Optimization of Experimental Variables to Modify Lignin from *Eucalyptus globulus* under Alkaline Catalysis

Marcela Norambuena,[a,]* Claudia Vidal,[a,b] Lissette Carrasco,[a,b] Pablo Reyes,[a]
Carolina Parra,[a] David Contreras,[a,c] and Regis Teixeira Mendonça [a,b]

Conditions to increase phenolic hydroxyl groups (OH-Phe) in organosolv lignin using alkaline catalysts (NaOH and KOH) were optimized with the purpose of increasing the reactivity of lignin and to evaluate the effect on the structure of the newly generated compounds as a result of the base-catalyzed depolymerization (BCD). The lignin-derived compounds could be used for the synthesis of bio-based polymers and nanomaterials. The maximal yield of OH-Phe reached values of 5270 and 3970 µmol per gram of lignin when NaOH (8.6%, 173 °C, and 33 min) and KOH (11.7%, 174 °C, and 58 min), defined as optimal conditions for BCD, were used, respectively, while for the control lignin, OH-Phe was 2830 µmol per gram of lignin. The molecular weight (M_w) and glass transition temperature (T_g) for lignin-NaOH were lower than those of lignin-KOH and lignin-control. Both lignin derivatives had greater thermal stability at high temperatures than lignin-control. In conclusion, the results showed higher modifications in the structure of lignin as a result of the oxidation process, primarily by cleavage of the β-O-4 ether bond and variations in thermochemical properties when NaOH is used as alkaline catalyst.

Keywords: Lignin; Alkaline catalysis; Experimental design; E. globulus

*Contact information: a: Laboratorio Recursos Renovables, Centro de Biotecnología, Universidad de Concepción, Casilla 160-C, Chile; b: Facultad de Ciencias Forestales, Universidad de Concepción, Casilla 160-C, Chile; c: Facultad de Ciencias Químicas, Universidad de Concepción, Casilla 160-C, Chile; *Corresponding author: mnorambu@udec.cl*

INTRODUCTION

The use of renewable lignocellulosic biomass (LCB) has become a growing area of research in recent decades, and today lignocellulosic materials have gained a special importance in product development because of their renewable nature (Asgher *et al.* 2013) and the possibility to develop new bio-based polymers and nanomaterials by replacing traditional synthetic materials. LCB has tremendous potential to reduce energy consumption/pollution, and presents the advantages of being eco-friendly, low cost, biodegradable, as well as its ease of processing and its impressive physico-mechanical properties (Thakur and Thakur 2014; 2015).

Among various biorenewable polymers, lignin has been considered a raw material with high potential because of its abundance of functional groups and reactive sites, which offer possibilities for chemical and enzymatic modifications. Lignin could play a central role as a new chemical feedstock in a biorefinery concept (Laurichesse and Avérous 2014) and can be used as a green alternative to many petroleum-derived products, such as oil-based resins, rubber additives, thermoplastic (Kaewtatip and Thongmee 2013), carbon fibers (Kadla *et al.* 2002; Eckert and Abdullah 2010), green hydrogels (Thakur and Thakur 2015), and activated carbon (Ragan and Megonnel 2011), as well as, for generation of vanillin, vanillic acid, dispersing agents, polymer filters, DMSO, phenol, and ethylene, *etc*. (Lora and Glasser 2002; Borges da Silva *et al.* 2009). The use of lignin as a wood adhesive has also been evaluated in several studies (El Mansouri *et al.* 2007a,b). It has been suggested that the high content of phenolic hydroxyl groups in organosolv lignin from softwoods could be a potential source for the production of phenolic, epoxy, and isocyanate resins (Sarkar and Adhikari 2000).

Lignin is an amorphous and three-dimensional polymer formed by the polymerization of three different cinnamyl alcohols (*p*-cumarilic alcohol, coniferyl alcohol, and sinapyl alcohol), which differ in their degree of methoxylation at positions 3- and 5- of the aromatic ring. Composition differs between softwood, hardwood, and herbaceous plants. In softwoods, lignin is composed of 95% guaiacyl (G) units, while in hardwoods, mostly syringyl (S) and guaiacyl units are found. In herbaceous plants and agricultural residues, *p*-hydroxyphenyl (H), guaiacyl, and syringyl units are present in different proportions (Sjostrom 1993). As is known, lignin macromolecules have multiple functional groups influencing the reactivity of lignin, such as methoxyl, phenolic and aliphatic hydroxyl, benzyl alcohol, noncyclic benzyl ether, and carbonyl groups (Pandey and Kim 2011). The use of lignin as a substrate for chemical functionalization demands a precise knowledge of its content in functional groups (Duval and Lawoko 2014) and could require some form of depolymerization or disintegration to generate smaller fragments or compounds more amenable to subsequent processing.

The controlled depolymerization of lignin is one of the major obstacles to generating new chemicals and composites, and it has become a main focus of current research. The use of unmodified lignin presents significant limitations that hinder its potential applications, and an increase in lignin reactivity is essential. The use of lignin for conversion into value-added chemicals requires a fine tuning of the depolymerization process to generate low molecular weight compounds that retain their oxyaromatic natures (Vigneault *et al.* 2007). Furthermore, chemical modifications based on structural features, such as the presence of free ortho positions on the phenolic ring, can also add reactive sites on C_3 positions, and the presence of multiple OH groups (aliphatic or phenolic) are strategies applied to modify lignin reactivity (Duval and Lawoko 2014). Several reviews have analyzed and presented research on lignin and on processes aiming at its valorization (Sannigrahi *et al.* 2010). These methods have ranged from classical chemical approaches such as pyrolysis (thermolysis) (Mohan *et al.* 2006; Pandey *et al.* 2011; Brebu and Vasile 2010) to hydrolysis (Sun and Cheng 2002), reduction (hydrogenolysis) (Eachus and Dence 2009; Son and Toste 2010), oxidation (Crestini *et al.* 2010), base-catalyzed depolymerization (BCD) reactions (Toledano *et al.* 2014), and newer biotechnological approaches (Van Dyk and Pletschke 2012).

The controlled cleavage of carbon–carbon and carbon–oxygen bonds in lignin represents a very selective depolymerization, which could produce a whole series of monomeric aromatic species (Lange *et al.* 2013). Technologies relying on selective bond cleavages in lignin also have the potential to yield new types of building blocks for block-polymers (Sannigrahi *et al.* 2010). Thus, selective modifications of lignin can suitably transform it into a structural base for complex copolymers with various potential applications (Toledano *et al.* 2012). Researchers have studied the effect of different base catalysts in the depolymerization of organosolv lignin from olive tree pruning at 300 °C and 4% (wt) of catalyst concentration, isolating the phenolic monomer compounds of the liquid fraction after the extraction with an organic solvent, and the main products generated were phenol, *o*-cresol, *m*-cresol, *p*-cresol, catechol, and 4-methylcatechol (Toledano *et al.* 2012).

The aim of this study was to examine the effect of alkaline catalysts on lignin depolymerization and on the structure of newly generated compounds, with the goal of optimizing conditions to increase phenolic hydroxyl groups in organosolv lignin. Organosolv lignin from *Eucalyptus globulus* was used as a starting raw material because of its low extent of condensation reactions in organic solvents and its structure, which is more similar to native lignin than that produced by other industrial processes. As alkaline catalysts, NaOH and KOH were used under various time, temperature, and concentration conditions. The conditions were optimized using chemometric tools, such as a central composite circumscribed design and quadratic and cubic models. Finally, the phenolic compounds obtained from the reaction at optimal conditions, defined by the chemometrics model, were characterized, and different analyses, such as size exclusion chromatography (SEC), nuclear magnetic resonance (^{31}P-NMR), differential scanning calorimetry (DSC), and thermal gravimetric analysis (TGA), were performed.

EXPERIMENTAL

Organosolv Lignin

Lignin was recovered from black liquor derived from organosolv cooking, which was carried out in a batch reactor (4550 Parr Instrument Company, Illinois, USA). The pulping conditions used were as follows: 2 kg of wood chips (3.0 × 3.0 cm) from *E. globulus* provided by a Chilean forest company, an ethanol to water ratio of 50:50, a temperature of 200 °C for 45 min, and an H-factor between 5000 and 7000. Organosolv lignin was recovered from ethanolic black liquor by precipitation with a liquor to water ratio of 1:3 and acidification with H_2SO_4 (3%) to a pH of 2 (Tejado *et al.* 2007). Afterward, the suspension was stored overnight at 4 °C, filtered, and washed with water until it achieved a neutral pH. The lignin was dried at 40 °C for 24 h and stored under dry conditions.

Alkaline Depolymerization of Organosolv Lignin

The methodology used to study the influence of different variables on depolymerization was a factorial design. The variables and ranges studied in the process were temperature (*T*: 150 to 170 °C), time (*t*: 40 to 60 min), and concentration of the alkaline catalyst (*C*: 5 to 10% w/w). The model was defined as a factorial design central composite with star points. The variables were coded and normalized with unit values of −1 (defined as the lowest value) and +1 (defined as the highest value). The focal point was coded as 0, and six star points were defined at a distance of 1.68 from the central point. Seventeen experiments were performed according to a circumscribed central composite model that included eight experiments of factorial design (codified as levels −1 and +1), six star points (codified as levels −1.68 and +1.68), and three central points (codified as level 0). The conditions defined for each experiment are described in Table 1. The experimental design was optimized to a response variable, defined as the free phenolic hydroxyl groups determined by phosphorous nuclear magnetic resonance (^{31}P-NMR), and the influence of each variable was determined using response surface methodology (RSM). Modde software (Umetrics, Sweden) was used for data processing.

Model equations included the first-order term to describe the main effects and the second order for interactions. An analysis of variance for experimental results was employed, and non significant effects were excluded from the model regression. Contour diagrams (two-dimensional response surfaces) from the model were employed to define the optimal conditions for lignin depolymerization. Finally, alkaline liquor was obtained as a product of the modification.

Table 1. Experimental Design used for Organosolv Lignin Depolymerization under Alkaline Catalysis

Experiment	XT	Xt	XC	T (°C)	T (min)	C (% w/w)
N-1	-1	-1	-1	150	40	5
N-2	+1	-1	-1	173	40	5
N-3	-1	-1	+1	150	40	10
N-4	+1	-1	+1	173	40	10
N-5	-1	+1	-1	150	60	5
N-6	+1	+1	-1	173	60	5
N-7	-1	+1	+1	150	60	10
N-8	+1	+1	+1	173	60	10
N-9	-1.68	0	0	142	50	7.5
N-10	+1.68	0	0	181	50	7.5
N-11	0	0	-1.68	161.5	50	3.3
N-12	0	0	+1.68	161.5	50	11.7
N-13	0	-1.68	0	161.5	33.2	7.5
N-14	0	+1.68	0	161.5	66.8	7.5
N-15	0	0	0	161.5	50	7.5
N-16	0	0	0	161.5	50	7.5
N-17	0	0	0	161.5	50	7.5

Analysis of Hydroxyl Groups *via* ^{31}P-NMR

Free phenolic hydroxyl groups *via* ^{31}P-NMR of the 17 lignin derivative samples modified were determined after acidic precipitation of the alkaline liquor. These were recovered from the liquor *via* acidic precipitation with hydrochloric acid until the pH equaled 2. The quantitative ^{31}P-NMR spectra of the lignin samples were obtained using published procedures (Granata and Argyropoulos 1995). Approximately 40 mg of dry lignin was placed into a sample vial, dissolved in 400 μL of pyridine and deuterated chloroform (1.6:1, v/v), and left at room temperature with continuous stirring. *N*-hydroxynaphtalimide (200 μL, 11.4 mg per mL^{-1}) and chromium (III) acetylacetonate (50 μL, 11.4 mg per mL^{-1}) were used as internal standards and relaxation reagents, respectively. Finally, 100 μL of 2-chloro-1,3,2-dioxaphospholane or 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane were added, and the mixture was transferred into a 5-mm-OD NMR tube. The spectra were acquired using a Bruker 400 MHz spectrometer (Bruker Company, Germany) equipped with a Quad probe dedicated to ^{31}P, and the amounts of the various hydroxyl groups (phenolic, aliphatic, and carboxyl) were calculated.

Molecular Weight Distribution

The molecular weight distribution of acetylated lignin was performed *via* size exclusion chromatography (SEC). The lignin samples were previously subjected to acetylation to increase their solubility in organic solvents (Tetrahydrofuran, Merck Millipore Corporation, Germany). Twenty milligram of each lignin sample was added to a solution of AcBr:AcOH (5.0 mL, 8:92 v/v), followed by stirring for 2 h at 50 °C. The solvent evaporated, and the resulting residue was immediately dissolved in THF (10 mL) (Guerra *et al.* 2006). Aliquots (10 µL) of each sample dissolved in 10 mL of THF were injected. The analyses were performed on a size exclusion chromatography system (Waters Corporation, USA) equipped with HR5, HR3, and HR1 columns (Waters Corporation, USA) and a UV detector set at 280 nm. Analyses were carried out at 40 °C using THF as an eluent at a flow rate of 0.60 mL/min. The SEC system was calibrated with polystyrene standards in the molecular weight range of 790 to 1.86×10^4 g/mol, and EMPOWER GPC software (Waters Corporation, USA) was used for data processing.

Differential Scanning Calorimetry (DSC) Analysis

Differential scanning calorimetry (DSC) was used to determine the glass transition temperature (T_g). The T_g of each lignin sample was determined using a DSC 204F1 (NETZSCH, Germany). Before analysis, the lignin samples were dried under a vacuum at 40 °C, and approximately 5.5 mg samples were weighed and encapsulated in a height to volume stainless steel pan. The samples were heated from 10 to 300 °C at 10 °C per min^{-1}, and the thermogram obtained was used to determine T_g. The T_g was defined as the midpoint of the temperature range at which the change in heat capacity occurred.

Thermogravimetric Analysis (TGA)

Thermogravimetric analysis were carried out in a TG 209F1 (NETZSCH, Germany) apparatus to determine the mass loss of samples as a function of temperature. The lignin samples, approximately 8.0 mg, were placed in appropriate pans and heated at a heating flow rate of 10 °C per min^{-1} from 30 to 550 °C under a nitrogen atmosphere. The TGA data were used to characterize the thermal decomposition of the samples.

RESULTS AND DISCUSSION

Influence of Factors on Free Hydroxyl Phenolic Group Generation in Alkaline Depolymerization of Lignin

The aim of this study is to evaluate the influence of three different factors and determine the optimal conditions for increasing free phenolic hydroxyl groups (OH-Phe) in organosolv lignin. The factorial design applied to optimize the response is described in Table 1, and the effects of catalyst concentration, temperature, and time of reaction have been assessed. The corresponding OH-Phe in modified lignin was determined by ^{31}P-NMR.

The response variable was defined by a surface model that describes the relationship between the dependent and the independent variable through regression. The corresponding polynomials for NaOH and KOH (Eqs. 1 and 2, respectively) were obtained using multiple linear regressions, where Y_{pOH} represents the independent variable (response), defined as OH-Phe in the models, and the linear coefficient, quadratic coefficient, and interaction coefficient for dependent variables X_1 (temperature), X_2 (time), and X_3 (concentration of alkaline catalyst) are described.

$$Y_{pOH(1)} = 5194.1(\pm 115.9) + 377.6[X_1](\pm 58.0) + 67.6[X_2](\pm 61.5) + 159.5[X_3](\pm 47.1) - 340.0[X_1]^2(\pm 76.4) - 572.1[X_3]^2(\pm 61.5) + 346.6[X_1X_3](\pm 61.5) - 90[X_1X_2](\pm 61.5)$$

$$Y_{pOH(2)} = 3848.0(\pm 88.5) + 88.8[X_1](\pm 36.6) - 1.9[X_2](\pm 36.6) + 94.1[X_3](\pm 36.6) - 200[X_1]^2(\pm 41.6) - 151.3[X_2]^2(\pm 41.6) - 126.6[X_3]^2(\pm 41.6) + 211.4\ [X_1X_3](\pm 50.1) + 151.4[X_2X_3](\pm 50.1)$$

When the different coefficients indicated in the equations were evaluated, all variables influenced the models, and interactions between them were also observed. The influence of temperature, time, and alkali concentration studied by analysis of variance (ANOVA) for a confidence level of 95% showed that both second-order polynomial models presented a high significance. In Table 2, the F- and P-values and correlation coefficients (R^2) for each model are described. These models were validated by ANOVA with a 95% confidence level, and the correlation coefficients (R^2) were 0.974 and 0.926 for sodium hydroxyl and potassium hydroxyl catalysts, respectively.

Table 2. Parameters of Validation by ANOVA for Both Models

Source	F-value	P-value	R^2
Model NaOH	26.909	0.001	0.974
Model KOH	9.426	0.007	0.926

Optimization of Free Hydroxyl Phenolic Group Generation by Alkaline Catalysis

In Fig. 1, the contour diagrams describing the estimated response surface for free OH-Phe for both models are shown, with the reaction time fixed at 50 min.

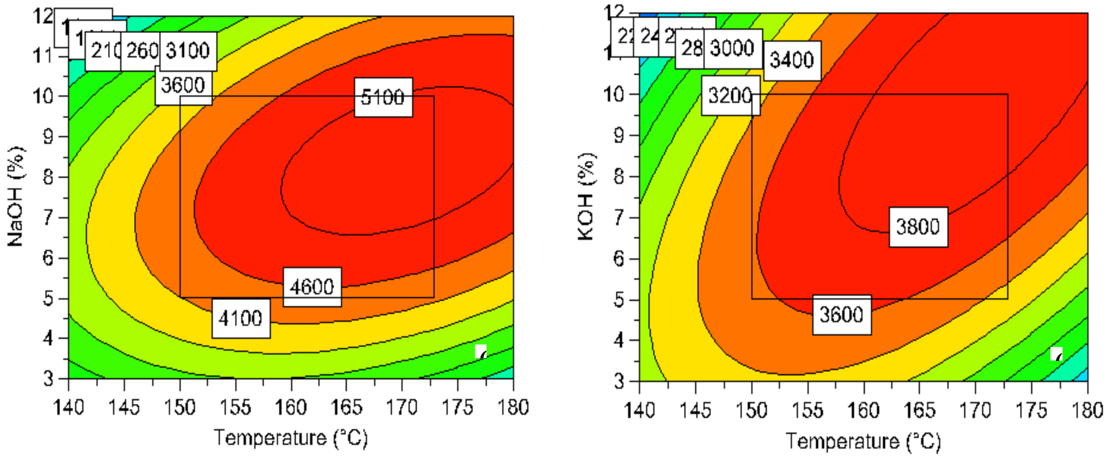

Fig. 1. Estimated response surface for free hydroxyl phenolic groups (μmol/g of lignin) obtained as a result of the optimization of the experimental design using (a) NaOH and (b) KOH as catalysts at a fixed reaction time of 50 min

The results indicated that the maximal region of OH-Phe, found close to 5100 (μmol/g of lignin), could be obtained using sodium hydroxide (Fig. 1a) as an alkaline catalyst, while with potassium hydroxide, the maximal region reached lower values of approximately 3800 μmol/g of lignin (Fig. 1b).

The optimal conditions obtained showed that at similar reaction temperatures but different concentrations and reaction times, the maximal free OH-Phe predicted by the models are different, as can be seen in Table 3, where the optimal conditions found for each variable are described. Under these optimal conditions, the maximal free OH-Phe values predicted by the models were 5383 and 4000 (μmol/g of lignin). Experimental values of 5270 and 3970 (μmol/g of lignin) were obtained when NaOH and KOH were used as catalysts, respectively (Table 3), where the optimal experimental conditions are indicated to reach the maximal free OH-Phe.

Moreover, these results showed that the increase in free OH-Phe groups in lignin derivatives was higher when NaOH is used as a catalyst in comparison to KOH at a similar temperature (173 °C) but with lower reaction time (33 min) and alkali concentration (8.6%).

Table 3. Free OH-Phe Groups Estimated and Obtained under Optimized Reaction Conditions

Variables/sample	Lignin-NaOH	Lignin-KOH
Alkali catalysts (%)	8.6	11.7
Temperature (°C)	173	174
Time (min)	33	58
Free phenolic groups predicted (μmol/g of lignin)	5383	4000
Free phenolic groups obtained (μmol/g of lignin)	5270	3970

Determination of Hydroxyl Groups in Lignin Derivatives

The determination of hydroxyl groups (aliphatic, carboxyl, and phenol) by ^{31}P-NMR was performed for the control lignin and lignin derivatives (lignin-NaOH and lignin-KOH) obtained after modification in the optimal conditions described in Table 3. The quantitative estimation of these groups was determined using cholesterol as an internal standard, and the chemical displacement was assigned as 145 ppm (Fig. 2). The integrated shift ranges (ppm) for each zone, indicated in Table 4, were reported according to Wu and Argyropoulos (2003).

The results shown in Fig. 2 and Table 4 indicate that an increase in the OH-Phe groups is primarily associated with an increase in OH-syringyl, condensed phenolic OH, and OH-guaiacyl when compared to the amount of hydroxyl groups obtained under alkaline catalyst modifications of organosolv lignin.

The lignin-control and lignin-KOH show a total of 2830 and 3970 (μmol per gram of lignin) of OH-Phe groups, while the lignin-NaOH presents a much higher value of 5270 μmol per gram of lignin. The higher value obtained when NaOH was used as a catalyst can be explain by the different linkages in each sample of lignin, where the β-O-4 ether bond is the most common linkage in lignin from *E. globulus* and is primarily associated with syringyl units.

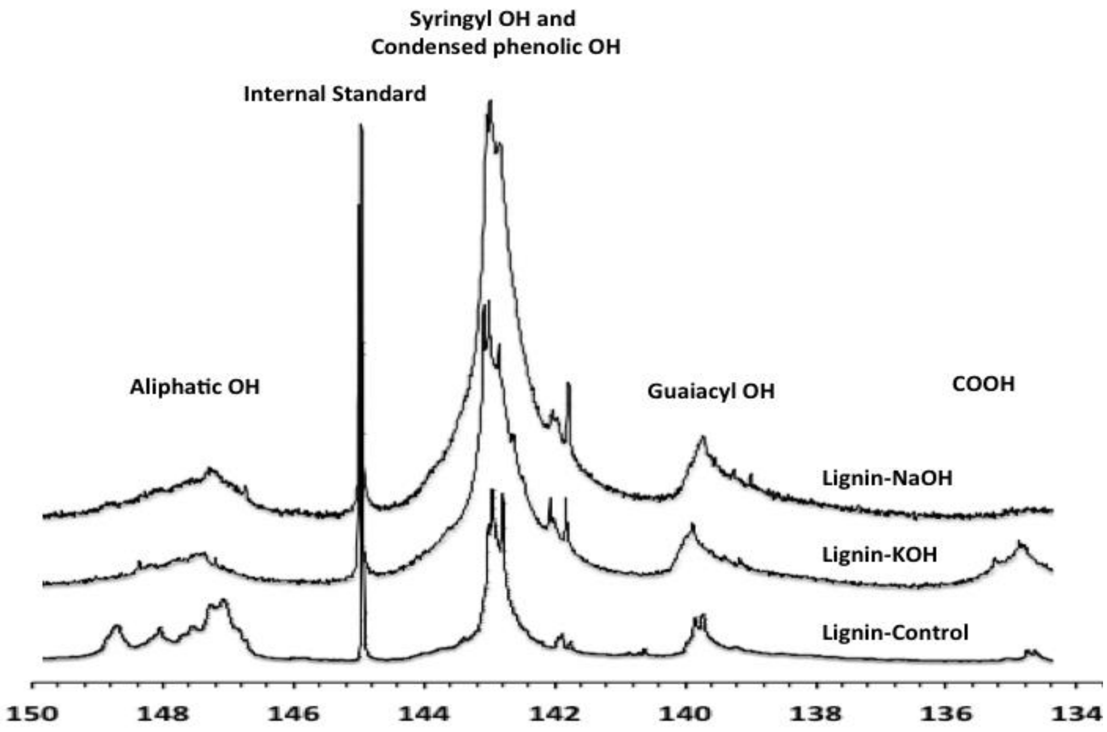

Fig. 2. ^{31}P-NMR spectra of control lignin and lignin derivatives

Table 4. Results of Hydroxyl Groups in Control and Modified Lignin Determined by ^{31}P-NMR

OH (μmol/g)						
	Aliphatic	Condensed and syringyl OH	Guaiacyl-OH	Carboxyl-OH	Total phenolic	Total OH
Integrated shift range (ppm)	150–146.5	144.4–141.0	140.4–139	135.6–134.2		
Lignin-control	1820	2130	700	90	2830	4740
Lignin-NaOH	540	3960	1310	120	5270	5930
Lignin-KOH	820	3280	690	170	3970	4960

The mechanism described by Zhao and Lercher (2013) suggests that during the BCD of lignin using NaOH as a catalyst, cleavage of the β-O-4 ether bond takes place heterolytically *via* cleavage of the polarized ether bond (A) with formation of a sodium phenolate derivative (B) and a carbenium (C) ion-like transition state, which is instantly neutralized by a hydroxide ion (Fig. 3). The cations catalyzed the reaction by forming adducts with lignin, thus polarizing the ether bond and increasing the negative partial charge of the oxygen.

As has been described, BCD generates the oxidation of lignin, primarily *via* cleavage of the most common bond in lignin, to produce phenolic compounds such as aldehyde, acid, and alcohols (Toledano *et al.* 2014) or polymers with low molecular weights.

BCD could also explain an increase in OH phenolic of syringyl type, preferably after treatment with both alkaline catalysts, because the mechanism takes place *via* carbenium ions, meaning syringylic carbenium provides a more stable transition state for carbenium ions than does guaiacylic carbenium, as it has more methoxy substitutes to disperse the positive charge (Erdocia *et al.* 2014; Toledano *et al*. 2014). Also, the results showed that the lignin-NaOH increase mainly in the OH-Phe guaiacyl type may be due to repolymerization occurring during alkaline reactions favoring C-C bonds in the G-type units available on the C5 position of the aromatic ring or because the dissociation of KOH was not very clear. As a result, the phenolate derivative and the carbenium ion were formed in a smaller proportion than when NaOH was used as a catalyst, indicating that basicity is one of the important factors to be considered for lignin depolymerization, as has been described by Toledano *et al.* (2012). Also, variations in aliphatic and carboxyl hydroxyl groups were also observed, indicating that different changes in the structure of lignin occur as a result of alkaline treatment, as described by Zhao and Lercher (2013).

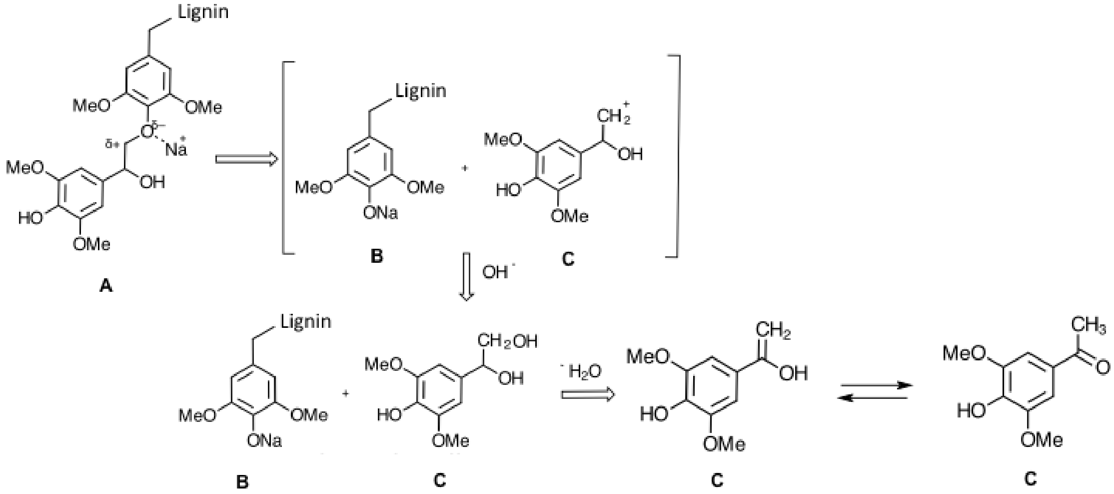

Fig. 3. Mechanism of the cleavage β-O-4 ether bond and formation of derivatives

Effect of Alkaline Depolymerization on the Molar Mass of Organosolv Derivative Lignin

An important feature for the development of lignin-based products is the molecular weight and polydispersity of lignin derivatives. Most research has supported the use of different fractions of lignin for the synthesis of several products (Tolbert *et al.* 2014). Thus, a lower molecular weight is suitable for condensates with phenol formaldehyde because they are more reactive than those with high molecular weights (Pizzi 1994; El Mansouri and Salvado 2006).

On the other hand, a high molecular weight offers improved performance in materials' mechanical properties through molecular entanglement and enhanced cohesive strength of the matrix, and it can be used for value-added lignin-based applications such as thermoplastic blends or copolymers (Saito *et al.* 2012). Thus, the determination of these characteristics in lignin is a useful tool to estimate chain lengths for future applications.

Figure 4 illustrates the nearly unimodal elution profiles of obtained for lignin and lignin derivatives after acetobromation with absence of molecular weight fractions.

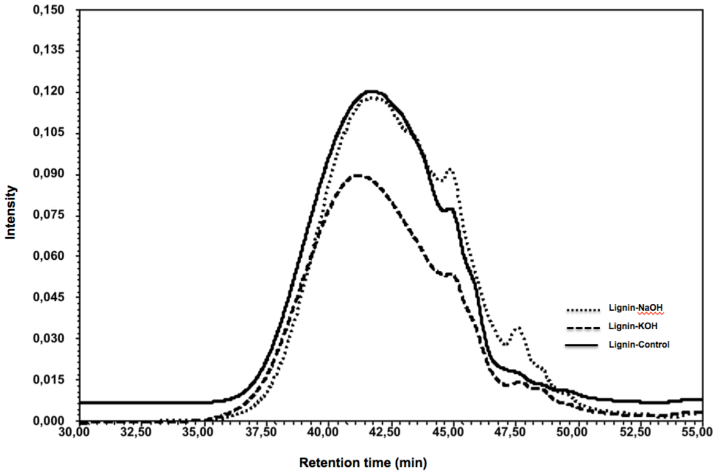

Fig. 4. Size exclusion chromatograms of control lignin and lignin derivatives

The results of molecular weight distribution obtained by SEC of acetylated lignin control and after modification are shown in Table 5. The molecular weight values must be considered relative because polystyrene was used as a calibration standard. These results indicate that the M_w and M_n for lignin modified in optimal conditions with NaOH were lower compared to lignin-control and lignin-KOH, and small differences in the polydispersity index were found. Fragmentation under these conditions would be associated with the fact that during the reaction process, the formation of phenolate cation and carbenium ion derivatives generated the fragmentation of lignin in smaller lignin derivative fragments and despite the fact that both catalysts are considered strong bases, it seems that NaOH could be more effective for the depolymerization of lignin despite small decreases in obtained molecular weight. This confirms the results shown before, that the repolymerization reactions could occur as a mechanism to stabilize the intermediate species formed during BCD or that KOH is less effective than NaOH in modifying lignin.

Table 5. Results of Acetylated Lignin of Weight-Average (M_w), Number-Average (M_n), and Polydispersity (M_w/M_n)

Sample	M_w (g/mol)	M_n (g/mol)	M_w/M_n
Lignin-control	1855	815	2.3
Lignin-NaOH	1581	639	2.5
Lignin-KOH	2021	749	2.7

Thermal Behavior of Lignin and Lignin-Derivatives

Differential scanning calorimetric analysis

Usually, lignin has a very interesting thermal behavior and can act as a thermoplastic as well as a thermoset material (Sen *et al.* 2015). Lignin is an amorphous polymer and, under specific temperatures, undergoes a transition from a glassy state to a rubbery state. This characteristic, which is specific for a polymer, is defined as the glass transition temperature, or T_g, and is useful to determine its possible applications and processing conditions. T_g is an indirect measure of crystallinity and degree of crosslinking and depends on the amount of water and polysaccharides, as well as molecular weight and chemical functionalization, but, in general, T_g will be lower with a greater mobility of lignin molecules (Doherty *et al.* 2011). The T_g values of different underivated lignin samples have been reported to be between 90 and 180 °C (Lora and Glasser 2002; Tejado *et al.* 2007), with higher values corresponding to softwood kraft lignin and lower values to organosolv lignin (Tejado *et al.* 2007). The corresponding glass transition temperatures are presented in Table 6, where the T_g of lignin-KOH is higher in comparison with the control and with lignin-NaOH. This difference in T_g can be explained by the free volume concept as described by Mohamad *et al.* (2011), which indicates that free volume is the space in a solid or liquid sample not occupied by polymer molecules, and T_g could be described by such activation energies through the use of free volume. Moreover, this property depends strongly on molecular weight because polymers with higher molecular weight could require more energy to reach this transition, thereby increasing T_g. As can be seen in Table 5, lignin-KOH showed a higher molecular weight, followed by lignin-control and lignin-NaOH. Thus, more energy was required, and the highest T_g value was observed for lignin-KOH in comparison with other lignin samples (Table 6).

Table 6. Comparison of Glass Transition Temperature (T_g), Maximum of Thermal Decomposition Temperature (DTGA $_{max}$)

Sample	T_g (°C)	TGA 50% (°C)	DTGA$_{max}$ (°C)	Residue (% w/w) (550 °C)
Lignin-control	135	398	374	35
Lignin-NaOH	131	500	362	46
Lignin-KOH	137	517	343	47

Thermogravimetric analysis

The thermal decomposition of different lignin derivatives (control, NaOH, and KOH) was investigated *via* thermogravimetric analysis. The thermogravimetric (TGA) and first derivative thermogravimetric (DTGA) curves are presented in Fig. 5 (a and b, respectively). The TGA curves show the weight loss of lignin in relation to the temperature of thermal degradation, and the DTG curves indicate the rate of weight loss of each lignin sample. The results obtained in the experimental data, which includes the degradation temperature at 50% weight loss (TG 50%), the degradation temperature at maximal weight loss (DTGA $_{max}$), and the final residues (% w/w), can be seen in Table 6. The thermal degradation of lignin occurred over a wide temperature range (200 to 400 °C) and can be explained by the fact that lignin presented a complex structure made up of phenolic hydroxyl, carbonyl groups, and benzylic hydroxyl, interlinked by various types of chemical linkages, which are connected by straight carbon-to-carbon links (Sjöström 1993; Domínguez *et al.* 2008). The results indicate that the initial weight loss for all samples could be associated with water evaporation, as shown in Fig. 3(a). The temperature for 50% mass loss was higher for lignin derivative samples modified with alkaline catalysts, at 500 and 517 °C for NaOH and KOH, respectively, whereas for the lignin control, the temperature was 398 °C.

The DTG curves show that DTGA $_{max}$ can be used to express the thermal stability characteristics for each lignin sample. The DTG with the maximum rate of weight loss for each lignin sample (control, NaOH, and KOH) appeared at 374.12, 362, and 342 °C, respectively, as shown in Table 6 and Fig. 3(b). The values of these lignin samples are similar that the values reported for other commercial lignin samples, such as Indulin AT lignin, from Wastvaco, Soda lignin (Protobind 2400), from Green Value Enterprises (Schorr *et al.* 2014), and soda-anthraquinone flax lignin (Tejado *et al.* 2007). Finally, after heating at 550 °C, the lignin NaOH and KOH samples that remained unvolatized were 46% and 47%, respectively, and 35% for the lignin control. These results reveal that both lignin samples modified by an alkaline catalyst had greater thermal stability at high temperatures than lignin that is not modified, which could be associated with a greater number of free hydroxyl phenolic groups for both types of lignin, and that the linkages between monomers are a preferred C-C type rather than β-O-4 type as a consequence of repolymerization reactions.

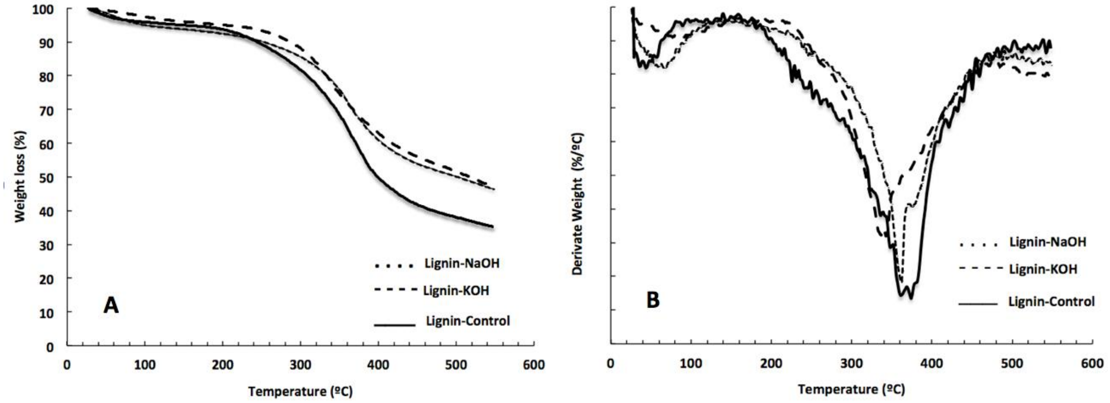

Fig. 5. The (a) thermogravimetric (TG) and (b) first derivative thermogravimetric (DTG) curves of all samples determined by TGA

CONCLUSIONS

1. The results showed that under defined conditions of alkali catalyst concentration, temperature, and reaction time, optimized using chemometric tools, it was possible to obtain chemical and thermal changes in organosolv lignin from *E. globulus*.
2. The base-catalyzed depolymerization (BCD) process causes a variation in the content of the various functional groups, such as an increase in free OH-Phe, specifically OH-syringyl and carboxyl-OH groups, and a decrease in aliphatic-OH groups.
3. As a catalyst, NaOH was more effective than KOH in modifying the lignin structure, demonstrated by a change in chemical characteristics. Also, the use of NaOH generated a decrease in the molecular weight distribution compared with unmodified lignin or treatment with KOH, confirming that despite the fact that both are considered strong bases, NaOH seems more appropriate for BCD.
4. Variations in thermal behavior properties were observed generated by possible structural changes occurring as a product of BCD, principally an increase in free OH-Phe groups for cleavage of the β-O-4 ether bond and repolymerization reactions.

5. Finally, the possibility of defining optimal conditions for the generation of derivatives of lignin with different chemical and thermal properties than original lignin could be an alternative method of valorizing waste lignin to produce new chemical compounds and materials.

ACKNOWLEDGEMENTS

Financial support was provided by Fondecyt (Grant 11121379), BioEnercel S.A. and Innova Chile Corfo 208-7302. The authors also thank the “Laboratorio de Recursos Renovables (LRR)” for allowing use the instrumental equipment, “CESMI” for NMR analysis and “Facultad de Ciencias Químicas” for thermal analysis.

REFERENCES CITED

Asgher, M., Ahmad, Z., and Iqbal, H. M. N. (2013). “Alkali and enzymatic delignification of sugarcane bagasse to expose cellulose polymers for saccharification and bio-ethanol production,” *Ind. Crop. Prod.* 44, 488-495. DOI: 10.1016/j.indcrop.2012.10.005

Borges da Silva, E. A., Zabkova, M., Araújo, J. D., Cateto, C. A., Barreiro, M. F., Belgacem, M. N., and Rodrigues, A. E. (2009). “An integrated process to produce vanillin and lignin-based polyurethanes from kraft lignin,” *Chem. Eng. Res. Des.* 87(9), 1276-1292. DOI: 10.1016/j.cherd.2009.05.008

Brebu, M., and Vasile, C. (2010). “Thermal degradation of lignin – A review,” *Cell. Chem. Technol.* 44(9), 353-363.

Crestini, C., Crucianelli, M., Orlandi, M., and Saladino, R. (2010). “Oxidative strategies in lignin chemistry: A new environmental friendly approach for the functionalisation of lignin and lignocellulosic fibers,” *Catal. Today* 156(1), 8-22. DOI: 10.1016/j.cattod.2010.03.057

Doherty, W., Mousavioun, P., and Fellows, C. (2011). “Value-adding to cellulosic ethanol: Lignin polymers,” *Ind. Crop. Prod.* 33(2), 259-276. DOI: 10.1016/j.indcrop.2010.10.022

Domínguez, J. C., Oliet, M., Alonso, M. V., Gilarranz, M. A., and Rodríguez, F. (2008). “Thermal stability and pyrolysis kinetics of organosolv lignins obtained from *Eucalyptus globulus*,” *Ind. Crop. Prod.* 27(2), 150-156. DOI: 10.1016/j.indcrop.2007.07.006

Duval, A., and Lawoko, M. (2014). “A review in lignin-based polymeric, micro-and nano-structured materials,” *React. Funct. Polym.* 85, 78-96. DOI: 10.1016/j.reactfunctpolym.2014.09.017

Eachus, S. W., and Dence, C. W. (2009). “Hydrogenation of lignin model compounds in the presence of a homogeneous catalyst,” *Holzforschung* 29(2), 41-48. DOI: 10.1515/hfsg.1975.29.2.41,

Eckert, R., and Abdullah, Z. (2010). “Carbon fiber from kraft softwood lignin,” US patent 7,678,358 B2.

El Mansouri, N., and Salvado, J. (2006). “Structural characterization of technical lignins for the production of adhesives: Application to lignosulfonate, kraft, soda-anthraquinone, organosolv and ethanol process lignins,” *Ind. Crop. Prod.* 24(1), 8-16. DOI: 10.1016/j.indcrop.2005.10.002

El Mansouri, N., Pizzi, A., and Salvado, J. (2007a). "Lignin-based wood panel adhesive without formaldehyde," *Holz Roh Werkst.* 65 (1), 65-70. DOI: 10.1007/s00107-006-0130-z

El Mansouri, N., Pizzi, A., and Salvado, J. (2007b). "Lignin-based polycondensation resins for wood adhesives," *J. Appl. Polym. Sci.* 103(3), 1690-1699. DOI: 10.1002/app.25098

Granata, A., and Argyropoulos, D. S. (1995). "2-Chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholate, a reagent for the accurate determination of the uncondensed and condensed phenolic moieties in lignins," *J. Agr. Food Chem.* 43(6), 1538-1544. DOI: 10.1021/jf00054a023

Guerra, A., Filpponen, I., Lucia, L. A., and Argyropoulos, D. (2006). "Comparative evaluation of three lignin isolation protocols for various wood species," *J. Agr. Food Chem.* 54(26), 9696-9705. DOI: 10.1021/jf062433c

Kadla, J., Kubo, S., Venditti, R., Gilbert, R. Compere, A., and Griffith, W. (2002). "Lignin-based carbon fibers for composite fiber applications," *Carbon* 40(15), 2913-2920. DOI: 10.1016/S0008-6223(02)00248-8

Kaewtatip, K. and Thongmee, J. (2013). "Effect of kraft lignin and esterified lignin on the properties of thermoplastic starch," *Mater. Design*. 49, 701-704. DOI:10.1016/j.matdes.2013.02.010

Lange, H., Decima, S., and Crestini, C. (2013). "Oxidative upgrade of lignin - Recent routes reviewed," *Eur. Polym. J.* 49(6), 1151-1173. DOI: 10.1016/j.eurpolymj.2013.03.002

Laurichesse, S., and Avérous, L. (2014). "Chemical modification of lignins: Towards biobased polymers," *Prog. Polym. Sci.* 39(7), 1266-1290. DOI: 10.1016/j.progpolymsci.2013.11.004

Lora, J., and Glasser, W. (2002). "Recent industrial applications of lignin: A sustainable alternative to nonrenewable materials," *J. Polym. Environ.* 10(1), 39-48. DOI: 10.1023/A:1021070006895

Mohamad, M., Zakaria, N., Sipaut, C., Sulaiman, O., and Hashim, R. (2011). "Chemical and thermal properties of lignins from oil biomass as a substitute for phenol in phenol formaldehyde resin production," *Carbohyd. Polym.* 86(1), 112-119. DOI: 10.1016/j.carbpol.2011.04.018

Mohan, D., Pittman Jr., C. U., and Steele, P. H. (2006). "Pyrolysis of wood/biomass for bio-oil: A critical review," *Energ. Fuel.* 20(3), 848-889. DOI: 10.1021/ef0502397

Pandey, M. P., and Kim, C. S. (2011). "Lignin depolymerization and conversion: A review of thermochemical methods," *Chem. Eng. Technol.* 34(1), 29-41. DOI: 10.1002/ceat.201000270

Pizzi, A. (1994). "Lignin-based wood adhesives," in: *Advanced Wood Adhesives Technology*, Pizzi, A (eds.), Marcel Dekker, New York, pp. 219-241.

Ragan, S., and Megonnel, N. (2011). "Activated carbon from renewable resources – lignin," *Cell. Chem. Technol.* 45(7-8), 527-531.

Saito, T., Brown, R., Hunt, M., Pickel, D., Pickel, J., Messman, J., Baker, F., Keller, M., and Naskar, A. (2012). "Turning renewable resources into value-added polymer: development of lignin-based thermoplastic," *Green Chem.* 14, 3295-3303. DOI: 10.1039/C2GC35933B

Sannigrahi, P., Pu, Y., and Ragauskas A. (2010). "Cellulosic biorefineries – Unleashing lignin opportunities," *Curr. Opin. Environ. Sust.* 2(5-6), 383-393. DOI: 10.1016/j.cosust.2010.09.004

Sarkar, S., and Adhikari, B. (2000). “Lignin-modified phenolic resin: Synthesis optimization, adhesive strength, and thermal stability,” *J. Adhes. Sci. Technol.* 14(9), 1179-1193. DOI: 10.1163/156856100743167

Sen, S., Patil, S., and Argyropoulos, D. (2015). “Thermal properties of lignin in copolymers, blends, and composites: A review,” *Green Chem*. DOI: 10.1039/C5GC1066G

Sjöström, E. (1993). “Lignin,” in: *Wood Chemistry: Fundamentals and Applications*, Academic Press, New York, pp. 71-89. DOI: 10.1016/B978-0-08-092589-9.50008-5

Son, S., and Toste, F. D. (2010). “Non-oxidative vanadium-catalyzed C-O bond cleavage: Application to degradation of lignin model compounds,” *Angew. Chem.-Ger. Edit.* 122(22) 3879-3882. DOI: 10.1002/ange.201001293

Sun, Y., and Cheng, J. (2002). “Hydrolysis of lignocellulosic materials for ethanol production: A review,” *Bioresour. Technol.* 83(1), 1-11. DOI: 10.1016/S0960-8524(01)00212-7

Tejado, A., Peña, C., Labidi, J., Echeverria, J., and Mondragon, I. (2007). “Physico-chemical characterization of lignins from different sources for use in phenol–formaldehyde resin synthesis,” *Bioresour. Technol.* 98(8), 1655-1663. DOI: 10.1016/j.biortech.2006.05.042

Thakur, V. K., and Thakur, M. K. (2014). “Processing and characterization of natural cellulose fiber/thermoset polymer composites,” 109, 102-117. DOI: 10.1016/j.carbpol.2014.03.039

Thakur, V. K., and Thakur, M. K. (2015). “Recent advances in green hydrogels from lignin: A review,” 72, 834-847. DOI: 10.1016/j.ijbiomac.2014.09.044

Tolbert, A., Akinosho, H., Khunsupat, R., Naskar, A., and Ragauskas, A. (2014). “Characterization and analysis of the molecular weight of lignin for biorefining studies,” *Biofuels Bioprod. Bioref.* 8(6), 836-856. DOI: 10.1002/bbb.1500

Toledano, A., Serrano, L., and Labidi, J. (2012). “Organosolv lignin depolymerization with different base catalysis,” *J. Chem. Technol. Biot.* 87(11), 1593-1499. DOI: 10.1002/jctb.3799

Toledano, A., Serrano, L., and Labidi, J. (2014). “Improving base catalyzed lignin depolymerization by avoiding lignin repolymerization,” *Fuel* 116(15), 617-624. DOI: 10.1016/j.fuel.2013.08.071

Van Dyk, J. S., and Pletschke, B. I. (2012). “A review of lignocellulose bioconversion using enzymatic hydrolysis and synergistic cooperation between enzymes – Factors affecting enzymes, conversion and synergy,” *Biotechnol. Adv.* 30(6), 1458-1480. DOI: 10.1016/j.biotechadv.2012.03.002

Vigneault, A., Johnson, D., and Chornet, E. (2007). “Base-catalyzed depolymerization of lignin: Separation of monomers,” *Can. J. Chem. Eng.* 85(6), 906-916. DOI: 10.1002/cjce.5450850612

Wu, S., and Argyropoulos, D. (2003). “An improved method for isolating lignin in high yield and purity,” *J. Pulp Pap. Sci.* 29(7), 235-240.

Zhao, C., and Lercher, J. (2013). “Catalytic depolymerization and deoxygenation of lignin,” in: *Role of Catalysis for the Sustainable Production of Bio-fuels and Bio-chemicals*, Triantafyllidis, K. S., Lappas, A. A., and Stöcker, M. (eds.), Elsevier, Amsterdam, pp. 289-320. DOI: 10.1016/B978-0-444-56330-9.00009-7

Article submitted: Sept. 10, 2015; Peer review completed: Dec. 2, 2105; Revised version received and accepted: Dec. 15, 2015; Published: January 6, 2016.
DOI: 10.15376/biores.11.1.1828-1842