignin; I_F is the integration of protons of the functional groups (δ 4.10−3.10 ppm for MeO, δ 2.50−2.17 ppm for the acetyl group corresponding to ArOH, and δ 2.17−1.70 ppm for the acetyl group corresponding to AlkOH); 3 is the number of protons of acetyl and methoxyl groups; 4 is the number of protons on NBA benzene ring; I_{NBA} is the integration of 4 protons on NBA benzene ring (δ 8.4 and 8.2 ppm); W_{NBA} is the weight of NBA, mg; 151 is the formula weight of NBA; W_L is the weight of the lignin acetate, mg; I_{Ac} is the integration of protons of total acetyl groups corresponding to ArOH and AlkOH (δ 2.50−1.70 ppm); 42 is the formula weight of acetyl group minus one (43 − 1).

The number-average and weight-average molecular weights (M_n and M_w, respectively) of the acetylated lignin samples were estimated by gel permeation chromatography (GPC) using an 1100 HPLC system (Agilent, Palo Alto, CA) equipped with an autosampler, an isocratic pump, a thermostated column compartment, a multiple wavelength detector (MWD), and two Styragel columns (HR5E and HR1) (Waters, Milford, MA) in tandem. Lignin acetate (1 mg) was dissolved in 1 mL of HPLC-grade tetrahydrofuran (THF) without stabilizer, and 50 μL of the solution was injected onto the GPC. THF was used as eluent at a flow rate of 1 mL/min. The column temperature was 50 °C. The columns were calibrated with polystyrene standards. Lignin samples and polystyrene standards were detected by MWD at 280 and 254 nm, respectively.

Evaluation of Lignin Antioxidant Activity. The antioxidant activity of the lignin preparations was determined based on the radical-scavenging capability. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was used as the radical generator. The method described by Dizhbite et al. (*33*) was adopted with minor modification in this study. Specifically, 320 μL of lignin solution (0.001−5.000 mg/mL) in 90% aqueous dioxane was mixed with 1180 μL of a 6.1 × 10^{-5} mol/L DPPH methanol solution at 25 °C for 16 min. The concentrations of DPPH radicals at 0 and 16 min were monitored at 515 nm (λ_{max}) using a Lambda 45 UV/VIS spectrometer (Perkin Elmer, Wellesley, MA). The inhibition percentage (IP) of the DPPH radical was calculated using eq 2.

$$\text{IP}(\%) = \frac{\text{absorbance}_{t=0\text{min}} - \text{absorbance}_{t=16\text{min}}}{\text{absorbance}_{t=0\text{min}}} \times 100 \quad (2)$$

The inhibition percentage was plotted as a function of the lignin concentration. From the graph the lignin concentration needed to obtain 50% IP was determined and defined as EC_{50}. The radical scavenging activity of the lignin was characterized using the term of radical scavenging index (RSI), which was defined as the inverse of EC_{50}. According to the definitions, higher antioxidant activity results in lower value of EC_{50} and higher value of RSI. The antioxidant activities of α-tocopherol (vitamin E) and 3,5-di-*tert*-butyl-4-hydroxytoluene (BHT) were determined using the same method as the references.

Statistical Analysis. A Statistical Analysis System (SAS, V9.0 for Windows, SAS Institute Inc., Cary, NC) was used to carry out the statistical computations of experimental data, including ANOVA, preparation of prediction profiler and surface plot, and prediction equations. Linear regression was conducted using Microsoft Excel 2002.

RESULTS AND DISCUSSION

Yield of Organosolv Ethanol Lignin. The effect of processing conditions on the yield of organosolv ethanol lignin from hybrid poplar is demonstrated in **Figure 1**. Within the temperature range investigated, 155−205 °C, lignin yield increased with increasing temperature and reached a maximum yield of 20.9%, based on wood, at 195 °C. High-temperature promoted delignification and thus enhanced the dissolution of lignin. However, further increase in temperature beyond 195 °C resulted in a decrease of lignin yield (**Figure 1A**). An explanation is that the excessive depolymerization caused by the high temperature reduces the recovery of lignin. This will be explained further below when the effect of time and catalyst on lignin yield is discussed. Ethanol concentration showed a similar effect on lignin yield, and a maximum lignin yield was observed at ∼65% ethanol. As delignification is a combination of lignin degradation and dissolution of degraded lignin fragments, ethanol concentration can have a dramatic impact on the delignification. Low ethanol concentration, which generates higher hydrogen ion concentration (lower pH value) at the same dosage of H_2SO_4, promotes acid-catalyzed cleavage of α- and β-ether linkages in lignin (*34*, *35*), while a minimum ethanol concentration is needed to reach a good lignin solubility (*36*, *37*). The reduced lignin yield at high ethanol concentration (75%) might be due to the depressed delignification at lower hydrogen ion concentration and the poor solubility of the lignin. Ni and Hu (*37*) reported that lignin solubility in ethanol−water mixture increased as the ethanol concentration increased and reached a maximum at an ethanol concentration of ∼70%. Further increase in the ethanol concentration resulted in a slight decrease in the lignin solubility. Extending the reaction time and increasing catalyst (sulfuric acid) concentration enhanced lignin yield (**Figure 1B**). Interestingly, when both the reaction time and catalyst were raised simultaneously, the lignin yield tended to decrease and a saddle was formed on the response surface (**Figure 1B**). An explanation is that the excessive degradation of lignin at high catalyst concentration and long reaction time resulted in the formation of small fragments of the lignin. The fragments were highly soluble in diluted ethanol and unable to be recovered by the precipitation operation, leading to low lignin yield.

Effect of Process Conditions on Lignin Structure. Figure 2 shows the effect of processing conditions on the contents of phenolic (**Figure 2A**) and aliphatic (**Figure 2B**) hydroxyl groups (ArOH and AlkOH, respectively) in the organosolv ethanol lignins. High temperature, long reaction time, and more catalyst led to more ArOH due to enhanced cleavage of α- and β-ether linkages between lignin structural units, which resulted in the formation of new ArOH. A similar dependence of ArOH on temperature and time was reported by Gilarranz et al. (*38*) during autocatalyzed methanol pulping of *Eucalyptus globulus* wood. As expected, low ethanol concentration generated more ArOH since low ethanol concentration, suggesting high hydrogen ion concentration, promoted acid-catalyzed cleavage of α- and β-ether linkages in lignin (*34*, *35*). This observation is consistent with the previous report regarding the effect of methanol concentration on ArOH content of organosolv lignin (*38*). Interestingly, all the process conditions affected the AlkOH in an opposite manner, compared to the ArOH. It was proposed that the cleavage of β-ether linkages during acid-catalyzed organosolv delignification is accompanied by the loss of terminal methylol groups on lignin side chain. It is the loss of the γ-methylol group as formaldehyde that forms the enol ether structure, subsequently resulting in the breakage of the β-ether (*34*). This mechanism was supported by the observation of a substantial increase of ArOH and loss of AlkOH during delignification (*34*). Since the breakage of β-ether linkages, leading to the formation of new phenolic hydroxyl groups, costs aliphatic hydroxyls, the process conditions generating more ArOH resulted in less AlkOH.

Figure 3 shows the effect of process conditions on the molecular weight (M_n and M_w, **Figure 3A,B**) and polydis-

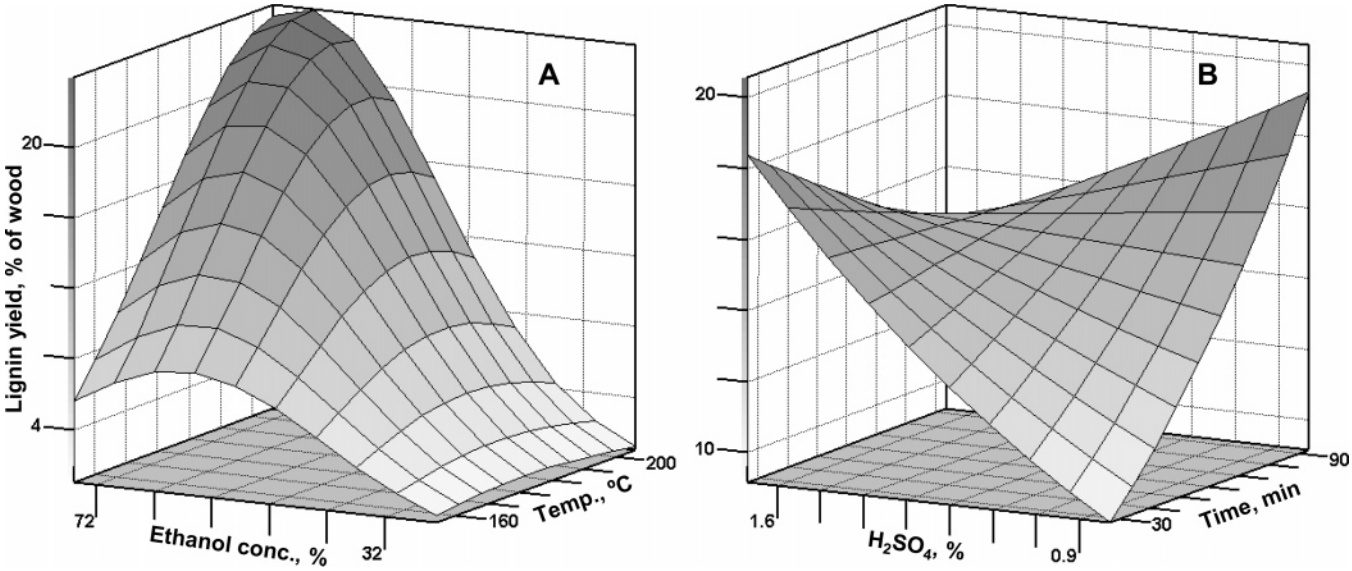


Figure 1. Effect of processing conditions on the yield of organosolv ethanol lignin (% of wood).

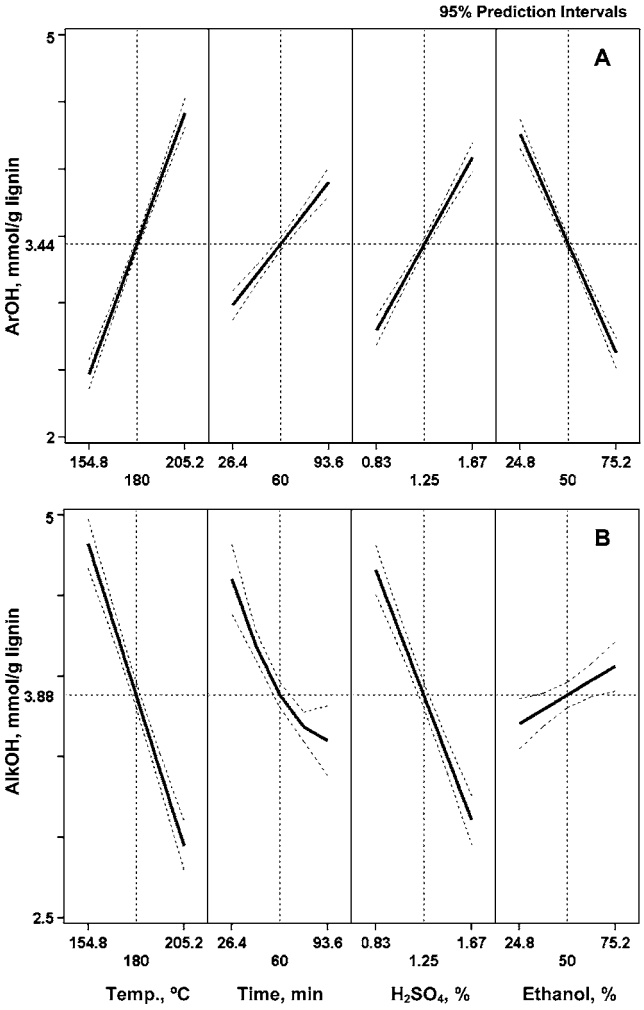


Figure 2. Effect of processing conditions on functional groups of organosolv ethanol lignin.

persity (M_w/M_n, **Figure 3C**) of the organosolv ethanol lignins. In general, increasing temperature and catalyst dose decreased molecular weight as an increase in the severity of the processing conditions leads to more extensive depolymerization (cleavage of ether linkages) of the lignin. The polydispersity of the lignin also became narrower with increased temperature and catalyst. Similar observations of the changes in lignin molecular weight and polydispersity during organosolv pulping were reported by others (*38*, *39*). Reaction time showed a slight effect on M_n but no effect on M_w or M_w/M_n, implying that the reaction time is not a significant factor affecting molecular weight. Since the fraction of low molecular weight has more significant influence on M_n than on M_w of a polymer, the effect of low molecular weight fragments of lignin formed from the extended reaction time was reflected only on M_n (**Figure 3A**) but not on M_w (**Figure 3B**). Ethanol concentration had a substantial effect on molecular weight of the lignin. Both molecular weight and polydispersity increased linearly with increasing ethanol concentration. As discussed above, low ethanol concentration promotes the cleavage of ether linkages between lignin units, consequently resulting in the depolymerization of the lignin. Typical organosolv ethanol lignins extracted at the center point conditions (EL 17−21) had a low molecular weight (M_w, 2000−2300) and narrow polydispersity (1.8−2.0) (**Table 1**), relative to other lignin types (*40*, *41*), consistent with results previously reported for Alcell organosolv lignin (*12*) and organosolv methanol lignins (*38*).

Relationship between Lignin Structural Features and Antioxidant Activity. Research into lignin model compounds (*24*, *33*, *42*) indicates that free phenolic hydroxyl groups are essential for antioxidant activity. **Scheme 1** demonstrates the trapping and stabilization of radicals by lignin, proposed by Barclay et al. (*24*). The radical scavenging ability of phenolic compounds depends not only on the ability to form a phenoxyl radical (i.e., hydrogen atom abstraction) but also on the stability of the phenoxyl radical. Phenolic structures with substituents that can stabilize the phenoxyl radicals have higher antioxidant activity than those that do not. For example, ortho substituents such as methoxyl groups stabilize phenoxyl radicals by resonance as well as hindering them from propagation. Conjugated double bonds can provide additional stabilization of the phenoxyl radicals through extended delocalization. However, a conjugated carbonyl group has a negative effect on antioxidant activity.

The radical scavenging index (RSI) values of the organosolv ethanol lignin preparations, as well as those of BHT and vitamin E, are summarized in **Table 1**. BHT and vitamin E are commonly used antioxidants. It can be found that the ethanol lignins had much higher RSI than BHT but significantly lower

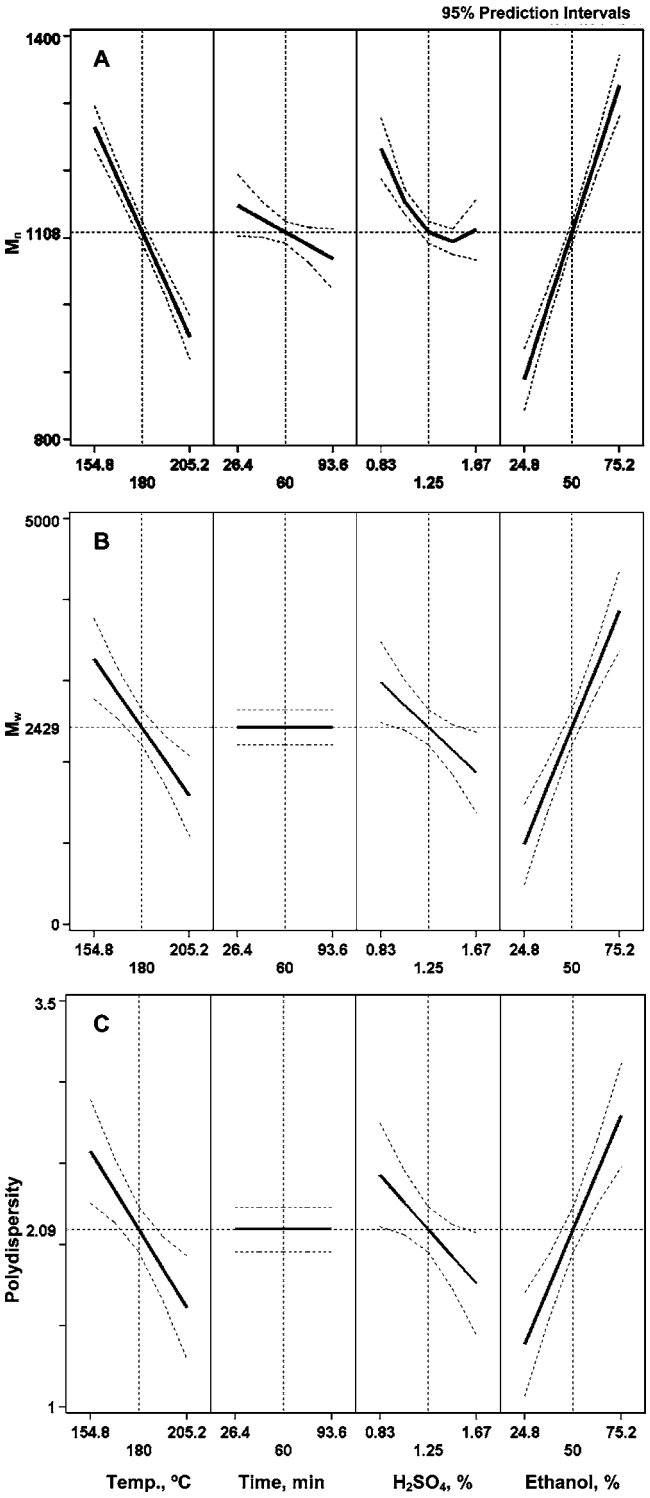
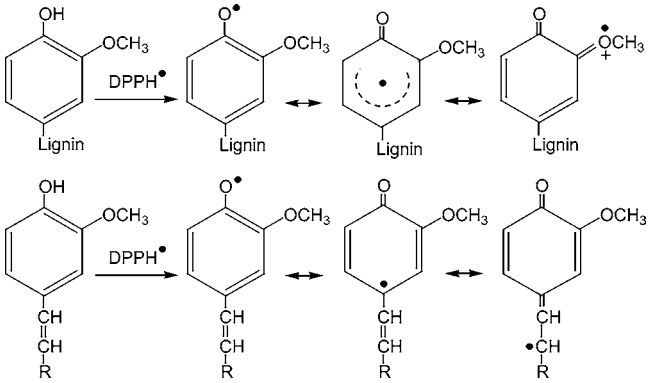


Figure 3. Effect of processing conditions on molecular weight and polydispersity of organosolv ethanol lignin.

RSI than vitamin E. When the radical scavenging index of the organosolv ethanol lignin samples was plotted against functional groups (**Figure 4**), it can be seen that RSI is positively correlated to phenolic hydroxyl group (ArOH) content (**Figure 4A**), consistent with the studies of lignin model compounds (*24*, *33*, *42*). Conversely, aliphatic hydroxyl group (AlkOH) content has a negative effect on antioxidant activity of the lignin (**Figure 4B**). The effect of AlkOH on radical scavenging activity of lignin is not very clear. Dizhbite et al. (*33*) reported that the AlkOH had positive influence on radical scavenging activity of lignin model compounds, but they did not observe the same

Scheme 1. Trapping and Stabilization of Radicals by Lignin



correlation on real lignins. Actually, some lignin samples they assessed had higher AlkOH content but lower radical scavenging activity. As no demethoxylation occurred during the organosolv ethanol pulping, there was no significant difference in methoxyl content among the 21 lignin preparations. The small variance in methoxyl group content did not show significant effect on lignin RSI, as shown in **Figure 4C**, although it was reported that methoxyl groups have a positive effect on lignin antioxidant activity (*24*, *33*).

Figure 5 shows the effect of molecular weight and polydispersity of the lignins on RSI. It is clear that the lignin preparations with low molecular weight had high antioxidant activity (**Figure 5A,B**). Meanwhile, it seems that the antioxidant activity benefits from the narrow distribution of the lignin molecular weight (**Figure 5C**). Similar observation was reported by Dizhbite et al. (*33*) that high molecular weight, enhancing heterogeneity, and polydispersity are factors decreasing the radical scavenging activity. Low molecular weight resulted from extensive depolymerization of lignin, i.e., cleavage of ether linkages, which led to the formation of new ArOH, the center to trap radicals. In other words, the low molecular weight fraction of the lignin possessed more ArOH than the high molecular weight fraction. This is the explanation for the high antioxidant activity of the lignin with low molecular weight.

Predictive Models of Lignin Yield and Characteristics. In the previous sections, the effect of process variables on the lignin yield, antioxidant activity, and lignin structural features was discussed in a qualitative way. To describe and predict the effect precisely and quantitatively, each of the lignin features was fitted using a second-order polynomial model of eq 3 using SAS software

$$Y = a_0 + \sum_{i=1}^{k} a_i X_i + \sum_{i=1}^{k} a_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=1}^{k} a_{ij} X_i X_j \qquad (3)$$

where Y is the estimate for a response, such as lignin yield, ArOH content, and RSI of the organosolv ethanol lignin; k is the total number of independent variables ($k = 4$ in the present study); X_i values are the independent variables (temperature, time, catalyst dosage, and ethanol concentration); X_i, X_i^2, and X_iX_j terms in the equation account for the linear, quadratic, and two-variable interaction effects, respectively, of the variables; a_0 is a constant; a_i, a_{ii}, and a_{ij} are linear, quadratic, and interaction coefficients, respectively.

The ANOVA (analysis of variance) was applied to the regression of the experimental results in **Table 1**. Based on the F (Fisher) distribution values, the factors with a significant level (Pr > F) (Pr stands for probability) lower than 5% were neglected for models regression. The regression models estab-

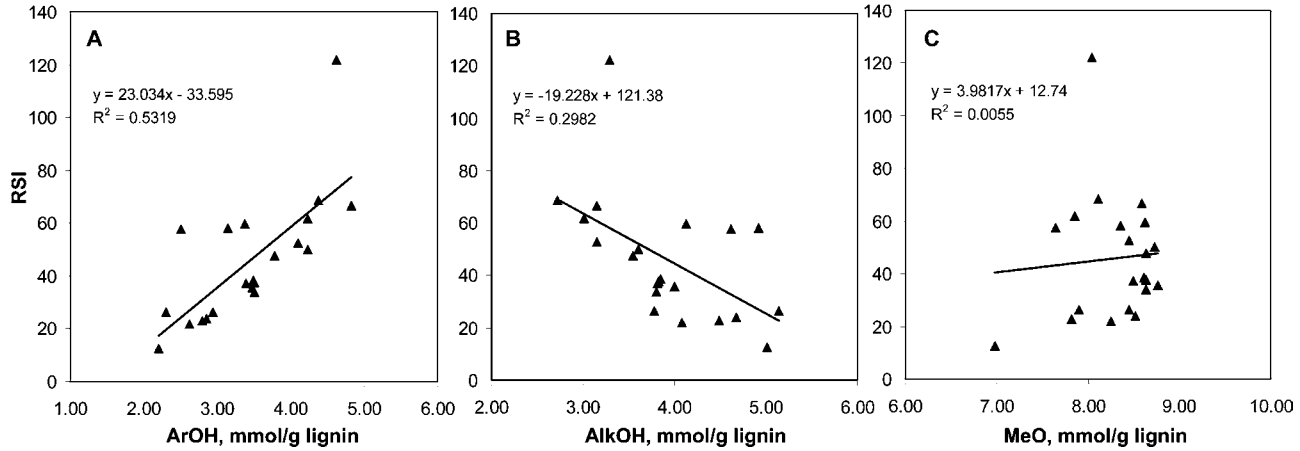


Figure 4. Correlation between functional groups and antioxidant activity of organosolv ethanol lignin (RSI, radical scavenging index).

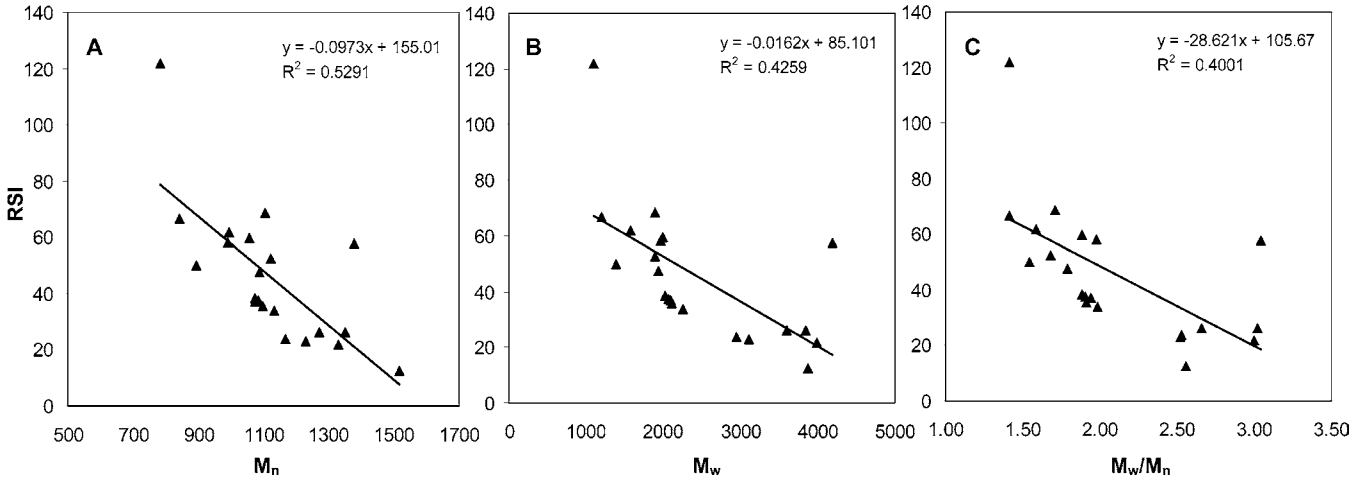


Figure 5. Correlation between molecular weight and antioxidant activity of organosolv ethanol lignin (RSI, radical scavenging index).

Table 2. Predictive Models of Lignin Yield and Lignin Characteristics[a]

response	equation	R^2	no.
yield (% of wood) =	$-272.116 + 2.569489T + 51.82181S - 0.153372C - 0.007105T^2 + 0.000313Tt - 0.243333TS + 0.008278TC - 0.010175C^2$	0.984	(4)
ArOH (mmol/g) =	$3.002901 - 0.00373T - 0.075635t - 5.125643S + 0.088568C + 0.000496Tt + 0.037TS - 0.000672TC$	0.989	(5)
AlkOH (mmol/g) =	$14.08571 - 0.037021T + 0.06035t - 1.847209S - 0.109884C - 0.000546Tt + 0.00065TC + 0.000192t^2$	0.975	(6)
MeO (mmol/g) =	$-44.2105 + 0.470447T + 0.113128t + 14.23279S - 0.084903C - 0.001187T^2 - 0.000529Tt - 0.024333TS + 0.000372TC - 0.031679tS + 0.000531tC - 2.88559S^2 - 0.000235C^2$	0.978	(7)
M_n =	$3030.446 - 6.193715T - 21.0625t - 1387.821S + 24.04762C + 15.89762tS + 362.5051S^2 - 12.31746SC$	0.984	(8)
M_w =	$7243.245 - 33.4461T - 1318.539S + 57.08548C$	0.802	(9)
M_w/M_n =	$5.133231 - 0.019115T - 0.797725S + 0.027961C$	0.728	(10)
RSI =	$-145.745 + 1.063848T$	0.303	(11)

[a] T = maximum temperature, °C; t = reaction time at the maximum temperature, min; S = sulfuric acid, % on oven dry wood chip (w/w); C = concentration of aqueous ethanol, % (v/v).

lished for each response are listed in **Table 2** as eqs 4−11. These equations can be used to predict lignin features within the investigated range of processing conditions. The reliability of the equations was assessed by comparing the experimental values of the responses at the center point conditions, average of five independent experiments (EL 17−21), with the calculated values using eqs 4−11 at the same process conditions. The results (not shown here) indicated that most of the predicted values agree well with the observed values except for RSI. As shown in **Table 2**, the prediction model for RSI (eq 11) contains only one process parameter, temperature. That means the temperature is the only significant factor affecting antioxidant activity of the lignin. However, the correlation coefficient (R^2) of the equation is only 0.3, implying relatively poor correlation. As discussed above, antioxidant activity of lignin is influenced by multiple factors (lignin features, e.g., functional groups and molecular weight) that are dependent on the extraction conditions of the lignin. In other words, the antioxidant activity of the lignin is indirectly correlated to the extraction conditions in eq 11. This might be the reason the equation has a low correlation coefficient.

In conclusion, the processing conditions used for the extraction of the organosolv ethanol lignins influence the structural features (functional groups and molecular weight) of the isolated lignins, and consequently the antioxidant activity of the lignins. In general, the lignin prepared at elevated temperature, extended reaction time, increased catalyst, and diluted ethanol shows high antioxidant activity due to more phenolic hydroxyl groups, low

molecular weight, and narrow polydispersity of the lignin. The antioxidant activity and structural features of the lignin are predictable using the regression models developed.

In a real biorefining process, the optimal conditions for the recovery of cellulose, hemicellulose, and lignin may not be the most favorable ones to antioxidant activity of the lignin. A fine-tuning of the process conditions is necessary to balance the recovery and quality of lignin with those of other wood components. The cellulose recovery and lignin yield were maximized for hybrid poplar at the fine-tuned processing conditions (190 °C, 70 min, 1.4% H_2SO_4, and 60% ethanol), as reported previously (*30*). Calculations using eqs 4 and 11 at this set of conditions give the yield and RSI of the lignin, 20.1% and 56.4, respectively. Compared with the results in **Table 1**, it seems that this set of the conditions gives a good compromise between yield and antioxidant activity.

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