Environ. Sci. Technol. **2006**, *40,* 3416−3422

Enzymatic Removal of Free and Conjugated Sterols Forming Pitch Deposits in Environmentally Sound Bleaching of Eucalypt Paper Pulp

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Free and conjugated sterols are among the main compounds responsible for pitch deposition in the manufacture of wood chemical pulps, making difficult the implementation of totally chlorine free bleaching (TCF) and closure of bleach plant circuits. In this work, the suitability of oxidative enzymes in efficiently removing sterols from eucalypt pulps is revealed. The enzymatic treatment was applied as an additional stage of an industrial-type TCF sequence for bleaching eucalypt kraft pulp. The pulp obtained after oxygen delignification was treated with a high-redox potential and thermostable fungal laccase using 1-hydroxybenzotriazole as an enzyme mediator. This pulp was further submitted to chelation and peroxide stages and compared with a control TCF pulp obtained using chemical reagents. The composition of the lipophilic extractives in the pulps and the corresponding liquids after the different stages was analyzed by gas chromatography and gas chromatography−mass spectrometry. Free sitosterol and sitosterol esters and glucosides, the major lipophilic compounds in eucalypt pulps, were completely removed during the laccase-mediator treatment. Only some intermediate products from sitosterol oxidation remained after the laccase stage, as well as in the final pulp. Pulp brightness was also improved due to the simultaneous removal of lignin by the laccase-mediator treatment.

Introduction

In the interest of reducing the environmental impact of paper pulp mill effluents, the removal of organochloride compounds has been one of the main issues for the chemical pulping industry during the past several years. Simultaneously, environmental legislation drives this industry toward the reduction in water usage and effluent discharge. As a result, the bleaching process is undergoing a dynamic development. At present, there are two strong trends in the processes of bleaching chemical pulps: (1) totally chlorine free (TCF) bleaching and (2) the closed-cycle mill concept for attaining zero-liquid effluent (ZLE) operation. Considerable progress has been made in minimizing the formation of chlorinated organics in pulp bleaching by replacing elemental chlorine, usually with chlorine dioxide (*1*). Moreover, there are mills already applying TCF bleaching using combinations of non-chlorine oxidizing chemicals, such as oxygen, hydrogen peroxide, and ozone. On the other hand, the attainment of ZLE operation is considered a serious proposition for many pulp and paper mills (*2*).

However, some problems have arisen or are being aggravated with the introduction of the environmentally sound technologies mentioned above. One of the major challenges in the closed-cycle TCF mill is the handling of lipophilic extractives from wood. These compounds accumulate in the circuits and are deposited in the equipment and final product, forming the so-called pitch deposits (*3*). Moreover, they exert a negative impact in the environment when released in effluents due to the toxicity of some extractives (*4*). During kraft pulp production (more than 50% of world pulp production), a large part of the lipophilic extractives is saponified and removed in the black liquor. However, some wood extractives, especially free and conjugated sterols that are abundant in eucalypt (*5*) and other woods, are difficult to remove. Therefore, they are carried over to the bleach plant where they react with the bleaching chemicals (*6*). Unfortunately, the TCF sequences using oxygen and hydrogen peroxide are not as effective as chlorine dioxide in removing these compounds.

Existing physicochemical technologies for pitch removal are far from satisfactory. As an alternative, biological removal of wood extractives has been suggested (*7*). Enzymes have been successfully applied to softwood mechanical pulping at mill scale (*8*). Nevertheless, the commercially available biotechnological preparations are not fully effective for pitch control. This is because they are based on enzymes or organisms mainly acting on triglycerides. Since triglycerides are easily hydrolyzed in kraft pulping, lipase treatment is not of interest in kraft pulp manufacturing. In addition to lipases, sterol esterases have been suggested for pitch control (*9*, *10*). However, free sterols need to be degraded because they are as problematic as sterol esters. Therefore, more efficient methods for solving pitch problems caused by non-easily biodegradable lipophilic extractives are still required.

Recently, the modification of some colloidal substances in process waters and pulps from softwood mechanical pulping has been suggested using laccases (*11*−*14*). This group of oxidative enzymes has been the object of high interest for the development of environmentally sound technologies (*15*). Moreover, the use of laccase in the presence of a redox mediator strongly expands their potential in degradation of lignin and other aromatic compounds (*16*). Much work has been done on the laccase-mediator system for delignification and bleaching of different paper pulps (*17*−*19*). In the work presented here, the suitability of this system for the removal of lipophilic extractives from eucalypt kraft pulps during TCF bleaching is investigated.

Materials and Methods

Eucalypt Pulp. *Eucalyptus globulus* kraft pulp was obtained from the ENCE mill in Pontevedra, Spain. The unbleached (brown) pulp had a kappa number of 14.2 and an ISO brightness of 41.2%, estimated by ISO methods (*20*).

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10.1021/es052547p CCC: $33.50

Published on Web 04/06/2006

Fungal Laccase and Mediator. The laccase preparation used was provided by Beldem (Andenne, Belgium) and includes a major protein (>99% after sodium dodecyl sulfate−polyacrylamide gel electrophoresis) with laccase activity (as revealed by 2,6-dimethoxyphenol staining of electrophoresis gels). No esterase activity was detected after incubation with several lipid standards. The enzyme was obtained from fermentor cultures of a laccase-hyperproducing monokaryotic strain (ss3) of the fungus *Pycnoporus cinnabarinus* (that does not produce extracellular peroxidases) provided by INRA (Marseille, France) (*21*) grown in the medium described by Lomascolo et al. (*22*). Its biochemical characteristics have been described previously (*23*). Activity was measured by oxidation of 5 mM 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) to its cation radical ($\epsilon_{436} = 29\ 300\ M^{-1}\ cm^{-1}$) in 0.1 M sodium acetate (pH 5) at 24 °C. One activity unit was defined as the amount of enzyme transforming 1 μmol of ABTS/min. 1-Hydroxybenzotriazole (HBT) was used as a mediator.

Pulp Treatment with the Laccase-Mediator System in a TCF Sequence. Pulp treatments were carried out in stainless steel pressurized reactors designed at ENCE with an operation volume of 4 L and automated stirring, pressure, and temperature controls, using 200 g of pulp (dry weight) at a consistency of 10%. The enzyme-mediator treatment, using laccase (20 units/g of pulp) and HBT (1.5% of pulp dry weight) at pH 4 for 2 h at 50 °C with stirring for 1 min (60 revolutions/min) every 30 min, was included in an industrial-type TCF sequence. The resulting sequence (O−O−L−Q−PoP) included two alkaline oxygen stages (O) using pressurized O_2 at 98 °C for 1 h, a laccase-mediator stage (L), a chelation stage (Q) using diethylenetriaminepentaacetic acid (0.3% of pulp dry weight), and an alkaline peroxide stage (PoP) using H_2O_2 (3% of pulp dry weight) for 2.3 h at 105 °C under pressurized O_2, and for 3 h at 98 °C (*24*). The pulps were separated from the treatment liquid by filtration and exhaustively washed with distilled water after each stage. To better identify the effects of the laccase-mediator treatment, a sequence that included a control stage under the same conditions but without addition of laccase and mediator (called stage a because of its mild acidic conditions) was applied. Controls including laccase without mediator, mediator alone, denaturized laccase (after 30 min at 100 °C), and denaturized laccase with mediator were also performed.

Extraction of Lipids from Pulps and Liquids. Two pulp samples from the enzyme-containing sequence (O−O−L and O−O−L−Q−PoP pulps) and two samples from the control sequence (O−O−a and O−O−a−Q−PoP pulps) were analyzed, together with the corresponding treatment liquids, and controls. Pulps were air-dried (40 °C until constant weight), and samples were Soxhlet-extracted with acetone for 8 h (*25*). The liquid filtrates were extracted three times in a separatory funnel with methyl *tert*-butyl ether (MTBE) (*26*) at pH 14. A mass balance of the lipophilic extractives present in pulps, bleaching filtrates, and total washing waters (that were not analyzed in other cases because of the large volume and high dilution rate) from a chemical TCF sequence was carried out. All extracts were evaporated to dryness and redissolved in chloroform for analysis of the lipophilic fraction by gas chromatography (GC) and gas chromatography−mass spectrometry (GC−MS).

GC and GC−MS Analyses of the Lipophilic Extracts. The extractives from pulps and liquids were analyzed by GC and GC−MS using short and medium-length high-temperature capillary columns as previously described (*27*). Bis(trimethylsilyl)trifluoroacetamide (BSTFA) silylation, in the presence of pyridine, was used when required.

Microscopy Studies. Pulp samples were stained with filipin and examined by fluorescence microscopy as previously described (*28*). Phase contrast images were taken for identification of pulp elements.

Pulp and Papermaking Evaluation. Pulp and paper analyses were carried out by ISO methods (*20*). The brightness and kappa number of pulps were evaluated following ISO 3688:1999 and ISO 302:1981, respectively. Before the papermaking evaluation, pulps were refined using a PFI refiner (ISO 5264/2:2002) operating at 1400 revolutions/min. The degree of refining was measured by ISO 5267/1:1999 using distilled water that yields Schopper-Riegler values approximately 2-fold higher than those obtained at the ENCE mill using tap water. After pulp disintegration (ISO 5263/1:2004), paper handsheets were prepared (ISO 5269/1:2005) with a grammage of ~65 g/cm^2. The tensile index (ISO 1924/2:1994), the tear index (ISO 1974:1990), the air porosity Gurley index (ISO 5636/5:2003), and the light scattering coefficient (ISO 9416:1998) were evaluated.

Results and Discussion

Eucalypt kraft pulp was treated with the laccase-mediator system in a laboratory-scale TCF sequence carried out in pressurized reactors (O−O−L−Q−PoP sequence). This sequence reproduced the industrial TCF sequence used for bleaching *E. globulus* kraft pulp, with the additional enzymatic treatment (stage L). The sequence started with oxygen delignification (two O stages) and was completed with a two-step peroxide bleaching (stage PoP, with the first step under pressurized O_2) after a chelation treatment (stage Q) to remove the metals that destroy the peroxide. Pulps and treatment liquids were collected at different stages of both the enzyme-containing sequence and the industrial-type control sequence. The lipophilic extractives were isolated from pulps and liquids and analyzed using GC, as well as GC−MS for compound identification. Pulp elements were examined by fluorescence microscopy after being stained with filipin, and some selected properties of the pulps and papers were evaluated.

Lipophilic Extractives in Eucalypt Pulps and Liquids in a TCF Sequence. The composition of the lipophilic extractives was first analyzed in samples (O−O−a and final pulps) from the industrial-type TCF sequence (O−O−a−Q−PoP), which included a control stage under the same conditions of stage L but without enzyme (stage a) (Figure 1A,B). The corresponding filtrates were also analyzed, and the abundances of the main lipophilic compounds identified in the pulps and liquids are listed in Tables 1 and 2, respectively. Analyses before stage a (data not shown) confirmed that this control stage did not affect the amount or composition of extractives. It can be observed that the major part of the lipids identified was present in the pulps, while only a minor fraction was released to the liquids.

Free sterols, sterol glycosides, and sterol esters were the main lipophilic compounds in the oxygen-delignified O−O−a pulp and the corresponding liquid, where they represent 80% of total lipids (Tables 1 and 2) together with minor amounts of steroid hydrocarbons. Sitosterol was the predominant sterol in both free and conjugated form. The presence of these lipophilic compounds in eucalypt pulps and process waters was previously reported (*25*, *29*, *30*). They have their origin in the lipophilic extractives present in eucalypt wood that survive the cooking and oxygen stages. On the other hand, some oxidized sterols were found in both pulp and liquids. The presence of oxidized steroids in oxygen-delignified eucalypt pulp was recently reported (*31*). A series of fatty acids was also found, together with minor amounts of ω-hydroxy fatty acids.

The analysis of pulp and liquids (Tables 1 and 2) at the end of the TCF sequence (O−O−a−Q−PoP) revealed that the content of lipophilic extractives (with the exception of stigmastanol) decreased with respect to that of the O−O−a pulp, although the composition was not strongly modified

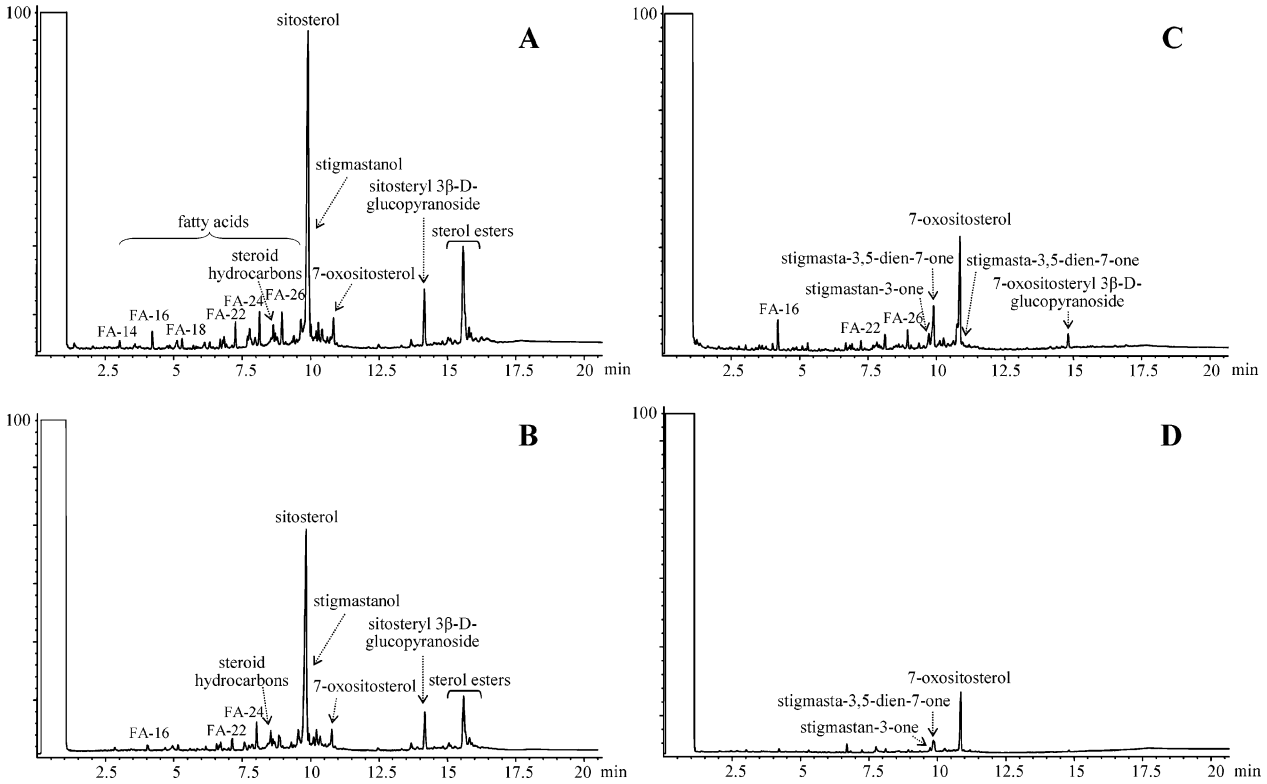

FIGURE 1. Chromatographic analysis of lipophilic extractives (as trimethylsilyl derivatives) in eucalypt pulps from different stages of the laccase-containing (right) and control TCF sequences (left): (A) pulp after double oxygen and control stage (O−O−a), (B) final pulp after the whole control sequence (O−O−a−Q−PoP), (C) pulp after double oxygen and laccase-mediator stages (O−O−L), and (D) final pulp after the whole enzymatic sequence (O−O−L−Q−PoP). All chromatograms correspond to the same amount of pulp.

(fatty and ω-hydroxy fatty acids were partially removed). This decrease is due more to dissolution and dispersion of lipophilic compounds under the alkaline conditions of the PoP stage than to the action of hydrogen peroxide (*32*). This was determined after a thorough mass balance of the lipophilic extractives present in pulps, bleaching filtrates, and total washing waters in a classical TCF sequence. This balance revealed that most (more than 90%) free and conjugated sterols were unaffected by the Q−PoP stages. The hydrogen peroxide stage had a minor influence on the composition of pulp steroids, in agreement with the results reported for industrial eucalypt pulps (*25*) and confirmed by reaction of pure sitosterol with these bleaching agents (*33*). In contrast, it has been reported that chlorine dioxide (in ECF bleaching) extensively degrades unsaturated sterols in eucalypt pulp (*25*, *32*, *34*).

Enzymatic Degradation of Lipophilic Extractives in Eucalypt Pulps. The laccase-mediator system was investigated for the degradation of lipophilic extractives in eucalypt kraft pulp in a TCF sequence. The laccase from *P. cinnabarinus* was selected after previous studies (*35*). In addition to its high redox potential (*19*), this enzyme exhibits good stability against temperature and mediator inactivation, as revealed by the fact that more than 65% of the initial activity remained at the end of the pulp treatment. On the other hand, HBT belongs to the group of −N(OH)− compounds that includes the most efficient laccase mediators that have been described (*36*). Oxygen-delignified pulp was treated with the laccase-mediator system and then subjected to peroxide bleaching after a chelation stage. The incorporation of the laccase-mediator treatment at this point of the sequence is the result of previous studies in which different points of incorporation of the enzymatic treatment for bleaching eucalypt kraft pulp were investigated (*24*). The GC analysis of the lipophilic extractives from the pulps collected after the combination of oxygen and laccase-mediator stages (O−O−L), and after the complete TCF sequence including the laccase-mediator stage (O−O−L−Q−PoP), is shown in panels C and D of Figure 1. When the pulps were treated with the *P. cinnabarinus* laccase alone (i.e., without HBT), no modification of the free and conjugated sterols, in the eucalypt pulp or treatment liquid, was observed. Negative results were also obtained in the controls with the denaturized enzyme or mediator alone, as expected.

The main lipophilic compounds described above were quantified in pulps after the laccase-mediator stage and at the end of the sequence, and their abundances are presented in Table 1, to be compared with that of the industrial-type sequence. The compounds present in the corresponding liquid filtrates were also analyzed, and the abundances are shown in Table 2. These analyses revealed that the main lipophilic compounds present in the oxygen-delignified eucalypt pulp, namely, sitosterol, sitosteryl 3β-D-glucopyranoside, and sitosterol esters, were completely removed (98%) by the laccase-mediator treatment, from both pulp and treatment liquid. The fatty acids and ω-hydroxy fatty acids observed in the pulp were also removed to a large extent. Only some oxidized steroids arising from sitosterol oxidation, namely, stigmastan-3-one, stigmasta-3,5-dien-7-one, and 7-oxositosterol, were identified in the enzymatically treated pulps (O−O−L and O−O−L−Q−PoP) together with minor amounts of stigmastanol. Oxidized sterols in the O−O−a pulp, such as 7α- and 7β-hydroxysitosterol and sitostanetriol, were also removed after the laccase-mediator treatment, and small amounts of 7-oxositosteryl 3β-D-glucopyranoside appeared. It is interesting to note that the saturated sterol (stigmastanol), which remained unaffected after oxygen and peroxide stages, was degraded (up to 75%) by the laccase-mediator treatment.

It is possible to conclude that the free and conjugated sterols surviving oxygen delignification were completely

TABLE 1. Composition of Lipophilic Extractives from Eucalypt Pulps after Different Stages of the Laccase-Containing (O−O−L−Q−PoP) and Control (O−O−a−Q−PoP) TCF Sequences (milligrams per kilogram of dried pulp)[a]

	O−O−a	O−O−L	O−O−a−Q−PoP	O−O−L−Q−PoP
total free sterols	140.3	5.5	98.2	3.9
sitosterol	103.1	–	69.3	–
stigmastanol	34.4	5.5	28.1	3.9
fucosterol	2.8	–	0.8	–
total oxidized sterols	11.9	98.5	11.7	49.3
stigmastan-3-one	0.6	3.6	0.2	3.7
stigmasta-3,5-dien-7-one	2.9	24.1	1.1	6.6
7α-hydroxysitosterol	0.8	0.2	2.2	0.2
7β-hydroxysitosterol	1.1	0.1	2.2	tr
sitostanetriol	1.0	1.0	0.5	0.5
7-oxositosterol	5.5	69.5	5.5	38.3
total sterol glycosides	17.1	6.9	5.1	0.9
sitosteryl 3β-D-glucopyranoside	17.1	–	5.1	–
7-oxositosteryl 3β-D-glucopyranoside	–	6.9	–	0.9
sterol esters	95.4	–	41.0	–
steroid hydrocarbons	14.2	2.6	6.1	1.8
total steroids	278.9	113.5	162.1	55.9
total fatty acids	44.5	18.1	3.9	0.7
tetradecanoic acid (FA-14)	2.1	1.7	–	–
palmitic acid (FA-16)	4.8	4.6	0.4	tr
linoleic acid (FA-18:2)	2.9	0.7	–	–
oleic acid (FA-18:1)	1.4	1.1	–	–
stearic acid (FA-18)	2.7	0.5	0.4	tr
eicosanoic acid (FA-20)	2.1	0.5	0.2	tr
docosanoic acid (FA-22)	6.9	2.5	1.2	0.5
tetracosanoic acid (FA-24)	9.4	3.0	1.5	0.2
hexacosanoic acid (FA-26)	9.2	2.8	0.2	tr
octacosanoic acid (FA-28)	3.0	0.7	tr	tr
total ω-hydroxy fatty acids	9.7	1.3	–	–
22-hydroxydocosanoic acid (OH-FA-22)	6.3	1.0	–	–
24-hydroxytetracosanoic acid (OH-FA-24)	1.5	0.3	–	–
26-hydroxyhexacosanoic acid (OH-FA-26)	1.9	–	–	–
total lipids	333.1	132.9	166.0	56.6

[a] Abbreviations: O, oxygen; L, laccase-mediator system; a, control (stage L without laccase and mediator); Q, chelation; PoP, double peroxide with the first step under pressurized oxygen; tr, traces; –, not detected.

removed by the laccase-mediator treatment. It should be mentioned that, although unsaturated lipids can in principle react with oxygen, the composition of eucalypt pulp lipophilic extractives is basically the same before and after the oxygen stage in the bleach plant (*25*). This is probably because oxygen was not able to penetrate into lipophilic aggregates in pulps (*37*). It has been reported that an oxygen partial pressure of >10 kg/cm^2 and temperatures of >150 °C are necessary for the complete degradation of lipophilic extractives in process waters using oxygen (*38*). Since laccases are oxidized by oxygen, which acts as the final electron acceptor (*15*), laccase-based bleaching can be considered as an enzyme-catalyzed oxygen delignification stage. In this sense, it is noteworthy that pulp treatment with the laccase-mediator system enables a removal of lipids under mild treatment conditions that is similar to that obtained using high temperatures and oxygen pressures. This is because the high redox potential of the activated copper species at the enzyme catalytic site enables the complete removal of free and conjugated sterols mediated by the mediator radicals.

The suitability of laccases alone (without a mediator) for the degradation and/or modification of some wood extractives had already been investigated using model compounds (*39*). Moreover, a partial modification of lipophilic extractives in the colloidal fraction from spruce thermomechanical pulping (*11*), the corresponding pulp (*13*), and a lipid dispersion containing unsaturated fatty acids (*12*, *14*) have also been reported, although some results are not conclusive. However, the studies presented here provide the first demonstration of the complete removal of free and conjugated sterols responsible for pitch deposition during TCF bleaching of eucalypt pulp, by an enzymatic treatment that is based on the laccase-mediator system. Moreover, no effect of the laccase alone on pulp free and conjugated sterols was observed.

Pulp and Paper Properties and Sterol Localization after the Enzymatic TCF Sequence. The effect of the laccase-mediator treatment on the distribution of free sterols in eucalypt pulp elements was analyzed by fluorescence microscopy after filipin staining that specifically reacts with 3-hydroxysterols presenting a double bond in carbon 5, such as sitosterol (*28*). This revealed that most of the sitosterol in the oxygen-delignified eucalypt pulp was located inside the ray parenchyma cells (Figure 2A,B), whereas fluorescent signals in fibers were less abundant, and smaller in size and intensity. Similar results were obtained with the final pulp, although a decrease in fiber fluorescence was observed (*28*). A large decrease in the amount of sitosterol by the laccase-mediator system was shown after filipin staining of the treated pulps (Figure 2C,D) compared with the controls (Figure 2A,B). This removal was especially evident in the sitosterol deposits of parenchyma cells that survived the whole TCF bleaching in the control sequence but were removed in the L stage of the enzymatic sequence.

TABLE 2. Composition of Lipophilic Extractives in Bleaching Liquids from Different Stages of the Laccase-Containing (O−O−L−Q−PoP) and Control (O−O−a−Q−PoP) TCF Sequences (milligrams per kilogram of dried pulp)[a]

	O−O−a	O−O−L	O−O−a−Q−PoP	O−O−L−Q−PoP
total free sterols	14.3	tr	11.8	2.8
sitosterol	9.5	–	7.3	tr
stigmastanol	3.5	tr	3.3	2.8
fucosterol	1.3	–	1.2	tr
total oxidized sterols	3.6	14.9	1.8	27.1
stigmastan-3-one	0.2	0.5	0.1	0.3
stigmasta-3,5-dien-7-one	0.2	0.9	0.1	2.2
7α-hydroxysitosterol	0.6	–	0.1	0.4
7β-hydroxysitosterol	1.0	–	0.6	0.2
sitostanetriol	0.8	tr	0.3	0.4
7-oxositosterol	0.8	13.5	0.6	23.6
total sterol glycosides	1.3	1.8	0.8	0.5
sitosteryl 3β-D-glucopyranoside	1.3	–	0.8	–
7-oxositosteryl 3β-D-glucopyranoside	–	1.8	–	0.5
sterol esters	1.1	–	5.3	–
steroid hydrocarbons	0.7	0.5	0.8	1.6
total steroids	21.0	17.2	20.5	32.0
total fatty acids	3.5	4.1	4.0	4.4
tetradecanoic acid (FA-14)	0.3	tr	0.2	0.1
palmitic acid (FA-16)	1.4	1.4	1.7	2.1
linoleic acid (FA-18:2)	tr	tr	0.2	0.2
oleic acid (FA-18:1)	tr	tr	0.3	0.9
stearic acid (FA-18)	0.8	0.8	0.8	0.4
eicosanoic acid (FA-20)	0.1	0.2	0.1	0.1
docosanoic acid (FA-22)	0.2	0.5	0.3	0.3
tetracosanoic acid (FA-24)	0.5	0.7	0.4	0.3
hexacosanoic acid (FA-26)	0.2	0.5	tr	tr
octacosanoic acid (FA-28)	tr	tr	tr	tr
total ω-hydroxy fatty acids	0.7	0.8	0.3	tr
22-hydroxydocosanoic acid (OH-FA-22)	0.1	0.2	tr	tr
24-hydroxytetracosanoic acid (OH-FA-24)	0.4	0.4	0.2	tr
26-hydroxyhexacosanoic acid (OH-FA-26)	0.2	0.2	0.1	tr
total lipids	25.2	22.1	24.8	36.4

[a] For abbreviations, see Table 1.

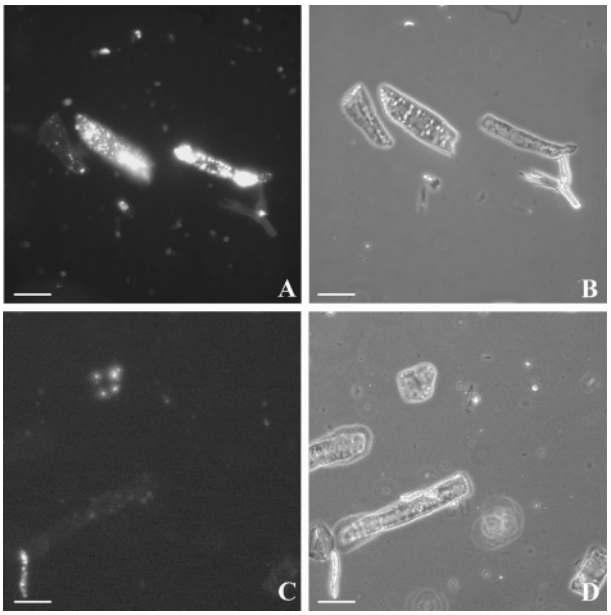

FIGURE 2. Fluorescence microscopy (left) and phase contrast (right) images of oxygen-delignified eucalypt kraft pulp (pitch-containing parenchyma cells) after the laccase-mediator stage (C and D) and the corresponding control without laccase and mediator (A and B). The scale bars are 25 μm.

Some selected properties of the pulps and papers that were obtained were assessed (Table 3). Comparison of the enzymatically treated and control pulps showed that the kappa number, an estimation of lignin in pulp, significantly decreased (from 6.7 to 5.2) after the laccase-mediator treatment, in agreement with previous studies (*24*). Simultaneously, an increase in brightness was produced, reaching 91.2% ISO. This can be considered a high brightness level for TCF-bleached eucalypt pulp. Most paper properties were only barely modified by the laccase-mediator treatment, even when refined at high Schopper-Riegler degrees (Table 3). An increase in air permeability was produced, with a concomitant increase in the scattering coefficient. A direct relationship between the light scattering coefficient and the porosity often exists, since both properties are functions of fiber surface characteristics (*40*). Lipophilic extractives are often responsible for the reduction in paper porosity and optical properties

TABLE 3. Pulp and Paper Properties of Eucalypt Pulps after the Laccase-Containing (O−O−L−Q−PoP) and Control (O−O−a−Q−PoP) TCF Sequences[a]

	laccase sequence	control TCF sequence
kappa number	5.2	6.7
brightness (% ISO)	91.2	87.9
tensile index (N m g^{-1})	92.0	92.5
tear index (mN m^2 g^{-1})	8.6	8.6
Gurley air porosity (s)	290	490
scattering coefficient (m^2/kg)	23.0	21.5

[a] The standard TCF pulp (O−O−Q−PoP) yielded properties similar to those of the control TCF pulp (O−O−a−Q−PoP). The paper properties were estimated in handsheets obtained after 4000 revolution PFI refining, which resulted in a similar refining degree (78−79 °SR) in all cases. For abbreviations, see Table 1.

since they are filling the pores in the paper (*12*). Therefore, an increase in air permeability, which is beneficial for paper drying at the paper machine, can be produced by the laccase-mediator treatment probably due to lipid removal.

Taking together the results of this and other studies (*24*), we are able to conclude that the enzymatic treatment of pulp using laccases in the presence of redox mediators allows the simultaneous removal of residual lignin responsible for pulp color and color reversion and detrimental lipids lowering pulp quality and causing shutdown of mill operation. Moreover, the results obtained here demonstrate that such enzymatic treatment could be integrated in the environmentally sound TCF sequences that represent the future trend in this industrial sector. However, more work is still needed to lower the prices of enzymes, including the use of genetic engineering tools (*22*, *41*), and mediators, including the search for easily available natural mediators among lignin-derived compounds or fungal metabolites (*42*, *43*), and adapt the dose and application conditions to the industrial needs before the industrial implementation of the laccase-mediator system becomes a reality.

Acknowledgments

This study has been funded by Spanish Projects AGL2002-393 and BIO2002-1166, EU projects "New environmentally-sound methods for pitch control in different paper pulp manufacturing processes" (QLK5-99-1357) and "Fungal metalloenzymes oxidizing aromatic compounds of industrial interest" (QLK3-99-590), and two ENCE-CSIC contracts. M. Asther (INRA, Marseille, France) is acknowledged for the *P. cinnabarinus* strain, and Beldem is acknowledged for laccase production. S. Camarero acknowledges a R&C contract of the Spanish MEC, and D. Ibarra and J. Rencoret thank the Spanish CSIC for I3P Fellowships.

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Received for review December 20, 2005. Revised manuscript received February 27, 2006. Accepted March 10, 2006.

ES052547P