

PUBLICATION II

**Reducing the content of VOCs of  
softwood kraft lignins for material  
applications**

In: BioResources 7(3), 2871–2882.

Copyright 2012 BioResources.

Reprinted with permission from the publisher.

## REDUCING THE CONTENT OF VOCs OF SOFTWOOD KRAFT LIGNINS FOR MATERIAL APPLICATIONS

Anna Kalliola,<sup>a,\*</sup> Anne Savolainen,<sup>b</sup> Taina Ohra-aho,<sup>a</sup> Greta Faccio,<sup>a</sup> and Tarja Tamminen<sup>a</sup>

Three laccases, functioning in mild acidic, and one in slightly alkaline conditions, were evaluated in order to reduce low-molecular phenolic VOCs of kraft lignins, which could be used in lignin/natural fibers composites. The potential of a sulfhydryl oxidase to catalyze the oxidation of sulfur containing VOCs (thiols) was also tested in combination with the laccase-catalyzed oxidation. In addition, oxidation at alkaline pH at room temperature that may induce polymerization of phenolics in an analogous manner to the laccase-catalyzed reaction was investigated. Enzyme reactivity towards lignin was evaluated as consumption of oxygen in the reaction solution. The effect of treatments on VOC reduction was determined both by sensing (odorimetry) and chemical (TD-GC/MS, SEC) analyses. Laccases, Lcc2, and MaL from *Thielavia arenaria* and *Melanocarpus albomyces*, respectively, showed potential in reducing odors. The most promising results were obtained by oxidizing lignin with O<sub>2</sub> at alkaline pH. However, the odor threshold values of the main VOC compounds are extremely low, which poses a challenge to VOC reduction.

*Keywords:* VOC; Kraft lignin; Composite; Laccase; Sulfhydryl oxidase; Oxygen; Oxidation

*Contact information:* a: VTT Technical Research Centre of Finland, VTT, P.O. Box 1000, FI-02044 VTT, Finland; b: Trelleborg Industrial Products Finland Oy, Kikkerlöntie 72, 38300 SASTAMALA, Finland.

\*Corresponding author: [anna.kalliola@vtt.fi](mailto:anna.kalliola@vtt.fi)

### INTRODUCTION

The potential of lignin to replace oil-based raw materials is being actively investigated for various material applications, such as composites. Based only on renewable resources, the Arboform<sup>®</sup> composites consist of isolated lignin, natural fibers, and natural additives, which are used in conventional thermoplastic processes such as injection molding (Naegele *et al.* 2002). However, there are several difficulties in applying lignin. One of them is the volatile organic compounds (VOCs), either present in the isolated lignin, or formed as they are processed at high temperature in thermoplastic processes. This drawback reduces the applicability of lignin-based composites, *e.g.* in the inner parts of cars.

VOCs in kraft lignin are typically lignin-originated phenolic structures, *e.g.* guaiacol (2-methoxyphenol), or reduced sulfur compounds formed in cooking. The odor threshold values of these VOCs are extremely low. For example, the odor threshold value for guaiacol in water solutions is reported to vary from 3 to 21 ppb (Fazzalari 1978; Buttery *et al.* 1988; Guth 1997). The great variation is due to different measurement techniques. For reduced sulfur compounds, dimethyl disulfide, and dimethyl trisulfide,

the threshold values are reported to be 5 and 0.2 ppb, respectively (Guth 1997; Zoeteman *et al.* 1973).

Recently, small amounts of organically modified montmorillonite (silicate clay-based) dispersed into the lignin/natural fibers system have been shown to improve the mechanical and thermal behaviors. Thermogravimetric analysis results suggested that exfoliated or intercalated layered aluminosilicates acted as a protective barrier against degradation of organic components. The thermooxidative degradation of the organic compounds (lignin, natural fibers, and additives) occurred in the temperature range of 180 to 420°C (Guigo *et al.* 2009).

Laccases catalyze the oxidation of a wide range of aromatic substrates, especially phenols, simultaneously with the reduction of molecular oxygen to water. Phenolic substrates are oxidized to phenoxyl radicals, which, depending on reaction conditions, can spontaneously polymerize via radical coupling or rearrange themselves leading to quinones, alkyl-aryl cleavage, C $\alpha$  oxidation, cleavage of C $\alpha$ -C $\beta$  bonds, or aromatic ring. Mediators, acting as intermediate substrates for laccases, enable laccase to indirectly oxidize large molecules, and even nonphenolic substrates (Giardina *et al.* 2010). Polymerization of lignosulfonates by laccases with and without mediators in varying reaction conditions has been confirmed recently (Areskogh *et al.* 2010; Prasetyo *et al.* 2010). Sulfhydryl oxidases catalyze the formation of de novo disulfide bonds from free thiol groups, with the reduction of molecular oxygen to hydrogen peroxide. These enzymes have been investigated in the food industry to improve the flavor of products such as ultraheat-treated milk and aromatic beverages such as coffee, tea, chocolate, wine, and beer (Starnes *et al.* 1986; Swaisgood 1977).

The objective of this study was to evaluate different approaches aiming to reduce VOCs of softwood kraft lignins. Laccase-catalyzed oxidation was tested as a potential means to polymerize the lignin-derived low-molecular phenolics such as guaiacol. Since the solubility of lignin increases significantly as pH increases and favors laccases that are active at higher pH, a laccase with the pH optimum at 8 was evaluated in addition to three other laccases functioning in mild acidic conditions. Sulfhydryl oxidase-catalyzed oxidation was tested as a means to reduce the odor from sulfur-containing compounds, *e.g.* thiols (methyl mercaptan, CH<sub>3</sub>SH). As a straightforward way of reducing VOCs, oxidations at alkaline pH and at room temperature were investigated. The idea of the treatment was based on the hypothesis that at ambient temperatures the reaction kinetics will be slower than at elevated temperatures, causing phenoxyl radicals to be relatively stable, which may induce polymerization of phenolics in an analogous manner to the laccase-catalyzed reaction. TD-GC/MS, is presented as a novel method to simulate and quantify the VOC formation in the temperature range of thermoplastic processing.

## EXPERIMENTAL

### Materials

Two softwood kraft lignins, commercial Indulin AT (Mead WestVaco), and non-commercial SE (Stora Enso) were used as raw materials.

Four laccases were tested: *Thielavia arenaria* Lcc1 (TaLcc1) and Lcc2 (TaLcc2) (Paloheimo *et al.* 2006a and b) produced as recombinant enzymes in *Trichoderma reesei*

by Roal Oy (Rajamäki, Finland), *Trametes hirsuta* (ThL) (produced in its native host by VTT), and *Melanocarpus albomyces* (MaL) (overproduced in *Trichoderma reesei* by VTT). TaLcc1, TaLcc2, and ThL are most active in mild acidic conditions, whereas MaL, in slightly alkaline conditions.

The mediator 1-hydroxybenzotriazole (HBT) was used with a dose of 5 percent with respect to lignin, in addition to ThL and TaLcc2 in two experiments. Sulfhydryl oxidase, *Aspergillus oryzae* (AoSOX1) (Faccio *et al.* 2010), produced in *Trichoderma reesei* by VTT was also tested.

## Methods

### *Lignin dissolution*

Lignin suspensions for enzyme treatments were prepared by dissolving lignin first into alkaline water, after which pH was decreased using 1 M HCl, and finally set to the target pH using 0.05 M sodium citrate buffer in the case of pH 5 and 6, and 0.05 or 0.2 M sodium phosphate buffer in the case of pH 8. In general terms, the lignin dissolution was performed according to Mattinen *et al.* (2008), however, using NaOH of higher molarities when increasing the lignin dry solids above 0.1 percent. For oxidation at alkaline pH, only a small amount of HCl was needed to decrease pH to 10 or 10.7 after dissolving lignin in NaOH.

### *Enzyme activity measurements*

2,2'-azino-bis-(3-ethylbenzthiazoline)-6-sulphonic acid (ABTS) (Roche) was used as a substrate for laccases in spectrophotometric enzyme activity measurement at pH 5 and 6, and guaiacol (Fluka) at pH 8. Katal units were used to express the catalytic activity of the laccase preparations (nkat/mL).

One katal of laccase is that amount of laccase which converts/oxidizes a mole of substrate per second under the specified conditions. Laccases were dosed on an activity basis with respect to the lignin content (nkat/g). 1 nkat ( $10^{-9}$  mol/s) corresponds to 0.0600 enzyme units (U, 1  $\mu$ mol/min), another commonly used unit expressing the catalytic activity. AoSOX1 was dosed on protein basis in respect to the sulfur content of lignin (2.2 percent).

### *Treatments*

At the analytical scale, the reactivity of enzymes towards the lignin was evaluated by monitoring oxygen consumption in the reaction solution. Lignin dry solids were 1, 2.5, or 5 percent. Monitoring was performed with an Oxy-10 mini sensor oxygen meter (PreSens, Germany) in a closed 1.9 mL vessel based on dynamic luminescence quenching. Parallel treatments were performed.

Selected treatments with promising enzyme dosages were repeated at a larger laboratory scale in a 2-L Parr reactor to allow the use of sensing analysis (odorimetry). Treatments were done at 5 or 10 percent lignin dry solids, and under 5 bars initial oxygen pressure at room temperature (RT) for 1 or 2 hours (Table 1).

Reference samples (pH 5, 6, and 8, samples 1, 7, 10, and 12, respectively), were prepared according to the dissolution-pH adjustment procedure at room temperature (not O<sub>2</sub> boosted).

**Table 1.** Experimental Set-up of the Oxidative Treatments Performed with and without Enzyme in 2-L Parr Reactor under 5 Bar Initial O<sub>2</sub> Pressure at RT. Reference samples (ref.) were not O<sub>2</sub> boosted.

| Sample | Substrate <sup>a</sup> | Lignin dry solids (%) | pH   | Enzyme <sup>b</sup> | Dosage <sup>c</sup> (nkat/g ; %) | HBT (%) | Treatment (h) |      |
|--------|------------------------|-----------------------|------|---------------------|----------------------------------|---------|---------------|------|
| 1      | SE                     | 5                     | 5    | -                   | -                                | -       | -             | ref. |
| 2      | SE                     | 5                     | 5    | -                   | -                                | -       | 2             |      |
| 3      | SE                     | 5                     | 5    | ThL                 | 100                              | -       | 2             |      |
| 4      | SE                     | 5                     | 5    | ThL                 | 100                              | 5       | 2             |      |
| 5      | SE                     | 5                     | 6    | TaLcc2              | 100                              | -       | 2             |      |
| 6      | SE                     | 5                     | 6    | TaLcc2              | 100                              | 5       | 2             |      |
| 7      | Ind AT                 | 5                     | 5    | -                   | -                                | -       | -             | ref. |
| 8      | Ind AT                 | 5                     | 5    | -                   | -                                | -       | 2             |      |
| 9      | Ind AT                 | 5                     | 5    | ThL                 | 100                              | -       | 2             |      |
| 10     | SE                     | 10                    | 8    | -                   | -                                | -       | -             | ref. |
| 11     | SE                     | 10                    | 8    | -                   | -                                | -       | 1             |      |
| 12     | SE                     | 10                    | 8    | MaL                 | 0.1                              | -       | 1             |      |
| 13     | SE                     | 10                    | 8    | MaL, AoSOX1         | 0.1, 0.1                         | -       | 1             |      |
| 14     | SE                     | 10                    | 10.7 | -                   | -                                | -       | 1             |      |

<sup>a</sup> Lignins: SE = Stora Enso lignin, Ind AT = Indulin AT

<sup>b</sup> Laccases: ThL = *Trametes hirsuta*, TaLcc2 = *Thielavia arenaria* Lcc2, MaL = *Melanocarpus albomyces*, Sulfhydryl oxidase: AoSOX1 = *Aspergillus oryzae*

<sup>c</sup> ThL and TaLcc2 activity measurements with ABTS; doses nkat/g. MaL and AoSOX1 doses on protein bases; MaL dose in respect to lignin, AoSOX1 dose in respect to sulfur in lignin.

### Analyses

In the odorimetry procedure, a wet sample volume containing 5 g of lignin dry solids was placed in a small crucible and stabilized inside closed glass containers (500 mL) for 48 hours at 40°C. Thereafter, the odor panel members (ten qualified experts) evaluated the odor intensity of each sample using a scale of 1 to 6, which was selected according to VDA-270 (1992) recommendations. The stabilized samples were also graded using numbers 0 to 10 by individual perception. The least odor-intense sample got number 0 and the most odor-intense was assigned number 10. The panel was able to evaluate a maximum 6 of the samples at a time. Three separate sessions were conducted. In general terms, the odorimetry method was a modification combining sensing analyses described by Söderhjelm and Pärssinen (1985) and Morvan *et al.* (2003).

The thermal desorption (TD) method was developed based on the earlier used method (Kleen *et al.* 2003) in order to simulate the formation of volatile degradation products at elevated temperatures prevailing in the injection molding. TD measurements were carried out with a PyroLab pyrolyzer unit (PyroLab2000<sup>®</sup>) connected to a Varian 3800 gas chromatography/Varian Saturn 2000 mass spectrometer. About 2.5 mg of the freeze-dried sample was weighed accurately and heated at 150 and 190°C for 5 minutes in a pyrolysis chamber. Thereafter, volatiles were led to the gas chromatography column (J&W, DB-1701, 30 m x 0.25 mm, film 1 µm) for separation. The oven was programmed as follows: initial temperature 100°C, rate of increase 4°C/min to 265°C and held for 18 min at the final temperature. A constant carrier gas flow of 0.9 ml/L was used. The mass spectra of the products were obtained using an ion trap mass spectrometer (EI 70 eV). The scan range of m/z 46-650 was used. Sulfur and phenol compounds were tentatively

identified using commercial mass spectra library Nist05. Quantification of guaiacol was performed with an external standard calibration (guaiacol, 98 percent, Aldrich).

Average lignin molecular size classes ( $M_n$ ,  $M_w$ ) of the freeze-dried samples were measured by size exclusion chromatography (SEC), using PSS MCX 1000 and 100 000 columns in 0.1 M NaOH eluent (25°C) with UV detection (280 nm). The molecular size distributions and average molecular sizes were calculated in relation to polystyrene sulfonate standards, using Waters Empower 2 software.

## RESULTS AND DISCUSSIONS

### Laccase Reactivity towards Lignins

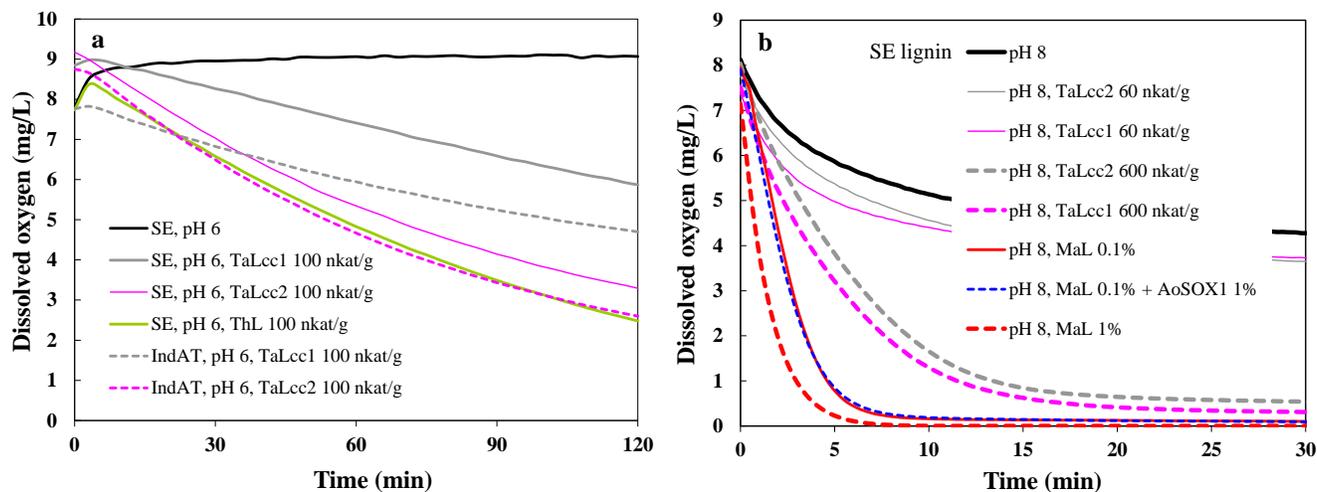
At analytical scale, the reactivity of laccases (ThL, TaLcc1, TaLcc2) functioning in mild acidic conditions was tested towards both kraft lignins by following the reduction of dissolved  $O_2$  in the reaction solutions. At pH 6, ThL and TaLcc2 showed higher reactivity towards lignins, as the curves of  $O_2$  consumption were steeper than in the case of TaLcc1 (Fig. 1a). Although the dissolution-pH adjustment procedure (Mattinen *et al.* 2008) is intended to reduce the agglomerates, lignin is mainly undissolved at pH 6, since the dissociation of guaiacyl- and syringyl-derived phenols occurs at higher pH levels (Ragnar *et al.* 2000). In order to increase the dissolution and the reactivity of lignin, the treatment was performed at pH 8 by inclusion of MaL with the respective pH optimum. At pH 8, lignin already reacted with the dissolved oxygen present in the reaction solution. However, more  $O_2$ -consuming reactions occurred in the presence of TaLcc1, TaLcc2, and MaL than in their absence (Fig. 1b). The enzyme dosing was based on guaiacol activity since lignin resembles guaiacol by its chemical structure and has similar behavior in terms of pH. Even if the activity of MaL (to guaiacol) was lowest, *e.g.* MaL 67.1 nkat/mL, TaLcc1 111.2 nkat/mL, TaLcc2 339.6 nkat/mL, it catalyzed lignin oxidation more efficiently than TaLcc1 or TaLcc2 at pH 8. Sulfhydryl oxidase (AoSOX1, 1 percent dose in respect to sulfur in lignin) was tested together with MaL.

However, the  $O_2$  consumption did not increase when compared to MaL treatment done without AoSOX1 (Fig. 1b). From the practical point of view, a 0.1 percent dose of MaL already showed potential in treating lignin.

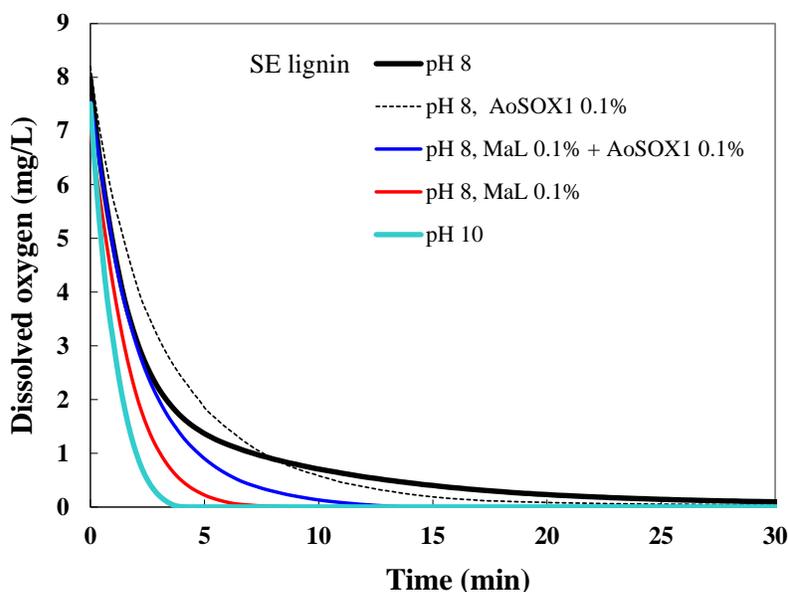
In addition to the laccase- (and AoSOX1) catalyzed oxidation, the oxidation of SE lignin was followed in alkaline conditions (pH 10), which causes the dissociation of the phenolic structures. Increasing pH from 8 to 10 increased  $O_2$  consumption and indicated improved lignin reactivity when compared to MaL-catalyzed oxidation (Fig. 2).

### Lignin Odor and Volatiles Formation

Based on the results obtained from the  $O_2$  consumption measurements, selected laccase treatments and oxidation at alkaline pH were performed at higher lignin dry solids and under oxygen pressure (Table 1). The pH level for the alkaline oxidation was set to 10.7, according to experimentally determined  $pK_0$  value of Indulin AT (at 21°C) (Norgren and Lindström 2000).

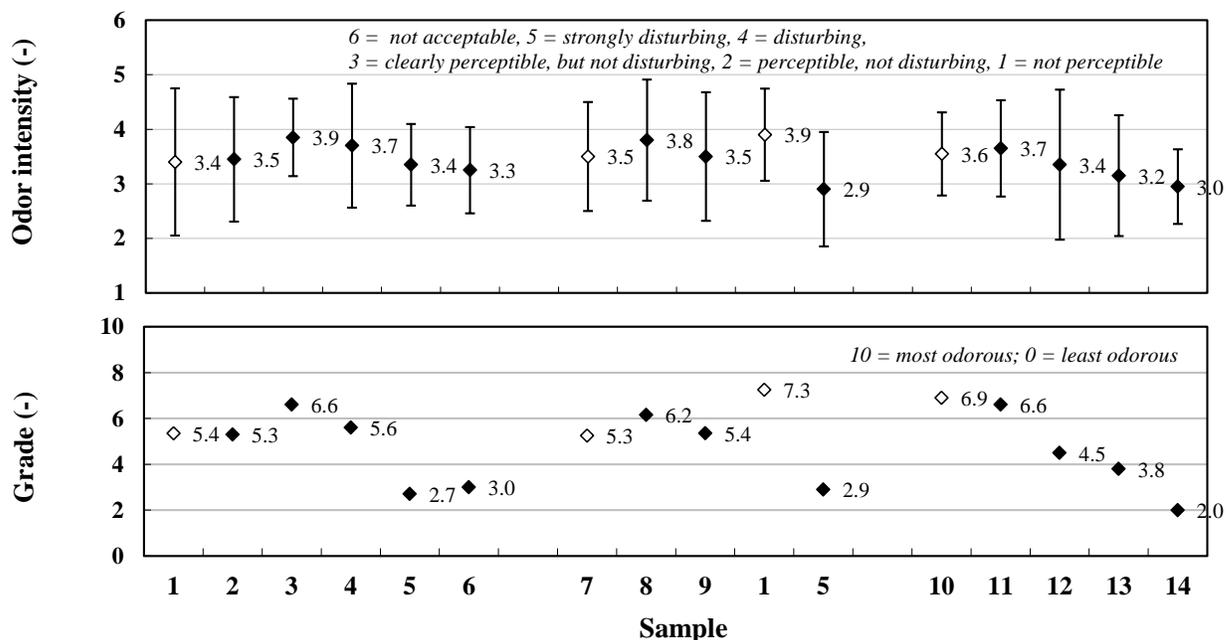


**Fig. 1. a)** Oxygen consumption of SE lignin and Indulin AT solution (ds. 1%) with and without laccase at pH 6. **b)** Oxygen consumption of SE lignin solution (ds. 2.5%) with and without laccase (and AoSOX1) at pH 8. 0.1% MaL dose equals to 31 nkat/g, and 1% MaL dose equals to 310 nkat/g. 1% AoSOX1 dose with respect to sulfur in lignin



**Fig. 2.** Oxygen consumption of SE lignin solution (ds. 5%) with and without laccase (and AoSOX1) at pH 8, and oxidation at pH 10. 0.1% MaL dose equals to 57 nkat/g

The overall odor intensity of the lignin samples (Table 1) was evaluated by ten human sniffers (the odor panel). The odor intensity of the samples varied between 2.9 and 3.9 (4 = disturbing, 3 = clearly perceptible), and standard deviations were rather large (Fig. 3 upper panel). Although the odor of the samples differed only slightly, the oxidation by TaLcc2 and MaL+ AoSOX1 and the oxidation at pH 10.7, indicated reduced VOC content, with the lowest odor intensity values *e.g.* 2.9 (Sample 5), 3.2 (Sample 13), and 3.0 (Sample 14), respectively. Grading the lignin samples using numbers from 0 to 10 (0 = the least odor intense, 10 = the most intense odor) supported the same observations (Fig. 3 lower panel).



**Fig. 3.** Odor intensity (upper panel) and grade (lower panel) of the wet SE lignin and Indulin AT samples treated with and without laccases (and AoSOX1) at different pH level. Samples are numbered according to Table 1; open dots refer to reference samples that were not O<sub>2</sub> boosted. Evaluation of samples 1 and 5 was conducted in the first and second separate odor panel

Volatile degradation products of the freeze-dried lignin samples (SE lignin treated with or without MaL/MaL+ AoSOX1, and at pH 10.7) were determined by TD-GC/MS at 150 and 190°C. Lignin softening takes place typically at 150°C, after which an increase in temperature induces depolymerization and recondensation (Bergmann *et al.* 2005). 190°C represents the temperature of the latter range, and also resembles the temperature at thermoplastic processing.

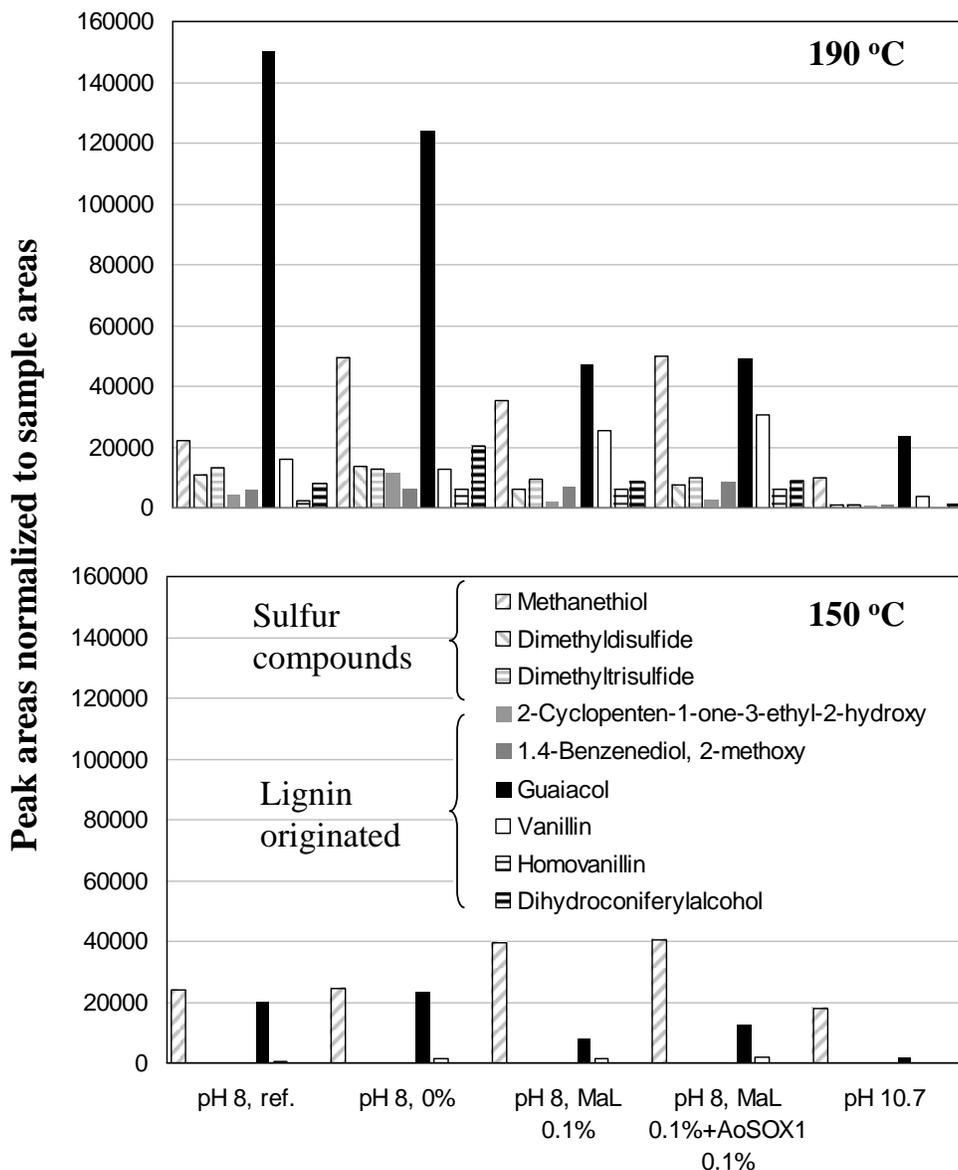
The main part of the volatiles had already evaporated within 5 minutes at both temperatures. The most abundant degradation product of lignin was guaiacol (Fig. 4). Clearly more volatiles were formed at 190°C than at 150°C. In the case of reference (pH 8, ref.) and O<sub>2</sub> treated (pH 8, 0%) samples, the sum of main volatiles was *ca.* 5-fold more at 190°C than at 150°C. In the case of MaL or MaL+ AoSOX1 treated lignin, it was *ca.* 3.5-fold more, and in lignin oxidized at pH 10.7 it was less than 2-fold more at 190°C than at 150°C.

At both temperatures, clearly less volatiles were formed as SE lignin was oxidized at pH 10.7, than treated with or without MaL/MaL+ AoSOX1 at pH 8. At least three explanations for reduced formation of volatiles of alkali-oxidized lignin may exist. Firstly, in the freeze-dried alkaline sample, the low-molecular phenolics most likely existed in sodium-salt form, which hindered their volatilization. Secondly, part of the phenolics might have degraded to non-volatile structures, which is unwanted, since in composite applications the molecular size presumably correlate with strength properties. Thirdly, polymerization/radical coupling of small-molecular phenolics, *e.g.* guaiacol, could have taken place.

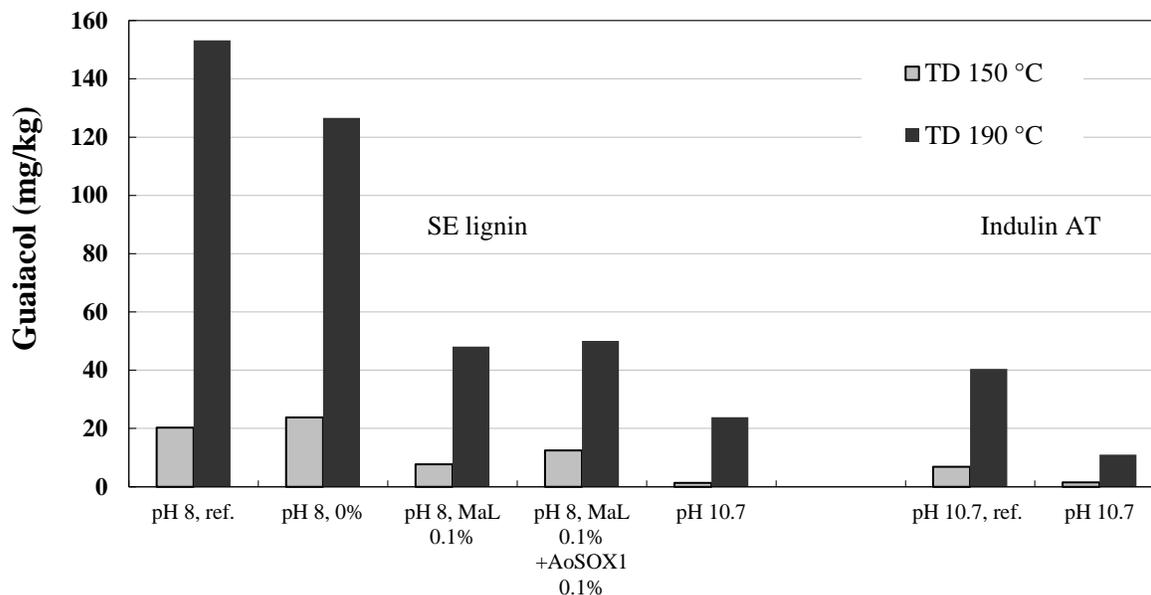
Also, the oxidation at pH 10.7 reduced the amount of sulfur containing VOCs. In these conditions, they might have degraded and further oxidized to sulfate. Furthermore,

the dissociation of thiols occurs at alkali pH levels, *e.g.* the  $pK_a$  value of methanethiol is 10.33 (Steward 1985), and thus the existence as sodium-salt may have hindered their volatilization.

The quantification of the main volatile, guaiacol, showed that its amounts in the SE lignin samples were still rather high when compared to the odor threshold values measured in water solutions, *i.e.* 1.3 ppm at 150°C and 24 ppm at 190°C (Fig. 5).



**Fig. 4.** Main volatiles of freeze-dried SE lignin samples at 190°C (upper panel) and 150 °C (lower panel). Reference sample (ref.) was not O<sub>2</sub> boosted



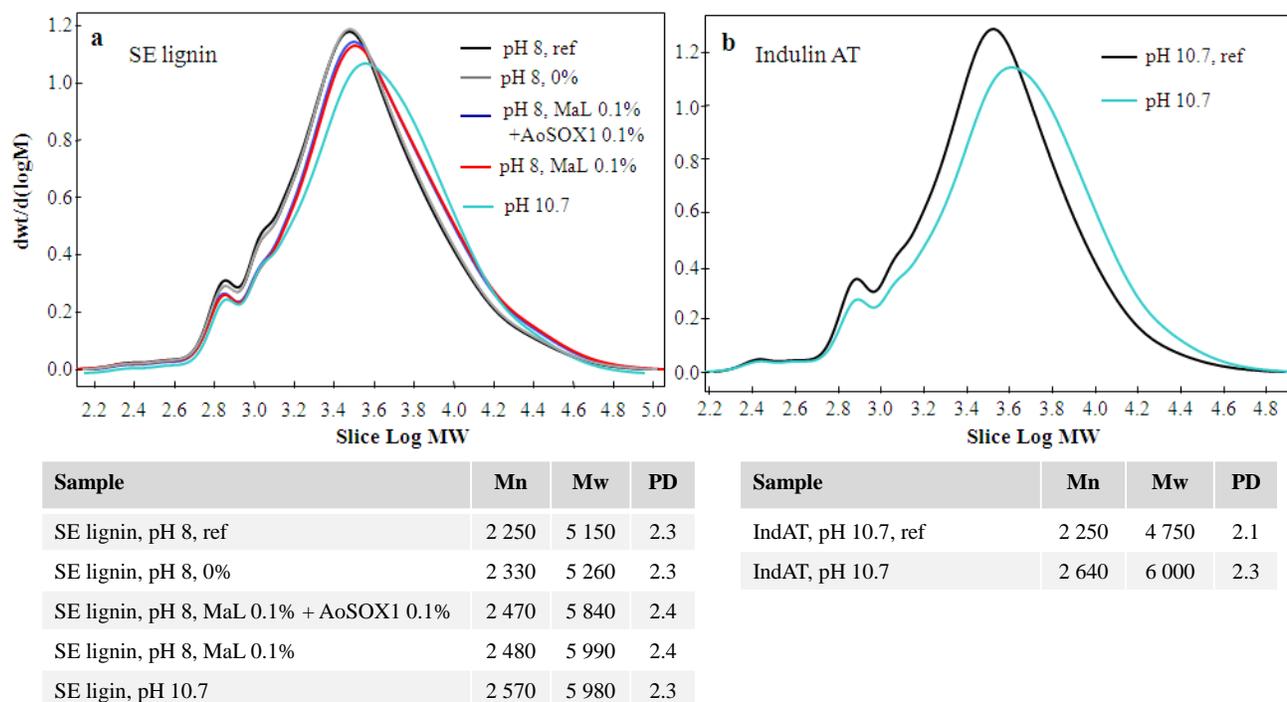
**Fig. 5.** Volatile guaiacol of freeze-dried SE lignin and Indulin AT samples at 150°C and 190°C. Reference samples (ref.) were not O<sub>2</sub> boosted

In order to investigate the importance of oxygen excess in the alkaline treatment, the pH 10.7 treatment was repeated to Indulin AT with and without oxygen boosting (5 bar initial O<sub>2</sub>). The results clearly showed that oxygen-pressurized alkali treatment decreased the volatile guaiacol formation at 150°C and at 190°C, when compared to the alkaline treatment performed without O<sub>2</sub> boosting (Fig. 5).

### Molecular Size Distribution of Lignin

The effect of the treatments on molecular size of both SE lignin and Indulin AT was analyzed using SEC. The results (molecular size distributions) indicated that the primary oxidation products, phenoxyl radicals, polymerized via radical coupling in the oxidation performed at room temperature and at pH 10.7. Also, MaL and MaL+AoSOX1 catalyzed oxidation caused minor polymerization of phenolics (Fig. 6a). Moreover, the experiments done with Indulin AT verified that at room temperature, the oxygen-pressurized alkali treatment caused lignin polymerization when compared to the alkali treatment done without O<sub>2</sub> boosting (Fig. 6b). However, the change in molecular size was rather small. Yet, the aim of this study was not to polymerize lignin in great extent, but to reduce the formation of low-molecular weight phenolic volatiles.

In alkaline oxygen oxidation (initially 5 bar O<sub>2</sub> excess) performed at elevated temperatures (90°C and 110°C), just the opposite takes place, as the Indulin AT macromolecule degrades fast, partly ending up to carbon dioxide (Kalliola *et al.* 2011). The degradation of lignin is desirable in pulp delignification, but likely unwanted in the composite applications.



**Fig. 6. a)** Molecular size distributions of freeze-dried SE lignin samples. **b)** Molecular size distributions of freeze-dried Indulin AT samples. Reference samples (ref.) were not O<sub>2</sub> boosted

## CONCLUSIONS

1. Laccase-catalyzed oxidation and oxygen oxidation in alkaline conditions were evaluated in order to reduce VOCs of kraft lignins for composite applications. Laccases Lcc2 from *T. arenaria* and MaL from *M. albomyces* with or without sulfhydryl oxidase from *A. oryzae*, as well as the alkaline oxygen oxidation (pH 10.7), showed potential in reducing odors. The importance of the oxygen excess in the alkali treatment was highlighted by the results. The analyses performed showed no clear effect of sulfhydryl oxidase.
2. Volatile degradation products of lignin were determined by TD-GC/MS method at temperatures prevailing in the thermoplastic processing. Also, the quantification of volatile guaiacol was conducted. The analysis method developed a possibility to study the effect of application processing under varying temperature and residence time, as well as distinct volatiles formed from different lignins from each other's.
3. From the practical point of view, the most promising results were obtained by oxygen oxidizing lignin at alkaline pH. The treatment would be straightforward to apply at an industrial scale.
4. Yet, the odor threshold values of the main VOC compounds are extremely low, which makes the reduction of VOCs challenging.

## ACKNOWLEDGMENTS

Tekes is acknowledged for financial support via project “VOC reduction of lignin containing materials” in the ERA-IB program. Stora Enso Corporation is thanked for providing the kraft lignin raw material. Marja Paloheimo, Roal Oy is thanked for providing Lcc1 and Lcc2 from *T. arenaria*. Mrs. Nina Vihersola and Mr. Juha Kaunisto are acknowledged for performing the experiments, Mrs. Eila Turunen for performing the TD-GC/MS and SEC analyses, Mrs. Tiina Liitiä for the determination of lignin molecular size distributions, and Mrs. Riitta-Maija Osmonen (VTT Expert Services) for arranging the sensing (odorimetry) analyses.

## REFERENCES CITED

- Areskog, D., Li, J., Gellerstedt, G., and Henriksson, G. (2010). “Investigation of the molecular weight increase of commercial lignosulfonates by laccase catalysis,” *Biomacromolecules* 11(4), 904-910.
- Bergmann, P. C. A., Boersma, A. R., Zwart, R. W. R., and Kiel, J. H. A. (2005). “Torrefaction for biomass co-firing in existing coal-fired power stations,” *Non-confidential ECN report, ECN-C--05-013*, The Netherlands.
- Buttery, R., Turnbaugh, J., and Ling, L. (1988). “Contribution of volatiles to rice aroma,” *J. Agric. Food Chem.* 36(5), 1006-1009.
- Faccio, G., Kruus, K., Buchert, J., and Saloheimo, M. (2010). “Secreted fungal sulfhydryl oxidases: sequence analysis and characterisation of a representative flavin-dependent enzyme from *Aspergillus oryzae*,” *BMC Biochemistry* 11, 31-42.
- Fazzalari, F.A. (1978). *Compilation of Odor and Taste Threshold Values Data*, Amer. Society for Testing and Materials, Philadelphia.
- Giardina, P., Faraco, V., Pezzella, C., Piscitelli, A., Vanhulle, S., and Sannia, G. (2010). “Review: Laccases: A never-ending story,” *Cell. Mol. Life Sci.* 67, 369-385.
- Guigo, N., Vincent, L., Mija, A., Naegele, H., and Sbirrazzuoli, N. (2009). “Innovative green nanocomposites based on silicate clays/lignin/natural fibres,” *Composites Science and Technology* 69, 1979-1984.
- Guth, H. (1997). “Quantitation and sensory studies of character impact odorants of different white wine varieties,” *J. Agric. Food Chem.* 45(8), 3027-3032.
- Kalliola, A., Kuitunen, S., Liitiä, T., Rovio, S., Ohra-aho, T., Vuorinen, T., and Tamminen, T. (2011). “Lignin oxidation mechanisms under oxygen delignification conditions. Part 1. Results from direct analyses,” *Holzforschung* 65, 567-574.
- Kleen, M., Ohra-aho, T., Tamminen, T. (2003). “On the interactions of HBT with pulp lignin during mediated laccase delignification – A study using fractionated pyrolysis-GC/MS,” *J. Anal. Appl. Pyrolysis* 70, 589-600.
- Mattinen, M., Suortti, T., Grosselink, R., Argyropoulos, D. S., Evtuguin, D., Suurnäkki, A., de Jong, E., and Tamminen, T. (2008). “Polymerization of different lignins by laccase,” *BioResources* 3(2), 549-565.
- Morvan, M., Talou, T., and Beziau, J-F. (2003). “MOS–MOSFET gas sensors array measurements versus sensory and chemical characterisation of VOC’s emissions from car seat foams,” *Sensors and Actuators B* 95, 212–223.

- Naegele, H., Pfitzer, J., Naegele, E., Inone, E. R., Eisenreich, N., and Eckl, W. (2002). "ARBOFORM – A thermoplastic, processable material from lignin and natural fibers," In: Hu, T.Q. (ed.), *Chemical Modification, Properties, and Usage of Lignin*, Kluwer Academic/Plenum, New York.
- Norgren, M. and Lindström, B. (2000). "Dissociation of phenolic groups in kraft lignin at elevated temperatures," *Holzforschung* 54(5), 519-527.
- Paloheimo, M., Puranen, L., Valtakari, L., Kruus, K., Kallio, J., Mäntylä, A., Fagerström, R., Ojapalo, P., and Vehmaanperä, J. (2006a). "Novel laccase enzyme and use thereof," *Patent WO/2006/032723*.
- Paloheimo, M., Valtakari, L., Puranen, L., Kruus, K., Kallio, J., Mäntylä, A., Fagerström, R., Ojapalo, P., and Vehmaanperä, J. (2006b). "Novel laccase enzymes and their uses," *Patent WO/2006/032724*.
- Prasetyo, E., Kudanga, T., Østergaard, L., Rencoret, J., Gutiérrez, A., Del Río, J., Santos, J., Nieto, L., Jiménez-Barbero, J., Martínez, A., Li, J., Gellerstedt, G., Lepifre, S., Silva, C., Kim, S., Cavaco-Paulo, A., Klausen, B., Lutnaes, B., Nyanhongo, G., and Guebitz, G. (2010). "Polymerization of lignosulfonates by the laccase-HBT (1-hydroxybenzotriazole) system improves dispersibility," *Bioresource Technol.* 101, 5054-5062.
- Ragnar, M., Lindgren, C. T., and Nilvebrant, N.-O. (2000). "pKa-values of guaiacyl and syringyl phenols related to lignin," *J. Wood Chem. Technol.* 20(3), 277-305.
- Söderhjelm, L., and Pärssinen, M., (1985) "The use of descriptors for the characterisation of odour in packaging materials," *Paperi ja Puu – Paper och Trä* 67, 412-416.
- Starnes, R. L., Katkocin, D. M., Miller, C. A., and Strobel Jr., R. J. (1986). "Microbial sulfhydryl oxidase," *US Patent US 1986/4632905, 06/738764*.
- Stewart, R. (1985). "The proton: Applications to organic chemistry," In: *Vol. 46 of Organic Chemistry, A series of Monographs*, Wasserman, H. H. (ed.), Academic Press, New York.
- Swaisgood, H. E. (1977). "Process of removing the cooked flavour from milk," *US Patent US 1977/4053644, 621631*.
- VDA-270 (1992). "Determination of the odour characteristics of trim materials in motor vehicles".
- Zoeteman, B. C. J., Piet, G. J., and van de Heuvel, R. (1973). *Threshold Odour Concentration in Water (and Air) of Chemical Substances*, Government Institute for Drinking Water Supply.

Article submitted: October 24, 2011; Peer review completed: January 13, 2012; Revised version received and accepted: May 10, 2012; Published: May 21, 2012.