RADICAL FORMATION ON TMP FIBERS AND RELATED LIGNIN CHEMICAL CHANGES

Luca Zoia,[a] Carmen Canevali,[b*] Marco Orlandi,[a] Eeva-Liisa Tolppa,[a] Jussi Sipila,[c] and Franca Morazzoni[b]

Oxidation of TMP fibers was compared at 298 K with molecular oxygen, in the presence of either [Co(salen)] in methanol or [Co(sulphosalen)] in water. Electron paramagnetic resonance (EPR) spectroscopy made it possible to reveal and quantify the formation of phenoxy cobalt radicals in the former case and of phenoxy radicals in the latter. These radicals reached the same concentration after 60 min from the onset of reaction. Fiber integrity was more preserved after oxidation in water than in methanol, as assessed by heteronuclear single quantum coherence - nuclear magnetic resonance (2D-HSQC-NMR) spectroscopy, nuclear magnetic resonance spectroscopy of carbon (^{13}C-NMR), and Gel Permeation Chromatography (GPC). These results suggest that efficient radical formation on fibers can be achieved also with water-soluble catalysts. Thus, it is proposed that treatment with molecular oxygen in the presence of [Co(sulphosalen)] in water represents a promising way to approach an environmentally sustainable radicalization of fibers, without heavy modification of the lignin structure.

Keywords: Lignocellulosic fibers, Phenols, [Co(salen)], [Co(sulphosalen)], Radicals, EPR, NMR

*Contact information: a: Dipartimento di Scienze dell'Ambiente e del Territorio, Università di Milano-Bicocca, Piazza della Scienza 1, 20126 Milano, Italy; b: Dipartimento di Scienza dei Materiali, Università di Milano-Bicocca, Via R. Cozzi 53, 20125 Milano, Italy; c: Laboratory of Organic Chemistry, University of Helsinki, P.O. Box 55 FIN-00014, Helsinki, Finland; *Corresponding author: carmen.canevali@unimib.it*

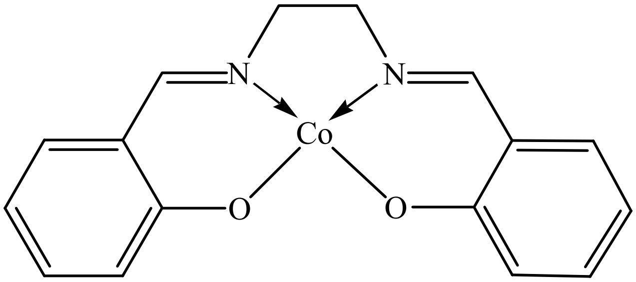
INTRODUCTION

In the field of packaging, materials with high barrier and mechanical properties are generally required. Wood fibers can achieve these properties, after proper modification. Attempts to modify fiber properties by grafting synthetic polymers onto the cellulose backbone started as early as the 1940´s. Radical centers at the cellulose backbone behave as grafting initiators, and they can be generated by high-energy irradiation, by oxygen reaction in the presence of transition metal complexes, by decomposition of peroxides, or by radical transfer reaction (Bledzki et al. 1998). Alternatively, radical active centers can be produced on lignin at the fiber surface. As an example, the reaction of wood fibers obtained from thermomechanical pulp (TMP) with molecular oxygen and laccase as catalyst, was demonstrated to produce the radicalic activation of the surface lignin phenols through the formation of phenoxy radicals (Lund et al. 2003):

$$4\ \mathrm{PhOH} + O_2 \xrightarrow{\text{laccase}} 4\ \mathrm{PhO}^{\bullet} + 2\ H_2O \qquad (1)$$

Under such treatment, glueless fiberboards were obtained, and the wet strength of paper was improved (Felby et al. 1997a; Lund and Felby 2001). The radical formation from surface lignin phenols can also improve other properties of interest for specific applications, such as hydrophobic or hydrophilic character (Buchert et al. 2005), as well as the improvement of paper strength properties (Chandra et al. 2004).

Besides by enzymatic treatment, phenoxy radicals on fibers can also be generated by reaction with molecular oxygen, using biomimic catalysts such as salen compounds. It was reported that molecular oxygen, in the presence of N,N'-ethylenebis-(salicylideneiminato) cobalt(II), [Co(salen)] (Scheme 1), efficiently formed radicals on CTMP and TMP fibers in methanol (Canevali et al. 2005).



Scheme 1.

The characterization of the intermediate paramagnetic species by electron paramagnetic resonance (EPR) spectroscopy suggested that the radicalization mechanism was the same as that proposed for lignin model compounds in homogeneous phase (Bolzacchini et al. 1997; Canevali et al. 2002), occurring through the following three steps:

$$[\mathrm{Co^{II}(salen)}] + \mathrm{ROH} + O_2 \rightleftharpoons [\mathrm{Co^{III}(salen)(ROH)}(O_2^{-})] \qquad (2)$$

$$[\mathrm{Co^{III}(salen)(ROH)}(O_2^{-})] + \mathrm{ROH} \rightleftharpoons [\mathrm{Co^{III}(salen)(ROH)(RO^{\bullet})}] + HO_2^{-} \qquad (3)$$

$$[\mathrm{Co^{III}(salen)(ROH)(RO^{\bullet})}] + HO_2^{-} \rightleftharpoons [\mathrm{Co^{III}(salen)(RO^{-})(RO^{\bullet})}] + H_2O_2 \qquad (4)$$

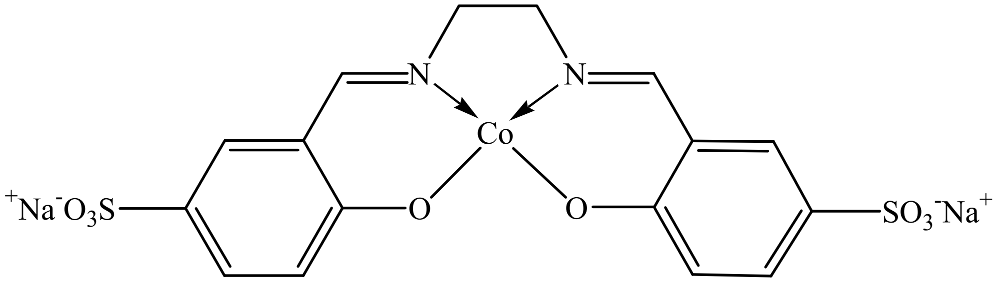
where ROH is the phenol unit and $\mathrm{RO}^{\bullet}$ the corresponding intermediate radical. In the first step of this mechanism, [Co(salen)] is co-ordinated by ROH and O_2, giving a superoxocobalt derivative, detected by EPR. In the second step, the superoxocobalt derivative reacts with another phenol ligand, giving an EPR active phenoxy cobalt radical, $[\mathrm{Co^{III}(salen)(ROH)(RO^{\bullet})}]$. In the third step, the phenoxy cobalt radical is in equilibrium with a phenoxy-phenate cobalt radical; both were detected by EPR.

After treatment with molecular oxygen in methanol in the presence of [Co(salen)], TMP fibers formed a higher amount of radicals and in parallel underwent deeper structural and morphological changes than CTMP (Canevali et al. 2005). By using

[Co(salen)] as catalyst, the absolute amount of radicals in fibers reaches very high values, 10 times higher than those reported in the literature for the treatment with laccase and molecular oxygen of TMP (Felby et al. 1997b) and of milled wood lignin (Ferm et al. 1972). These results are probably due to the smaller molecular dimension of [Co(salen)] compared with laccase, which allows the biomimic catalyst to interact also with subsurface lignin phenol groups.

However, [Co(salen)] does not allow an environmentally sustainable radical formation on fibers and a water soluble catalyst should be used instead.

With the aim of developing an eco-friendly process for maximizing the radical amount, while preserving the fiber integrity, in the present paper the treatments of unbleached TMP fibers with molecular oxygen, in the presence of either [Co(salen)] in methanol or [Co(sulphosalen)] (Scheme 2) in water were compared.



Scheme 2.

The best conditions for radical formation on TMP fibers using salen catalysts were assessed in the proper solvent by varying the reaction time, under the experimental conditions which were found to maximize the formation of radicals in the presence of [Co(salen)] (Canevali et al. 2005).

The radicals formed on fibers during oxidation were identified and quantified by EPR spectroscopy. The radical formation data were correlated to the changes in lignin chemical structure, achieved by lignin units under oxidative treatments, as assessed by heteronuclear single quantum coherence - nuclear magnetic resonance (2D-HSQC-NMR) spectroscopy, nuclear magnetic resonance spectroscopy of carbon (^{13}C-NMR) and Gel Permeation Chromatography (GPC).

EXPERIMENTAL

Materials

Pulps

The softwood unbleached thermomechanical pulp (TMP) was provided by Stora Enso Oyj. The amount of lignin in pulp was evaluated using the Klason method (Dence 1992) and resulted 27.1 %. %. The amount of extractives in pulp evaluated by Stora Enso, is reported in Table 1:

Table 1. Amounts of Extractive Components in TMP

Percentage of total extractives (w/w)	Fatty acids (mg/g)	Resin acids (mg/g)	Lignans and sterols (mg/g)	Sterylesters (mg/g)	Triglycerides (mg/g)
1.39	0.94	0.88	0.42	0.64	0.65

Reagents

N,N'-ethylenebis(salicylideneiminato) cobalt(II), [Co(salen)] (99%), was supplied by Aldrich. (Bis[(5-sulphonatosalicylaldehyde)ethylenediiminato] cobalt(II)) disodium, [Co(sulphosalen)], was synthesized according to the literature (Sippola and Krause 2003). Methanol and deuterated DMSO-d_6 (Fluka) were used as received. Mill-Q water was used. Oxygen (99.99%) was supplied by Technogas.

Methods

Radical formation on fibers

Fibers and fines, hereafter named "fibers", were obtained by suspending pulp in dichloromethane for 30 min, then in methanol for 60 min, under mechanical stirring, in order to eliminate extractives. Then, lignocellulosic fibers were recovered by filtration and dried in air at 353 K.

The best conditions for radical formation on TMP fibers using salen catalysts were assessed in the proper solvent by varying the reaction time, under the experimental conditions which were found to maximize the formation of radicals in the presence of [Co(salen)]: 298 K; fiber/[salen] ratio 10:1 w/w, corresponding to a molar ratio phenol/[salen)] ~ 0.8; fiber concentration in the solvent 5.0 mg/ml; oxygen pressure 1 bar (Canevali et al. 2005). Thus, radicals were formed by suspending fibers (150 mg) in 30 ml of either methanol containing [Co(salen)] (15 mg) or water in the presence of [Co(sulphosalen)] (15 mg), then fibers were allowed to react for the required time with molecular oxygen (1 bar pressure) at 298 K.

After reaction, fibers were recovered by filtration, washed either three times with 30 ml of ethyl acetate and three times with 20 ml of acetone (after [Co(salen)] treatment) or three times with 20 ml of water (after [Co(sulphosalen)] treatment). The washed fibers were allowed to dry in air, then subjected to spectromagnetic and structural characterization.

Solvents and washing liquids were collected in order to check the presence of paramagnetic species by EPR spectroscopy. In all cases paramagnetic species were absent in these samples.

EPR measurements

Immediately after drying in air, fibers were inserted into the EPR tube and frozen at the liquid nitrogen temperature, in order to inhibit further reaction before the spectromagnetic investigation.

The EPR spectra were recorded at 123 K on a Bruker EMX spectrometer working at the X-band frequency, equipped with a variable temperature BVT 2000 unit (Bruker).

The g values were determined by standardization with α,α'-diphenyl-β-picryl hydrazyl (DPPH). The amount of paramagnetic species, expressed as area/mg, was calculated with a ± 10 % accuracy by double integration of the resonance lines and by accurately determining the weight of dry fibers filling 1 cm length of the EPR tube (sensitive part of the EPR cavity).

NMR analyses

NMR analyses were performed on lignin extracted from TMP fibers by a modification of the acidolysis method developed in the literature (Gellerstedt et al. 1994): dried fibers (5 g) were suspended in 175 ml of dioxane/water 82:18 v/v (0.1 M HCl) and refluxed under nitrogen for 3 h. The fibers were filtered and washed 3 times with 15 ml of dioxane/water 82:18 v/v, then with distilled water to reach a neutral pH. The filtrate was then evaporated under reduced pressure at 313 K until dioxane had been removed. The aqueous solution was kept overnight in a refrigerator to induce coagulation of lignin; the precipitate was collected by filtration through a fine porous glass filter and washed with distilled water. After drying in air at 353 K for 2 h, lignin was refluxed with hexane in a Soxhlet extractor for 8 h in order to remove low molecular weight compounds. The yield of lignin, evaluated as (extracted lignin) / (lignin in pulp) w:w %, was around 35-40%.

The extracted lignin was acetylated with acetic anhydride:pyridine 1:1 v/v and each sample, approximately 60 mg, dissolved in 0.75 ml DMSO-d_6. The inverse detected ^{1}H-^{13}C correlation (2D-HSQC-NMR) spectra were recorded on a Bruker 500 MHz instrument at 308 K. The spectral width was set 5 kHz in F2 and 25 kHz in F1. Altogether 128 transients in 256 time increments were collected. The polarization transfer delay was set at the assumed coupling of 140 Hz and a relaxation delay of 2 s was used. Spectra were processed using Π/2 shifted squared sinebell functions in both dimensions before Fourier transform. The ^{1}D-^{13}C spectra were recorded using a Varian Mercury 400 MHz instrument at 308 K. The chemical shifts were referred to the solvent signal at 39.5 ppm. A relaxation delay of 10 s was used between the scans. Line broadening of 2-5 Hz was applied to FIDs before Fourier transform. For each spectrum, typically about 8000 scans were accumulated.

The number of primary, secondary and phenolic OH groups per aromatic ring was calculated (Cyr and Ritchie 1989; Faix et al. 1994; Robert and Brunow 1984; Landucci 1985) by multiplying the intensity of signals due to acetylic carboxyl groups divided by the intensity of signal due to methoxyl group, with the average number of methoxyl groups per aromatic ring in TMP lignins, this last number being evaluated by elemental and gas-chromatographic analyses (Girardin and Metche 1983).

GPC analyses

The investigation was carried out on acetylated lignin extracted from fibers. Before analysis, the acetylated lignin samples were dissolved in THF.

Analyses were performed using a Waters 600 E liquid chromatograph connected with an HP 1040 ultraviolet diode array (UV) detector set at 280 nm. The GP-column was an Agilent PL 3 μm MIXED gel E MW 220-400W. Polymer standards of poly(styrene) (PS) from Polymer Laboratories were used for calibration. The PS-calibration curve was tested using acetylated dimeric, tetrameric, and hexameric lignin model compounds. Analysis were performed at a flow rate of 0.8 ml/min

The evaluation of both the number-average molecular weight (M_n) and the weight-average molecular weight (M_w) was performed following the methodology developed in the literature (Himmel et al. 1989).

RESULTS AND DISCUSSION

Radical Formation on Fibers

Radicals formed on TMP fibers after reaction at 298 K with molecular oxygen in the presence of either [Co(salen)] in methanol or [Co(sulphosalen)] in water, were identified and quantified by EPR spectroscopy at 123 K (see Experimental).

In water an isotropic signal (g = 2.004 ΔHpp = 9 G) was observed (Fig. 1), very similar to that formed on fibers after treatment with laccase (Hon 1992) and attributable to phenoxy radicals.

In methanol an eight-resonance line signal was detected (Fig. 2a), very similar in shape to that observed (Bolzacchini et al. 1997; Canevali et al. 2002) in frozen solution during the oxidative degradation of lignin model compounds (Fig. 2b), which is attributable to the phenoxy cobalt radical, $[Co^{III}(salen)(ROH)(RO^{\bullet})]$.

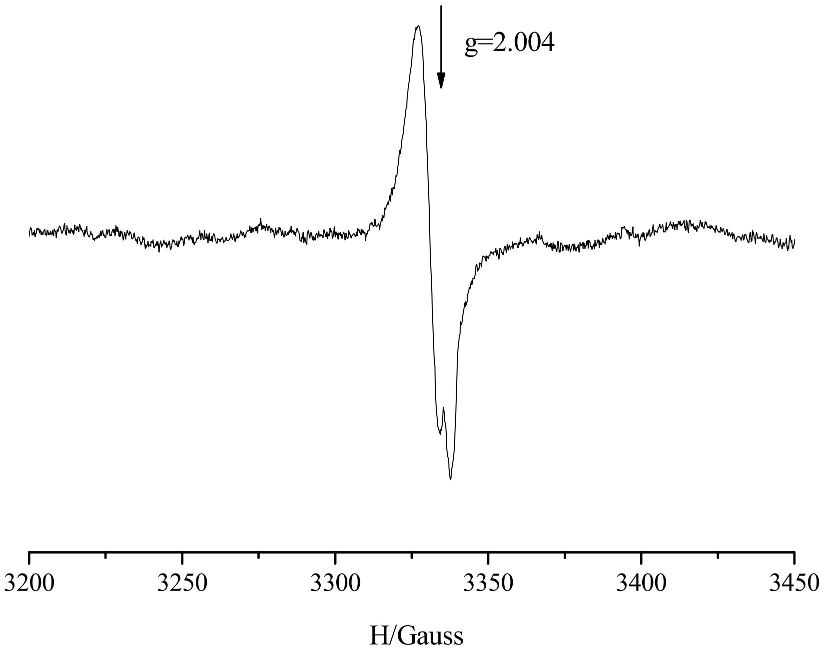


Fig. 1. X-band EPR spectrum recorded at 123 K on TMP fibers after oxidation in water in the presence of [Co(sulphosalen)] at 1 bar O_2 pressure for 60 min.

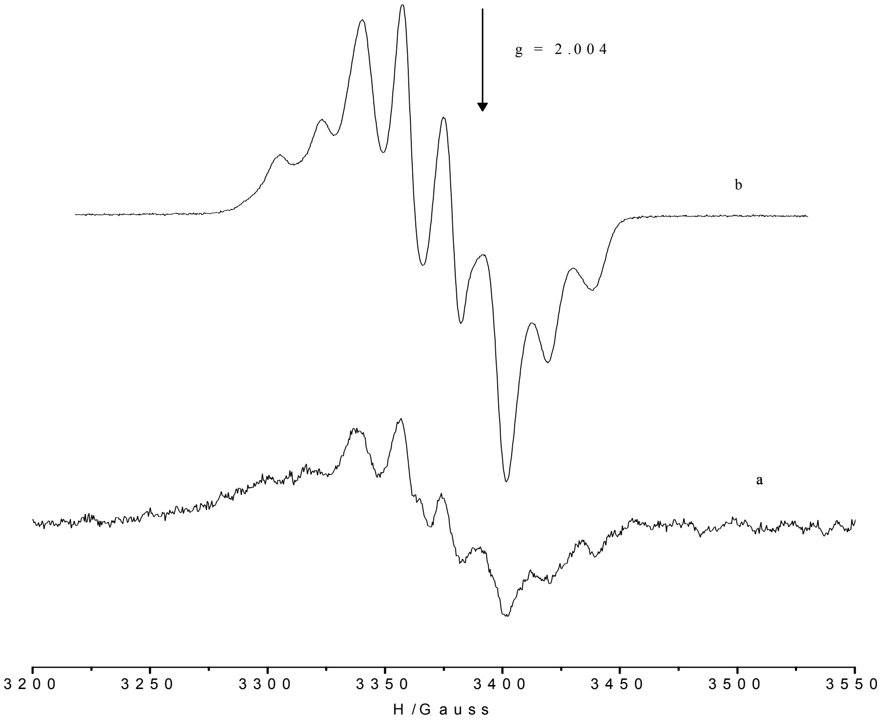


Fig. 2. X-band EPR spectra recorded at 123 K after oxidation in methanol in the presence of [Co(salen)] at 1 bar O_2 pressure for 30 min a) on TMP fibers and b) on E-methyl ferulate.

The radical signal formed by lignin model compounds in solution was deeply investigated in previous studies, both by X-band and high frequency (HF) EPR spectroscopy and showed axial magnetic anisotropy of g and A (^{59}Co) tensor components, with $g_{\perp} > g_{||}$ and $A_{||} > A_{\perp}$. The hyperfine coupling constant values vary with the ROH molecule (Canevali et al. 2002). In the case of lignocellulosic fibers, the observed signal is probably the envelope of several phenoxy cobalt radicals, which are [Co^{III}(salen)(ROH)(RO$^{\bullet}$)]-like, originated by the co-ordination of [Co(salen)] to different phenols present at the lignin surface. This causes a higher width of resonance lines with respect to the species containing a unique ROH ligand (Canevali et al. 2005).

The difference in the paramagnetic species formed during oxidation in the presence of [Co(salen)] and [Co(sulphosalen)] needs further investigation in order to be explained, which goes behind the scope of the present paper.

The best conditions for radical formation on TMP were assessed by evaluating the amount of paramagnetic species formed at different times of reaction (5, 15, 30, 60 min) in the presence of [Co(sulphosalen)] in water (Fig. 3a) or in the presence of [Co(salen)] in methanol (Fig. 3b).

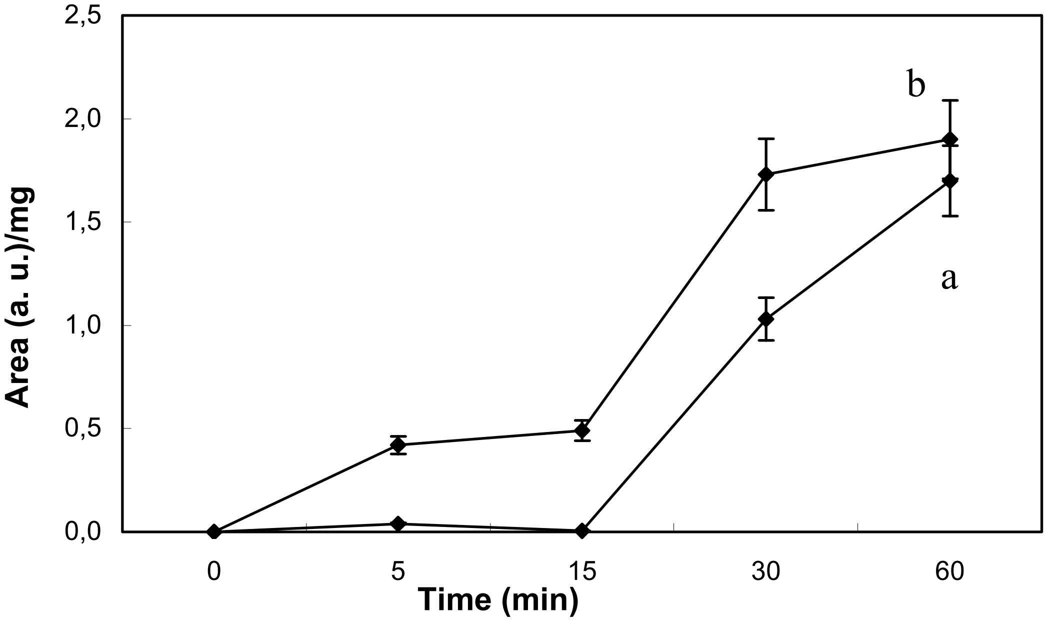


Fig. 3. Amounts of radicals, reported as area of EPR resonance lines (arbitrary units) per TMP fiber weight (mg), formed at different reaction times: a) phenoxy radicals formed in the presence of [Co(sulphosalen)] in water and b) phenoxy cobalt radicals formed in the presence of [Co(salen)] in methanol.

During both treatments, the amount of radicals gradually increased, [Co(salen)] in methanol being more reactive than [Co(sulphosalen)] in water at each time of reaction, except after 60 min of reaction, when the amounts of radicals formed in the two ways do not significantly differ.

Change in Lignin Structure

In order to evaluate changes in lignin chemical structure induced by catalytic oxidations in the presence of [Co(salen)] or [Co(sulphosalen)], lignins extracted by acid hydrolysis of fibers were characterized by 2D-HSQC-NMR spectroscopy, to identify the main intermonomeric units, and by ^{13}C-NMR spectroscopy, to quantify the principal intermonomeric units and the amount of alcoholic and phenolic groups. Spectra were run in DMSO-d_6 on the acetylated samples for the following three reasons: i) to avoid lignin fractionation before NMR analysis (Sipila 2002), ii) to increase the lignin solubility in DMSO-d_6 and iii) to enhance the chemical shift dispersion of the side chain units (Adler et al. 1987). As investigated lignins were obtained from both unreacted and oxidized fibers, the structure modifications due to the oxidative treatment were unequivocally distinguished from those due to the isolation method.

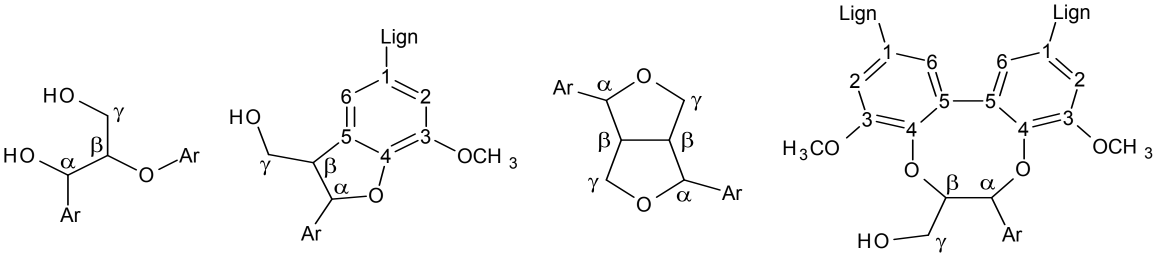
A preliminary evaluation by Klason method (Dence 1992) of the lignin amount in fibres after oxidation treatments and a preliminary evaluation of methoxyl content in lignin by the Zeisel method (Girardin and Metche 1983) allowed us to exclude relevant delignification effects. The data are reported in Table 2.

Table 2. Lignin Amount in TMP Fibres Before and After Treatment with [Co(salen)] and [Co(sulphosalen) and Amount of Methoxyl Content In Lignin Extracted from Fibres Before and After Treatment with [Co(salen)] and [Co(sulphosalen)].

Type of fibers	% lignin amount in fibers *	μmol OCH_3 / mg lignin**
Untreated TMP	27,1	4,06
[Co(salen)]-treated TMP	27,0	4,05
Co(sulphosalen)]-treated TMP	27,1	4,07

*Klason method, ** Zeisel method

The assignment of predominant signals in 2D-HSQC-NMR spectra was based on the chemical shift data of lignin model compounds and of milled wood lignin (MWL), as reported in the literature (Drumond et al. 1989; Ralph 1996; Kilpelainen et al. 1994). The predominant intermonomeric units found in TMP lignins extracted from fibers are reported in Scheme 3: arylglycerol-β-aryl ether (β-O-4 unit), phenylcoumaran (β-5 unit), pinoresinol (β-β unit) and dibenzodioxocine (5-5’-O-4 unit).



β-O-4 β-5 β-β 5-5’-O-4

Scheme 3.

The quantitative evaluation of the predominant intermonomeric units by ^{13}C-NMR showed that unreacted TMP lignin and TMP lignin treated with [Co(sulphosalen)] were rich in β-O-4 units and also contained significant amounts of β-5 units (Table 3). Instead, after [Co(salen)] treatment, the amount of β-O-4 and β-5 units in acetylated lignin approximately reduced to a half, with respect to untreated TMP. The amount of β-β units did not change after both treatments, while the 5-5’-O-4 units were not observed in lignin extracted from [Co(salen)]-treated TMP.

Table 3. Relative Amounts of the Predominant Intermonomeric Units in TMP Lignins Extracted from Fibres Before and After Treatment with [Co(salen)] and [Co(sulphosalen)].

Structural units	Untreated TMP	[Co(salen)]-treated TMP	[Co(sulphosalen)]-treated TMP
β-O-4	++++	++	+++
β-5	++	+	++
β-β	+	+	+
5-5'-O-4	+	traces	+

In addition to the elucidation of the structural changes in lignin intermonomeric composition, a quantitative evaluation of alcoholic and phenolic groups in TMP fibers, before and after the oxidative treatments, was also performed. It was shown that treatment in the presence of [Co(salen)] induced significant changes in the amount of alcoholic and phenolic groups per aromatic ring, while in the presence of [Co(sulphosalen)] the number of hydroxyl groups did not significantly change (Table 4). These results agree with the observed relative amounts of the predominant intermonomeric units, detected by ^{13}C-NMR and suggest that chemical lignin structure is better preserved after treatment with [Co(sulphosalen)] in water than with [Co(salen)] in methanol.

Table 4. Number of Primary, Secondary and Phenolic OH per Aromatic Ring in TMP Acetylated Lignins, Before and After Treatment with [Co(salen)] and [Co(sulphosalen)].

OH Type/C_6H_6	δ/ppm	Untreated TMP	[Co(salen)]-treated TMP	[Co(sulphosalen)]-treated TMP
primary	169.9-171.0	0.52	0.36	0.52
secondary	169.5	0.28	0.24	0.29
phenolic	168.6	0.20	0.15	0.19

The effects of salen treatments on lignin structure were also elucidated by evaluating the molecular weight distribution changes in the extracted acetylated lignins. Results show that the changes in M_n, M_w and polydispersity (M_w/M_n) of acetylated lignins, after [Co(sulphosalen)] treatment, are not significant, whereas in the case of [Co(salen)] treatment a small increase for M_n (from 5600 to 7500) and for M_w (from 13600 to 18000) was observed (Table 5). These results agree with those obtained by NMR investigation.

Table 5. M_n, M_w and Polydispersity Ratio (M_w/M_n) Values of TMP Acetylated Lignins, Before and After Treatment with [Co(salen)] and [Co(sulphosalen)].

Molecular weight distribution	Untreated TMP	[Co(salen)]-treated TMP	[Co(sulphosalen)]-treated TMP
M_n	5600 ± 500	7500 ± 500	5800 ± 500
M_w	13600 ± 1000	18000 ± 1000	14100 ± 1000
M_w/M_n	2.43 ± 0.04	2.40 ± 0.04	2.43 ± 0.04

CONCLUSIONS

1. The results of EPR investigation showed that the oxidation of unbleached TMP fibers by molecular oxygen, catalyzed by [Co(sulphosalen)] in water, induced the formation of phenoxy radicals that were very similar to those reported in the literature for the treatment of the same fibers with molecular oxygen and laccase (Lund et al. 2003). By contrast, in the presence of [Co(salen)] in methanol, phenoxy cobalt radicals similar in structure to those formed during the oxidation of lignin model compounds (Bolzacchini et al. 1997; Canevali et al. 2002) were observed.
2. After 60 min of reaction, the amount of radicals formed on TMP fibers in the presence of either [Co(salen)] or [Co(sulphosalen)] did not significantly differ. This result suggests that that efficient radical formation on fibers can be achieved also with water-soluble catalysts
3. The modification of lignin chemical structure was lower after oxidation with [Co(sulphosalen)] in water than with [Co(salen)] in methanol, as assessed by 2D-HSQC-NMR, ^{13}C-NMR and GPC.
4. The obtained results showed that the treatment with molecular oxygen in the presence of [Co(sulphosalen)] in water represents a promising way to approach an environmentally sustainable radical formation on fibers, without an heavy modification of the lignin structure.

ACKNOWLEDGMENTS

This contribution was an original presentation at the *ITALIC 4 Science & Technology of Biomass: Advances and Challenges* Conference that was held in Rome, Italy (May 8-10, 2007) and sponsored by Tor Vergata University. The authors gratefully acknowledge the efforts of the Conference Organizers, Prof. Claudia Crestini (Tor Vergata University, Rome, Italy), Chair, and Prof. Marco Orlandi (Biococca University, Milan, Italy), Co-Chair. Prof. Crestini also is Editor for the conference collection issue to be published in *BioResources.*

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Article submitted: Sept. 18, 2007; First round of peer review completed: Nov. 1, 2007; Revised article received and approved: Nov. 26, 2007; Published, Nov. 27, 2007.