RECENT ADVANCES IN PITCH CONTROL USING THE LACCASE-MEDIATOR SYSTEM

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ABSTRACT

Lipophilic extractives from wood and nonwood fibers exert a negative impact in pulp and paper manufacture causing pitch deposits. We have shown the effectiveness of the laccase-mediator system in removing pulp lipids regardless the pulping process and the raw material used. The enzymatic treatments were performed using a fungal laccase from *Pycnoporus cinnabarinus* and 1-hydroxybenzotriazole (HBT) as mediator. Gas chromatography-mass spectrometry of extracts from the enzymatically-treated pulps revealed that most of the lipids (including free and conjugated sitosterol, fatty and resin acids, and triglycerides) were removed. Improved pulp brightness and decreased kappa number were also observed. Then, the chemistry of the reactions of lipophilic extractives with laccase-HBT was studied using model alkanes, fatty alcohols, fatty acids, resin acids, free sterols, sterol esters and triglycerides. The laccase alone modified some unsaturated lipids, however, the most rapid and extensive removal was obtained in the presence of mediator. Different degradation patterns were observed, and several oxidation products were identified. Lignin-related phenols were tested as alternative to synthetic mediators for lipid removal from eucalypt pulp. Over 90% removal of free and conjugated sitosterol, similar to that attained with HBT, was obtained with some of them. A positive effect on pulp brightness and kappa number was also obtained, especially after a peroxide stage.

BACKGROUND

Lipophilic extractives, such as fatty and resin acids, fatty alcohols, alkanes, steroids and triglycerides (Fig. 1) cause pitch deposits along the pulp and paper manufacturing processes [1]. Pitch deposition is a serious problem in the pulp and paper industry since it is responsible for reduced production levels, higher equipment maintenance costs, higher operating costs, and an increased incidence of defects in the finished products, which reduces quality and benefits [2]. Furthermore, process effluents containing wood extractives may also be toxic and harmful to the environment [3,4].

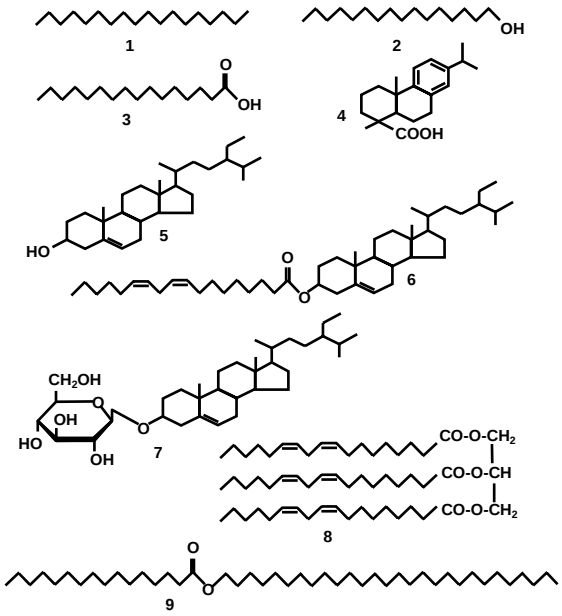

Figure. 1: Chemical structures of main classes of lipophilic extractives from woody and nonwoody plants: 1, octadecane; 2, 1-hexadecanol; 3, palmitic acid; 4, abietic acid; 5, sitosterol; 6, sitosteryl linoleate; 7, sitosteryl 3β-D-glucopyranoside; 8, trilinolein; and 9, octacosyl hexadecanoate.

Besides physicochemical methods, enzymes and microorganisms have been investigated to solve pitch problems [5]. Lipases, which hydrolyze triglycerides, are successfully applied in softwood (mainly pine) mechanical pulping at mill scale [6]. However, pitch problems in most of the chemical and mechanical processes using other raw materials have not been solved yet. Other compounds, such as free and esterified sterols, resin acids, fatty alcohols and alkanes, are responsible for pitch problems in these processes [5,7]. Laccase modification of lignans and other colloidal substances in process waters and pulps from softwood pulping has been reported [8,9]. In contrast to lipases, laccases are oxidative enzymes whose action is directed toward phenols, anilines and related compounds. The interest on laccases as industrial biocatalysts has, however, increased after discovering the effect of some synthetic compounds [10,11] expanding the action of laccases to non-phenolic aromatic substrates and, therefore, increasing their potential in degradation of lignin and other recalcitrant compounds. Moreover, the use of laccases in the presence of redox mediators has very recently been described for the removal of lipophilic extractives responsible for pitch deposits from wood and nonwood paper pulps [12-14]. Further investigations on the chemistry of the reactions of the laccase-mediator system with several model compounds representative for the main lipophilic extractives from different pulp types, have been carried out to better understand the degradation patterns observed in pulps [15]. Here, we report a summary of the main results obtained with the laccase-mediator system in the removal of lipophilic extractives.

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EXPERIMENTAL

Pulps. Unbleached kraft pulp from eucalypt (*Eucalyptus globulus*) was obtained from ENCE (Spain), thermomechanical pulping (TMP) spruce (*Picea abies*) pulp after the primary refiner was provided by UPM-Kymmene (Finland), and unbleached soda-anthraquinone (AQ) pulp from flax (*Linum usitatissimum*) was supplied by CELESA (Spain).

Model lipophilic compounds. Alkanes (octadecane), fatty alcohols (1-hexadecanol), fatty acids (palmitic, oleic and linoleic acids), resin acids (abietic acid), free sterols (sitosterol), sterol esters (cholesteryl palmitate, cholesteryl oleate and cholesteryl linoleate) and triglycerides (triheptadecanoin and trilinolein), were used.

Laccase and mediators. The laccase preparation was provided by Beldem (Andenne, Belgium). The enzyme was obtained from fermentor cultures of a laccase-hyperproducing strain of the fungus *Pycnoporus cinnabarinus* [13,14]. Laccase activity was measured by oxidation of 5 mM 2,2´-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) to its cation radical (ε_{436} 29300 M^{-1} cm^{-1}) in 0.1 M sodium acetate (pH 5) at 24ºC. One activity unit was defined as the amount of enzyme transforming 1 µmol of ABTS per min. 1-Hydroxybenzotriazole (HBT) and syringaldehyde (4-hydroxy-3,5-dimethoxybenzaldehyde), acetosyringone (4-hydroxy-3,5-dimethoxyphenylethanone) and *p*-coumaric acid (3-(4-hydroxyphenyl)-2-propenoic acid) were assayed as mediators.

Enzymatic treatments of paper pulps. Pulp treatments with laccase-mediator were carried out in 50 mM sodium tartrate (pH 4), 20 U/g of laccase, 6.75 mM concentration of syringaldehyde, acetosyringone and *p*-coumaric acid, and 3.33 mM of HBT [13,14]. The treatments were carried out in flasks with O_2 bubbling, placed in a thermostatic shaker at 170 rev/min and 50 ºC. In a subsequent step, pulps at 5% consistency (w:w) were submitted to: i) an alkaline extraction stage using 1.5% NaOH (w:w; referred to pulp dry weight) at 60 ºC for 1 h; or ii) a bleaching stage using 3% (w:w) H_2O_2 and 1.5% (w:w) NaOH (both referred to pulp dry weight) at 90 ºC for 2 h. Controls including laccase without mediator, mediator alone, and denaturized laccase (after 30 min at 100 ºC) were also performed. Treated pulp samples were extracted with acetone (8 h), and the extracts obtained were evaporated and redissolved in chloroform for gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) analyses.

Enzymatic treatments of model compounds. The enzymatic treatments (five replicates) of the different model lipids (1 mg) were performed using laccase (0.5 U/mg lipid), HBT (1 mg/mg lipid), and Tween 20 as dispersant (1% v/v) at pH 4, 50ºC, and different reactions times (5, 15, and 30 min, 1, 2, and 8 h). Oxygen was bubbled through the reaction flasks. In control experiments, lipids were treated under the same conditions but without laccase and mediator. Additional controls including laccase alone and boiled laccase were also performed. Mixtures of the saturated lipids (octadecane, hexadecanol, palmitic acid or triheptadecanoin) with linoleic acid or cholesteryl linoleate were treated (2 h) with laccase-HBT under the same conditions described above. After the enzymatic treatments, the lipid dispersions were immediately evaporated, and the reaction products recovered with chloroform:methanol (1:1), dried and redissolved in chloroform for GC and GC-MS analyses. Bis(trimethylsilyl)trifluoroacetamide in the presence of pyridine was used to prepare trimethylsilyl derivatives, before and after sodium borohydride reduction.

GC and GC-MS analyses of lipids. These analyses were performed using short and medium length capillary columns as previously described [14-16]. Longer columns (30 m) were also used.

Pulp evaluation. Pulp brightness, kappa number and intrinsic viscosity were analyzed following ISO 3688:1999, ISO 302:1981 and ISO 5351/1:1981 standard methods, respectively [17].

RESULTS AND DISCUSSION

We have demonstrated the high efficiency of the laccase-mediator system for the removal of lipophilic extractives present in pulps from different origins regardless the pulping process, the raw material or the chemical nature of the compound to be degraded [13] and a patent application was filed [18]. In these studies, eucalypt kraft pulp, spruce thermomechanical pulp, and flax soda-anthraquinone pulp were treated with laccase from the basidiomycete *P. cinnabarinus* in the presence of HBT as mediator. This treatment was very efficient in removing free and conjugated sterols (95-100% decrease) from eucalypt kraft pulp; triglycerides, resin acids and sterols (65-100% decrease) from spruce TMP pulp; and fatty alcohols, alkanes and sterols (40-100% decrease) from flax soda pulp. The removal of lipids by laccase-HBT resulted in the formation of several oxidized derivatives that were absent or presented low abundances in the initial pulps. In spite of this, the total lipid content in pulps decreased significantly, and the most problematic compounds were completely removed. In another work this enzymatic treatment was applied as an additional stage of an industrial-type TCF sequence for bleaching eucalypt kraft pulp [12] showing the complete removal of free and conjugated sitosterol. Pulp brightness was also improved due to the simultaneous removal of lignin by the laccase-mediator treatment.

Further investigations on the chemistry of the reactions of the laccase-HBT system with model lipids representative for the main lipophilic extractives present in hardwood, softwood and nonwood paper pulps (including alkanes, fatty alcohols, fatty acids, resin acids, free sterols, sterol esters and triglycerides) were carried out, and the reaction products were identified and quantified during the treatment, to better understand the degradation patterns observed in pulps [15]. These studies evidenced that a 60-100% decrease of the initial amount of unsaturated compounds such as abietic acid, trilinolein, linoleic and oleic acids, sitosterol, cholesteryl palmitate, oleate and

linoleate, was found at the end of 2-h laccase-HBT treatment. Likewise, a decrease of 20-40% of these unsaturated lipids was observed after treatment with laccase alone except in the cases of abietic acid that decreased 95%, and cholesteryl palmitate and sitosterol that were not affected.

The above study confirmed that laccase alone decreased the concentration of some unsaturated lipids [19,20]. However, the most rapid and extensive lipid modification was obtained with the laccase-mediator system. Model unsaturated lipids were largely oxidized and the dominant products detected were epoxy and hydroxy-fatty acids from fatty acids, and free and esterified 7-ketosterols and steroid ketones from sterols and sterol esters. In the case of sterol linoleate, breakdown of the fatty acid chain is produced releasing the so-called core aldehydes. The enzymatic reaction on sterol esters largely depended on the nature of the fatty-acyl moiety, i.e. oxidation of saturated fatty-acid esters started at the sterol moiety, whereas the initial attack of unsaturated fatty-acid esters was produced on the fatty-acid double bonds. In contrast, saturated lipids were not modified, although some of them decreased when the laccase-mediator reactions were carried out in the presence of unsaturated lipids suggesting participation of lipid peroxidation radicals.

Since some issues concerning the use of HBT and related synthetic mediators (such as the high cost and possible toxicity) difficult its industrial application, the search for natural compounds that could act as laccase mediators has been objective of scientists. The existence of some laccase natural mediators synthesized by fungi [21,22] or derived from lignin [14,23] has been suggested. In this respect, it has been reported for the first time that three cost-effective phenolic compounds related to lignin can act as laccase mediators for the removal of lipophilic compounds from paper pulp in the frame of a TCF sequence [14]. These natural mediators represent an alternative to synthetic mediators, such as HBT. In this study, unbleached eucalypt kraft pulp was treated with a fungal laccase in the presence of syringaldehyde, acetosyringone and *p*-coumaric acid as mediators. The enzymatic treatment using syringaldehyde as laccase mediator caused the highest removal (over 90%) of free and conjugated sitosterol, similar to that attained with HBT (Fig. 2), followed by acetosyringone (over 60% removal), whereas *p*-coumaric acid was barely effective. Moreover, recalcitrant oxidized steroids surviving laccase-HBT treatment could be removed when using these natural mediators.

In these treatments, pulp brightness was also improved (from 57% to 66% ISO brightness) by the laccase treatment in the presence of the above phenols followed by the peroxide stage due to the simultaneous removal of lignin [14,23]. The use of natural compounds as laccase mediators makes these enzymatic treatments more feasible to be applied in the pulp and paper industry. However, more knowledge is needed before this enzymatic treatment for (simultaneous) removal of pulp pitch and lignin can be considered as a serious proposition to be implemented in the pulp and paper industry.

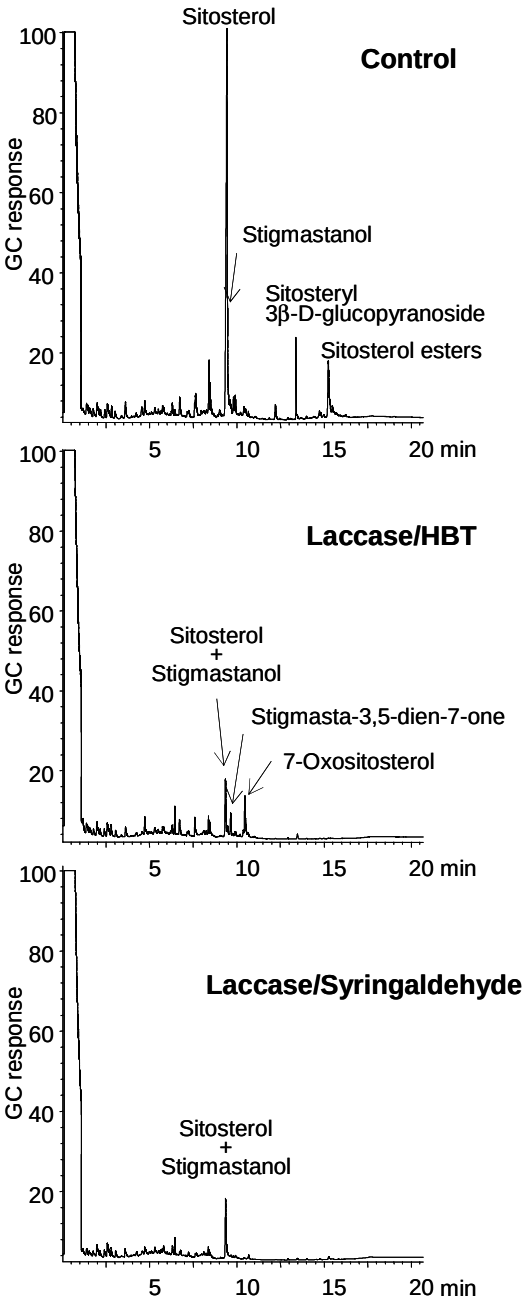

Figure. 2: GC analysis of lipophilic extractives during eucalypt pulp treatment with laccase-mediator followed by peroxide treatment: Control pulp after H_2O_2 bleaching; pulp after treatment with laccase in the presence of HBT, and subsequent H_2O_2 stage; and pulp after treatment with laccase in the presence of syringaldehyde, and subsequent H_2O_2 stage.

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