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Studying the effects of laccase treatment in a softwood dissolving pulp: Cellulose reactivity and crystallinity

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ABSTRACT

An enzymatic biobleaching sequence (L_{VA}QPO) using a laccase from *Trametes villosa* in combination with violuric acid (VA) and then followed by a pressurized hydrogen peroxide treatment (PO) was developed and found to give high bleaching properties and meet dissolving pulp requirements: high brightness, low content of hemicellulose, satisfactory pulp reactivity, no significant cellulose degradation manifested by α-cellulose and HPLC, and brightness stability against moist heat ageing. The incorporation of a laccase–mediator system (LMS) to bleach sulphite pulps can be a good alternative to traditional bleaching processes since thermogravimetric analysis (TGA) showed that the laccase treatment prevented the adverse effect of hydrogen peroxide on fibre surface as observed during a conventional hydrogen peroxide bleaching treatment (PO). Although VA exhibited the best results in terms of bleaching properties, the performance of natural mediators, such as *p*-coumaric acid and syringaldehyde, was discussed in relation to changes in cellulose surface detected by TGA.

1. Introduction

Dissolving pulps consisting of wood derived celluloses are the main source for the manufacture of viscose rayon and cellulose derivatives, such as cellulose esters and cellulose ethers (Gehmayr, Schild, & Sixta, 2011). Dissolving pulps are defined by high cellulose content and small amounts of residual lignin, extractives and minerals, as well as high brightness and very uniform molecular weight distribution (Ibarra, Köpcke, & Ek, 2010; Ibarra, Köpcke, Larsson, et al., 2010; Sixta, 2006). Besides the mentioned characteristics, reactivity is one of the most important quality parameters of dissolving pulps (Ibarra, Köpcke, Larsson, et al., 2010; Köpcke, Ibarra, & Ek, 2008; Schild & Sixta, 2011). During the last decade, the dissolving pulp production has notably been increased due to a growth of regenerated cellulose fibre production, particularly in Asian countries. Moreover, a declining cotton production due to environmental and agricultural restrictions and the development of new ways of obtaining cellulosic fibres from forest resources are gaining worldwide attention (Sixta, Iakovlev, Testova, & Roselli, 2013) and contributing in this production growth. In this scenario, the introduction of new technologies to bleach dissolving pulps using enzymatic treatments and complemented with treatments based on oxygen-derived compound can be an alternative to traditional bleaching processes, such as elemental chlorine free (ECF) and totally chlorine free (TCF). The laccase–mediator system (LMS) has been widely applied in alkaline pulps (Andreu & Vidal, 2011; Aracri & Vidal, 2011; Bourbonnais, Paice, Freiermuth, Bodie, & Borneman, 1997; Chakar & Ragauskas, 2004; Valls, Cadena, & Roncero, 2013) but has not attracted widespread attention on sulphite pulps.

Recently, laccase–mediator systems have been found to interact with fibre surfaces in various ways; thus, they can deposit onto fibres through condensation, promote grafting of the mediator

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on lignin surfaces or cause oxidative degradation of lignin (Barneto, Aracri, Andreu, & Vidal, 2012). Also, as shown by Saarinen et al. (2009) using a quartz crystal microbalance, and by Barneto, Valls, Ariza, & Roncero (2013) using X-ray photoelectron spectroscopy (XPS) and thermogravimetric analysis (TGA), laccase remains adsorbed on fibre surfaces after the enzymatic treatment. In fact, XPS revealed the presence of increased amounts of nitrogen on fibre surfaces after the enzyme treatment, and TGA large amounts of cellulose that underwent degradation at a low temperature. Thermal degradation of bleached pulp occurs in two stages, namely: degradation of amorphous cellulose at around 300 °C and subsequent degradation of crystalline cellulose with rapid mass loss at a higher temperature level close to 350 °C. The ensuing mass loss derivative curve (DTG) exhibits a typical sharp peak. A chemical or enzymatic treatment (e.g., a laccase bleaching treatment) increases the amount of cellulose that is degraded at a low temperature (i.e., amorphous cellulose) or even splits the peak for crystalline cellulose into two peaks. In this situation, crystalline cellulose in microfibrils loses its identity and behaves as amorphous or "paracrystalline" cellulose (Ioelovich, Leykin, & Figovsky, 2010), even though glucosyl units in microfibrils remain in their original location (Barneto et al., 2013). That is, laccase bleaching modifies the surface but has no effect on micrifibril core, which remains crystalline. The differential behaviour of surface and inner cellulose during thermal degradation make interesting the use of thermogravimetric analysis (TGA) in order to monitor changes in cellulose fibre surfaces during pulping and bleaching (Barneto, Valls, Ariza, & Roncero, 2011). In the present work, TGA is used to monitor changes in fibre surfaces of softwood sulphite pulp with high cellulose content resulted from the action of an enzymatic bleaching treatment. In our previous work (Quintana, Valls, Vidal, & Roncero, 2013), it was demonstrated that violuric acid (VA) in combination with *Trametes villosa* laccase and complemented with a multiple sequential hydrogen peroxide stage reinforced with oxygen, was able to bleach softwood sulphite pulp obtaining excellent bleaching properties. Besides studying cellulose surface modification, the main interest of this study was to examine the resulted biobleached sulphite pulps (L_{VA}QPO) in terms of dissolving pulp characteristics in order to satisfy the market-like requirements.

2. Materials and methods

2.1. Raw material

Unbleached sulphite cellulose, cooked at Domsjö mill (Sweden), was used as raw material. The initial pulp was a mixture of 60% spruce (*Picea abies*) and 40% pine (*Pinus sylvestris*). Prior to bleaching treatments, fibre samples were conditioned at pH 4 adjusted with H_2SO_4, stirred at 2% (w/w) pulp consistency for 30 min and washed with de-ionized water in a glass filter funnel.

2.2. Laccase–mediator treatments and hydrogen peroxide treatment (P stage)

Laccase from *Trametes villosa* was used in combination with the natural mediators syringaldehyde (SA) and *p*-coumaric acid (pCA), and also with the synthetic mediators 1-hydroxybenzotriazole (HBT) and violuric acid (VA). The enzyme was supplied by Novozymes® (Denmark) and had a laccase activity of 588 U/mL. The mediators were purchased from Sigma–Aldrich and were used as received. Unbleached sulphite cellulose was treated with a laccase–mediator system (LMS) consisting of the enzyme and either a synthetic or a natural mediator. A laccase control treatment (KL, without mediator) and a control treatment (K, without mediator and laccase) were conducted in parallel under the same conditions. All treatments were performed in an oxygen pressurized reactor (0.6 MPa) at stirring rate of 30 rpm, using 50 mM sodium tartrate buffer (pH 4) to adjust 5% (w/w) pulp consistency. The enzyme dose was 20 U/g odp (oven-dry weigh of pulp) of laccase and 1.5% odp of each mediator at 50 °C for 4 h. After treatment, pulp samples were filtered and extensively washed for further processing. Then, a simple alkaline hydrogen peroxide stage was performed after each laccase–mediator treatment. The conditions used were 2% odp H_2O_2, 1.5% odp NaOH, 1% odp DTPA (diethylenetriaminepentaacetic acid) and 0.2% odp $MgSO_4$ in a Datacolor Easydye AHIBA oscillating individual reactor at 90 °C for 2 h at 5% consistency (Quintana et al., 2013). After the bleaching stage, pulp samples were filtered and extensively washed for further processing. A conventional hydrogen peroxide bleaching treatment (i.e., a P stage directly applied to the initial pulp) was also performed in order to compare the bleaching efficiency of the laccase–mediator system in combination with a hydrogen peroxide bleaching stage.

2.3. Biobleaching sequence (LQPO)

Based on the results of the preliminary bleaching treatments, a complete biobleaching sequence using only violuric acid as mediator was studied in order to more accurately assess the performance of LMS in combination with a pressurized hydrogen peroxide treatment. The biobleaching sequence was designated LQPO, where L denotes an enzymatic stage, Q a chelating stage and PO a pressurized hydrogen peroxide bleaching treatment. The enzymatic stage (L) was carried out with the laccase–violuric acid system (Lac–VA), using the same conditions described in Section 2.2. The enzymatic treatment was followed by a Q stage involving the use of chelating agents to reduce the contents in metal ions (Fe^{2+}, Cu^{2+}, Mn^{2+}) capable of degrading the bleaching agents and cellulose during the subsequent peroxide bleaching treatment (Heijnesson, Simonson, & Westermark, 1995). The Q stage was performed with 1% odp DTPA at 5% consistency at pH 5–6 (adjusted with H_2SO_4 1 N) in polyethylene bags at 85 °C for 1 h. The biobleaching sequence was completed with a chemical bleaching stage involving an alkaline hydrogen peroxide bleaching procedure (PO) which consisted of a multiple sequential steps. PO was carried out at 5% consistency in oxygen pressurized (0.6 MPa) reactor, using a stirring rate of 30 rpm under the following conditions: 1.5% odp NaOH, 0.3% odp DTPA and 0.2% odp $MgSO_4$ at 90 °C for 4 h. This stage was performed in three consecutive steps (PO_1 = 1 h reaction, PO_2 = 1 h reaction, PO_4 = 2 h reaction) each involving the addition of 10% odp H_2O_2 and no interstep washing. A small amount of pulp was withdrawn after each PO step. The residual liquors were collected and the pulps were extensively washed with de-ionized water in a filter funnel for subsequent analysis. The results were compared with those for a control sequence (KQPO) without laccase and mediator in order to confirm that neither the buffer suspension nor the incubation time had any effect on the process. The operating conditions of each sequence are described in detail elsewhere (Quintana et al., 2013). In addition, the effect of an enzymatic stage was assessed via a conventional treatment; thus, the enzymatic stage was omitted and a chemical hydrogen peroxide (PO) treatment was directly applied to the initial pulp under the same conditions followed in the enzymatic sequence.

2.4. Characterization of enzyme-treated pulp: surface and bleaching properties

The effects of the bleaching process produced on fibre surfaces were examined by thermogravimetric analysis. Thermogravimetric runs were carried out on a Mettler Toledo TGA/SDTA851e/LF1600 thermobalance, using samples of ca. 5 mg. Pyrolysis and combustion runs were performed in nitrogen and synthetic air (N_2:O_2 4:1),

respectively. Samples were heated from 25 °C to 900 °C at a rate of 5, 10 or 20 °C/min.

XRD measurements were performed on a SIEMENS D-500 BRAG-BRENTANO θ/2θ geometry X-ray diffractometer with Cu $K\alpha_1$ radiation (λ = 0.15418 nm) at 40 kV and 30 mA. A divergence aperture of 0.3° and a reception aperture of 0.05° were used. Sweeps of 5° to 5° 2θ were made with a step size of 0.05° and a step time of 10 s. The experimental XRD signal was fitted to Gaussian distributions, which include an amorphous background. Pulp crystallinity was assessed from the ratio between the area of the crystalline cellulose peaks and the total area, which included the amorphous background contribution (Andersson, Wikberg, Pesonen, Maunu, & Serimaa, 2004).

Fibre optical properties were measured in terms of brightness and in terms of the CIE $L^*a^*b^*$ colour coordinates, namely: lightness (L^*), red–green (a^*) and yellow–blue (b^*). Chroma (C^*), which is the perpendicular distance of a point from the lightness axis [$C^* = (a^{*2} + b^{*2})^{1/2}$] and represents the amount of colour "saturation" of a sample, was also used to characterize the bleaching process.

Initial and treated pulp samples were characterized in terms of kappa number, brightness and viscosity conducted in a dilute cupriethylendiamine (CED) solution according to ISO 302:2004, ISO 2470:2009 and ISO 5351:2004, respectively. All analyses were carried out in duplicate. Biobleached fibre samples were subjected to accelerated thermal ageing treatment by moist heating (at 80 °C and 65% RH) in a HC 2020 Heraeus-Vötsch climatic chamber according to ISO 5630-3:1996. The resulting Brightness Loss Index (BLI) was calculated from the following equation (Eq. (1)), where Br was the brightness value:

$$BLI(\%) = \frac{Br_{oh} - Br_{144h}}{Br_{oh}} \quad (1)$$

2.5. Characterization of biobleached pulp: chemical properties

The cellulose reactivity of the pulp samples was determined according to slightly modified version of Fock's method (Fock, 1959; Ibarra, Köpcke, Larsson, et al., 2010). This is a micro-scale process simulating the industrial viscose process for manufacturing regenerated cellulose. Prior to analysis, the samples were dried at 50 °C and conditioned in a climate room at 23 °C and 50% RH overnight. In the first step, cellulose is soaked in alkali media and then transformed to cellulose xanthate with CS_2, which makes the cellulose polymer soluble. Then, $-CS_2$ groups were removed in dilute sulphuric acid to obtain re-precipitated cellulose fibres. Finally, regenerated cellulose was oxidized with $K_2Cr_2O_7$, refluxed and titrated with $Na_2S_2O_3$.

Reactivity was expressed as regenerated cellulose yield (Eq. (2)):

$$R = (100) \cdot 9.62^a \frac{M\left(V_1C_1 - (V_2C_2 100/40^b)/6\right)}{4Y} \quad (2)$$

where R denotes reacted cellulose (%), Y sample weight (g), M the molecular mass of glucopyranosyl residues ($C_6H_{10}O_5$, 162 g/mol), V_1 the volume of titrant ($Na_2S_2O_3$, L), C_1 the concentration of $K_2Cr_2O_7$ (mol/L), C_2 that of $Na_2S_2O_3$ (mol/L), a the first dilution to 100 g and withdrawal of 10 mL (10.4 g = 100/10.4 = 9.62), and b the second dilution of the sample to 100 mL and withdrawal of 40 mL = 100/40. The respective reactivity results were calculated from ten repetitions for each sample.

α-, β- and γ-celluloses were determined according to TAPPI method T 203 cm-09. Biobleached pulp samples were extracted with 17.5% and 9.45% sodium hydroxide solutions at 25 °C. The soluble fraction, consisting of β- and γ-cellulose, was analyzed volumetrically by oxidation with potassium dichromate, and the α-cellulose, as an insoluble fraction, was calculated by difference. Alkali resistance (R10 and R18) was determined according to TAPPI standard T 235 cm-09. All determinations were performed in duplicate.

The carbohydrate composition of the initial pulp and the respective treated samples was determined by high performance liquid chromatography (HPLC). Two replicates of the resulting samples were hydrolysed by using a modified version of the TAPPI test method T 249 cm-09. The hydrolysis process involved the following two steps: (*a*) *Pre-hydrolysis with concentrated sulphuric acid.* Approximately 50 mg of sample was placed in a test tube and soaked with 5 mL of 72% w/w sulphuric acid, after which the tube was placed in a water bath at 30 ± 0.5 °C for 1 h with occasional stirring. (*b*) *Final hydrolysis with dilute sulphuric acid.* The tube contents were washed in a 250-mL flask in order to obtain a final solution 4% w/w in sulphuric acid and the flask was placed in an autoclave at 103 ± 7 kPa for 1 h. Once the reaction was completed, the specimen solution was cooled at room temperature and passed through a Gooch filter No. 4 to remove lignin insoluble in sulphuric acid, which was taken to represent Klason lignin. Prior to HPLC analysis, the solution was filtered through a Whatman membrane of 0.45 μm pore size. The equipment used consisted of an Agilent Technology 1200 series equipped with a refractive index detector (RID) with an Aminex HPX-87H column operated at 60 °C with a mobile phase consisting of 6 mM sulphuric acid pumped at a rate of 0.7 mL/min. Measurements were interpolated into calibration curves run from standards of glucose, rhamnose, arabinose and xylose (all purchased from Sigma–Aldrich). Because the column failed to resolve xylose, mannose and galactose, their combined content was expressed as xylose (Garrote et al., 2001).

The determination of calcium, iron and manganese in pulps was conducted by atomic absorption spectroscopy according to SCAN-CM 38:87 and was carried out in duplicate. The samples were incinerated and charred at 575 °C for a period of 3 h. The charred residue was treated with 6 M hydrochloric acid and the acid-soluble element content in the solution was determined by flame atomic absorption spectroscopy. Previously to the measurements, standard solutions of calcium, iron and manganase at five different concentrations were used for the construction of the calibration graph of each metal.

3. Results and discussion

3.1. Characterization of biobleached pulp: bleaching properties

As reported in Quintana et al. (2013), violuric acid (VA) proved to be the most efficient mediator for a biobleaching treatment, among other studied mediators such as hydroxybenzotriazole (HBT), syringaldehyde (SA) and *p*-coumaric acid (pCA). Moreover, Lac-VA treated pulps exhibited a potential application for dissolving pulp or cellulose derivatives production. In view of these results, in the present work a complete biobleaching sequence designated L_{VA}QPO was conducted and compared with a simple hydrogen peroxide treatment (PO). A control sequence (KQPO) was used as a reference treatment. The pulp resulting from each sequence was characterized in terms of dissolving pulp characteristics (viz., α-cellulose, Fock's solubility, alkali resistance (R18 and R10), content in metal ions and carbohydrate composition). The changes that each bleaching stage produced in cellulose fibres were assessed by thermogravimetric analysis. In addition, in order to evaluate the effectiveness of bleaching process, standard bleaching properties (viz., kappa number (KN), viscosity and brightness loss by moist heat ageing treatment) were also studied.

Fig. 1a illustrates the delignification effect of each bleaching stage. The greatest delignification was obtained with the enzymatic sequence (L_{VA}QPO), followed by the control sequence (KQPO) and then the conventional sequence (PO). The low delignification achieved with the buffer treatment (13%) in the control

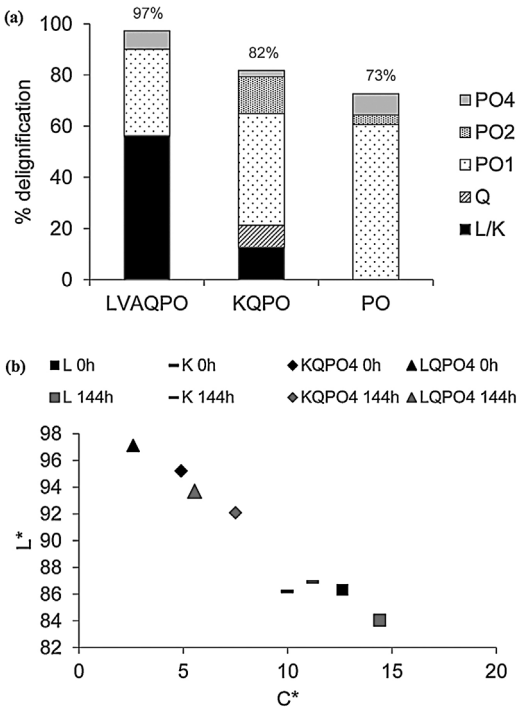

Fig. 1. (a) Contribution of each bleaching stage to delignification for the different bleaching sequences: $L_{VA}QPO$ (enzymatic treatment), KQPO (control treatment) and PO (conventional treatment). Percentages indicate the total degree of delignification obtained with each bleaching sequence with respect to initial pulp. (b) Effect of accelerated ageing by moist heating treatment on the CIE L^*C^* colour coordinates for pulps obtained with the enzymatic (L), control (K), $KQPO_4$ and $L_{VA}QPO_4$ treatments. Black and grey symbols represent values before and after a moist heating ageing treatment, respectively.

treatment suggested a marked boosting effect of LMS on lignin removal – the enzyme–mediator stage accounted for 43% of delignification. The hydrogen peroxide treatment enhanced delignification in all sequences and especially during the first addition of the reagent. Interestingly, the low lignin content after the enzymatic stage (KN = 2.5) led to substantially improved brightness. Laccase in combination with HBT or VA causes that hydrogen peroxide is consumed mainly to oxidize chromophoric groups during the P stage, thereby leading to increased brightness (Moldes & Vidal, 2008). No such effect was observed in the first hydrogen peroxide step of the conventional sequence since the pulp contained more amount of lignin (KN = 5.3); therefore, hydrogen peroxide added in the PO_1 step acted as a delignifying agent rather than as a bleaching agent.

Regarding cellulose integrity during the bleaching process, the enzymatic sequence resulted in higher cellulose losses than the conventional sequence. The final viscosity for $LQPO_4$, $KQPO_4$ and PO_4 was 343 ± 12.5, 420 ± 31.5 and 455 ± 15 mL/g, which corresponds to a cellulose preservation at the end of the bleaching process of 62%, 76% and 82%, with respect to the initial viscosity value; but, the final pulp ISO brightness differed from 5% between sequences, which was considered a significant dissimilarity (89.14%, 84.40% and 84.00%, respectively). Thus, on a similar brightness level (e.g., about 84%ISO), pulp viscosity fell in the range 413–455 mL/g with all bleaching sequences. However, it should be noted that the reference value of 84% ISO brightness was obtained under different operating conditions (viz., H_2O_2 dose and reaction time) for each bleaching sequence. Overall, at a given value of 84%ISO brightness, the use of laccase–VA system saved about 70% of hydrogen peroxide and 2 h of reaction time with respect to the conventional bleaching sequence (PO) (Quintana et al., 2013).

Accelerated pulp ageing was conducted in order to assess the effect of an enzymatic treatment on the stability of optical properties. Pulp brightness was reduced by 10.1% with the L_{VA} treatment, but only by 0.3% with the buffer stage (K). Cadena, Vidal, & Torres (2010) postulated that hexenuronic acids (HexA) contributed to 69% brightness reversion in eucalyptus TCF pulp during an accelerated ageing. However, the pulp used in this study contained no HexA, so the brightness loss observed after the L_{VA} stage suggests that the enzymatic treatment produces chromophores or oxidizable structures that boost reversion of optical properties. In fact, the chroma coordinate was increased an 18% by L treatment while after the buffer stage (K) was reduced by a 3.5%, with respect to initial chroma value and before the ageing treatment (Fig. 1b). Other authors also related (Aracri, Colom, & Vidal, 2009; Moldes & Vidal, 2008) the changes in the CIE L^*C^* colour coordinates to the formation of chromophoric groups. In addition, Chakar and Ragauskas (2001) studied the relative amounts of quinones in residual lignins isolated from a softwood kraft pulp before and after LMS treatments; and the P NMR spectral analyses confirmed the formation of quinones from $LMS_{VA,NHA,HBT}$. Importantly, the total brightness loss detected for $L_{VA}QPO_4$ and $KQPO_4$ samples by effect of accelerated ageing was 13.0% and 12.2%, respectively. This result indicates that chromophoric groups formed during the laccase treatment were removed or altered during the hydrogen peroxide stage. Interestingly, the complete biobleaching sequence ($L_{VA}QPO$) led to the highest value of brightness without a detriment to brightness stability. Fig. 1b represents the CIE L^*C^* colour coordinates before and after the accelerated ageing treatment. After 144 h of ageing treatment, chroma (C^*) and lightness (L^*) coordinates of control-treated pulp (K) remained constant while the enzymatic-treated pulp (L_{VA}) suffered an increase in C^* and a diminution in L^* due to the quinones or carbonyl species generated during the L stage. As observed with brightness loss index, although the enzymatic sequence ($L_{VA}QPO_4$) led to lower L^* and higher C^* values than control sequence ($KQPO_4$), both sequences experimented similar changes. As a conclusion, it can be said that the introduction of an L stage had no negative impact on the final brightness result of the whole bleaching sequence.

In general, the enzymatic treatment presented satisfactory results in terms of bleaching-related pulp properties and the introduction of an L stage in the bleaching process provided a reduction in hydrogen peroxide dose with respect to conventional bleaching treatment. However, the results were not conclusive enough as regards dissolving pulp characteristics, so further study was needed. In the following part of the work, the quality of dissolving pulp was assessed mainly via carbohydrate composition, viscosity, Fock solubility, alkali resistance (R18 and R10) and metal ion content. Therefore, $L_{VA}QPO_4$, $KQPO_4$ and PO_4 samples were selected and subjected to an extended study.

3.2. Characterization of biobleached dissolving pulp: chemical properties

As can be seen from Table 1, the carbohydrate composition of the bleached pulp samples was similar in all cases and essentially the same as in the initial pulp. This result was to be expected since, unlike cellulases or xylanases, a laccase–mediator system acts on the diminution of lignin content rather than on the hemicellulose content of the pulp (Gübitz, Lischnig, Stebbing, Saddler, & Gÿbitz, 1997; Ibarra, Köpcke, & Ek, 2010; Köpcke et al., 2008). However, it is important to emphasize that the hemicellulose content fell in the acceptable range for commercial dissolving pulp (<10%) (Christov, Akhtar, & Prior, 1998; Köpcke, 2010) in all cases.

Another important property of dissolving pulp is reactivity, which was previously quantified after the application of a cellulase or xylanase treatment by several authors (Ibarra, Köpcke, & Ek, 2010; Ibarra, Köpcke, Larsson, et al., 2010; Köpcke et al., 2008; Köpcke, 2010). In this work, the Fock's solubility of

Table 1
Mean values (± standard deviation) of carbohydrate composition, ISO brightness and kappa number for the different bleaching treatments.

		Initial	L_{VA}	$KQPO_4$	$L_{VA}QPO_4$	PO_4
Glucan	%	91.1 ± 0.53	91.9 ± 0.44	91.9 ± 1.48	92.6 ± 0.15	92.7 ± 0.14
Xylan	%	6.5 ± 0.09	6.9 ± 0.35	7.03 ± 1.11	6.5 ± 0.12	6.5 ± 0.17
Arabinan	%	–	0.13 ± 0.02	0.33 ± 0.28	0.06 ± 0.00	0.08 ± 0.01
Glucuronic acid	%	0.76 ± 0.01	0.66 ± 0.17	0.60 ± 0.05	0.03 ± 0.26	0.48 ± 0.15
ISO brightness	%	58.75 ± 0.6	55.43 ± 0.4	84.40 ± 0.2	89.14 ± 0.1	84.00 ± 0.1
Kappa number	–	5.3 ± 0.1	2.33 ± 0.1	0.97 ± 0.21	0.15 ± 0.0	1.46 ± 0.0

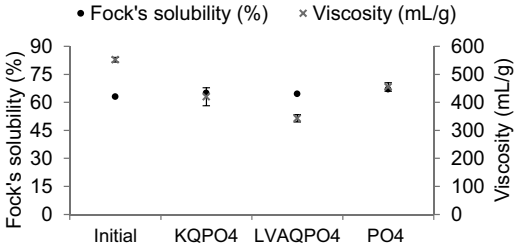

Fig. 2. Viscosity (± standard deviation) and Fock's solubility obtained with the different bleaching treatments: initial (unbleached sulphite pulp), $KQPO_4$ (control treatment with no laccase or mediator), $L_{VA}QPO_4$ (enzymatic stage plus pressurized hydrogen peroxide stage) and PO_4 (pressurized hydrogen peroxide stage or conventional treatment). (The confidence interval of reactivity values was <8, with and alpha of 0.05).

Table 2
Characteristic parameters used to describe market-like dissolving pulp. Initial (unbleached sulphite pulp), $L_{VA}QPO_4$ (enzymatic stage plus pressurized hydrogen peroxide stage), $KQPO_4$ (control treatment without laccase and mediator) and PO_4 (pressurized hydrogen peroxide stage or conventional treatment). The standard deviations were below ±0.7 in all cases.

		Initial	$L_{VA}QPO_4$	PO_4
R10	%	88.7	86.1	85.2
R18	%	90.3	86.6	90.2
S18	%	9.7	13.4	9.8
		Initial	$KQPO_4$	$L_{VA}QPO_4$
α-Cellulose	%	88.3	88.1	87.6
β-Cellulose	%	7.5	7.0	5.8
λ-Cellulose	%	4.2	5.4	6.1

laccase-bleached pulp was analyzed. As can be seen from Fig. 2, no substantial differences in the amount of reacted cellulose between bleaching treatments were observed. According to Gehmayr and Sixta (2012), reactivity measured with Fock's test represents the amount of pulp that is dissolved in the viscose-like solution. Consequently, the results are strongly influenced by pulp viscosity and by the amount of alkali-soluble hemicellulose present, which might be misleading in terms of cellulose reactivity (Gehmayr & Sixta, 2012). Therefore, the little change observed can be explained in this way. Therefore, accurately comparing our bleaching sequences in this respect was made difficult by the fact that viscosity differed markedly although alkali solubility only moderately between bleaching sequences. In particular, the enzymatic sequence experimented a 2.5% of reactivity increase, followed by the control treatment with a 3.0% and then the conventional hydrogen peroxide treatment with a 6.1% of reactivity improvement, with respect to initial pulp. However, this trend should be taken in caution since enzymatic sequence suffered the highest viscosity loss, 38%, while the control sequence a 24% and the conventional treatment only an 18%, with respect to initial pulp. However, all the Fock's solubility values are similar to those obtained by Köpcke et al. (2008) for commercial dissolving pulp. In general, the conventional hydrogen peroxide bleached pulp (PO_4) had similar chemical properties (lignin content, brightness and viscosity) as control treated pulp ($KQPO_4$) and also a comparable Fock's solubility. On the other hand, Fock's solubility and viscosity results obtained with the enzymatic sequence ($L_{VA}QPO_4$) were slightly lower than PO_4, $KQPO_4$ but the enzymatic treated pulp reached the highest final brightness value.

Table 2 summarizes other interesting properties of dissolving pulp. Among chemical properties, alkali solubility is a measure of cellulose degradation, and also a loss or retention of hemicellulose during pulping and bleaching processes. In general, in 18% NaOH (S18) the hemicellulose is soluble, whereas in 10% NaOH (S10) low-molecular weight cellulose (degraded cellulose) and the hemicellulose are dissolved (Tappi T-235 cm-00). In other words, alkali resistance can be defined as the fraction of pulp that is insoluble in sodium hydroxide at different concentrations (R18 = 100–S18 and R10 = 100–S10). On the other hand, α-cellulose is defined as the residual portion which is insoluble in 17.5% NaOH. This α-cellulose has a high molecular weight. Likewise, β-cellulose is the alkali-soluble fraction of pulp, which consists mainly of hemicelluloses that are insoluble under slightly acidic conditions (pH 2.5). Based on the foregoing, a relationship between α-cellulose and alkali resistance (R18) can be established; similarly, alkali solubility (S18) can be related to hemicellulose and β-cellulose fraction. However, as can be seen from Tables 1 and 2, the concentration of hemicellulose (xylan and arabinan) failed to match pulp solubility in 18% NaOH (S18), but did the β-cellulose fraction with slight difference in $L_{VA}QPO_4$ treatment. Therefore, solubility of the pulps was mainly influenced by hemicellulose and cellulose of short chain. In addition, the small differences in β-cellulose fraction, and hence in S18, during the bleaching process, can be ascribed to differences in carboxylic acid content. By contrast, α-cellulose contents were similar with all bleaching treatments and consistent with carbohydrate composition (particularly with glucan fraction).

The X-ray diffraction (XRD) technique measures the proportion of crystalline material in cellulose. Deconvoluting X-ray signals and isolating the contribution of amorphous cellulose led to crystallinity values of 64 ± 2% for all treated pulp samples (data not shown). A high value of crystallinity was obtained since crystallinity in bleached pulp typically ranges from 52% to 65% (Andersson et al., 2004; Leppänen et al., 2009). The X-ray results confirmed that an enzymatic bleaching treatment was essentially a surface process (Roncero, Torres, Colom, & Vidal, 2000) that causes virtually no change in fibre chemical composition or crystalline cellulose integrity. In other words, there was no alteration of the planes of anhydroglucose units inspected by the X-ray technique, so pulp crystallinity remained unchanged during the bleaching treatment.

The content in metal ions of dissolving pulp is also very important because a high concentration can affect the processability of cellulose derivatives (Table 3). The fact that the calcium and iron contents of the control and enzymatically treated pulp samples were similar suggests that the L stage does not alter pulp composition. Also, none of the samples was found to contain manganese at any stage during the bleaching process.

Table 3
Contents in metal ions (± standard deviation) of pulp subjected to different bleaching treatments. Nd: not detected. $KQPO_4$ (control treatment without laccase and mediator); $L_{VA}QPO_4$ (enzymatic stage plus pressurized hydrogen peroxide stage.

		$KQPO_4$	$L_{VA}QPO_4$
[Ca]	mg/kg pulp odp	177 ± 110	189 ± 0
[Fe]	mg/kg pulp odp	479 ± 186	519 ± 0
[Mn]	mg/kg pulp odp	Nd	Nd

3.3. Effect of the biobleaching sequence on fibre surface

Pulps biobleached with the laccase–VA system and complemented with a hydrogen peroxide treatment resulted in good dissolving pulp characteristics meeting market-like requirements. The surface changes of the pulp during the biobleaching treatment were assessed using thermogravimetric analysis.

TGA is sensitive to chemical changes in fibre surfaces because such changes can alter the thermal degradation path of pulp. Consequently, if a bleaching process results in enzyme adsorption or surface oxidation, then the hydrogen bond network which protected the surface of crystalline cellulose will be partially broken. In order to understand the effect of laccase adsorption on the thermal degradation of pulp requires considering that fibres contain both amorphous and crystalline cellulose. Cellulose is organized as long bundles of cellulose polymer chains called microfibrils, which includes crystalline and amorphous zones (Tsuji, Nakao, Hirai, & Horii, 1992). In the crystalline cellulose region, cellulose chains form planes where equatorial hydroxyl groups in pyranose rings are hydrogen-bonded. The amorphous regions, which consists of non-ordered cellulose chains, are located mainly between cellulose crystallites (Nishiyama et al., 2003).

As can be seen from Fig. 3a and b, the pulp underwent surface changes in the course of biobleaching process. A comparison of the initial and PO_4 curves in Fig. 3a reveals that the hydrogen peroxide treatment damaged fibre surfaces considerably. In fact, this bleaching step caused a substantial decrease in the crystalline cellulose peak, which suggested alteration of fibre crystalline surfaces. In addition, the region from 300 to 325 °C in the PO_4 curve fell above the curve for the initial pulp, thus indicating that the hydrogen peroxide treatment increased the amount of cellulose volatilizing at low temperatures (i.e., amorphous and paracrystalline cellulose). These alterations of pulp may have resulted from alkaline depolymerization by effect of β-elimination reactions breaking bonds between adjacent glucosyl units (Barneto et al., 2011). These results differed from with others obtained for treated eucalyptus and kenaf pulp, where a hydrogen peroxide treatment had a substantial cleaning effect (viz., removing deposits on the cellulose surface) and led to pulps giving sharper peaks than the initial or LMS-treated pulps (Andreu, Barneto, & Vidal, 2013; Barneto et al., 2013). The absence of residual lignin and low content of hemicellulose manifested by KN and HPLC analyses, may account for such an important difference. As viscosity values showed, hydrogen peroxide caused a marked degradation of cellulose chains and TGA detected the actual surface changes since no deposits were removed. However, although an alkaline hydrogen peroxide treatment damages fibre surfaces, when pulps were previously treated with a laccase–mediator system or buffer solution resulted in some peculiarities. Contrasting the $KQPO_4$ and $L_{VA}QPO_4$ curves in Fig. 3b with the PO_4 curve revealed that the former two fell below the latter at low temperatures but above it at high temperatures. These results mean that the reduction in crystalline cellulose by effect of the hydrogen peroxide was less marked when the pulp was previously treated with laccase–VA–tartrate or tartrate alone. As noted earlier, the buffer seemingly has a surface protective effect on this type of pulp that is strong enough to prevent a subsequent adverse effect of hydrogen peroxide.

Table 4
Delignification and colour (as defined in terms of chroma) of pulp samples from the L and P stages. K, control treatment with no laccase or mediator; KL, laccase control treatment; P, hydrogen peroxide stage directly applied to the initial pulp. (–) The analysis was not performed.

	L stage		P stage	
	Delignification (%)	C^*	Delignification (%)	C^*
K	13	10.70	–	–
KL	4	9.53	55	6.71
PCA	9	13.26	69	7.52
SA	16	13.36	60	9.43
HBT	26	12.26	79	6.67
VA	74	12.21	86	6.61
P	–	–	17	8.83

Putting together TGA results and the above-described chemical properties, the initial pulp exhibited the highest viscosity value but also the lowest Fock solubility result. These observations can be explained by the fact that initial pulp contained more amount of crystalline cellulose than treated pulps, as TGA curves demonstrated. Although the PO_4 and $KQPO_4$ treatments led to pulp with similar chemical properties, $KQPO_4$ fibres had a "cleaner" surface judging by the TGA results. On the other hand, treated biobleached pulp ($L_{VA}QPO_4$) exhibited a good Fock solubility value, an acceptable viscosity result and, even more important, better surface properties than PO_4-treated pulp and bleached commercial dissolving pulp.

3.4. Influence of the mediator on fibre surfaces

As demonstrated in Quintana et al., 2013, the bleaching efficiency of *Trametes villosa* laccase in combination with the natural mediators, *p*-coumaric acid (PCA) and syringaldehyde (SA), on unbleached sulphite pulp was very low and did not present potential capacity for meeting dissolving pulp characteristics. The differences in bleaching properties between natural and synthetic mediators can be explained by particular changes on fibre surface. Therefore, this part of the work intends to elucidate the changes that cellulose underwent due to the action of LMS during a bleaching process, using a thermogravimetric technique.

As can be seen from Fig. 4, the initial pulp exhibited a sharp peak around 300–350 °C indicating a high content in crystalline cellulose. The peak was sharper than the typical peaks for unbleached kenaf (Andreu et al., 2013) and unbleached eucalyptus pulps (Barneto et al., 2013), and suggestive of dissolving pulp composition. Clean and crystalline cellulose are thermally degraded at high temperatures spanning a narrow range because ordered cellulose chains yield crystallites that are protected from external attack by a surface hydrogen bond network (Barneto et al., 2011). The control treatments, which included (KL) or excluded laccase (K), caused no change in chroma value as observed in Table 4. Also, based on the TGA results, fibres resulted from control treatment appeared more "crystalline" since the mass loss rate was higher at high temperatures. This fact contrasts with that obtained with kenaf pulp and eucalyptus pulp (Andreu et al., 2013; Barneto et al., 2013), where laccase or buffer were adsorbed onto fibre surfaces and increased the amount of "paracrystalline" cellulose. Seemingly thus, tartrate buffer can be adsorbed on pulp surfaces and alter their thermal degradation path by effect of the formation of new hydrogen bonds between lone electron pairs in oxygen atoms from the reagents (carboxyl group) and hydrogen atoms in hydroxyl groups from cellulose molecules (Barneto et al., 2013). Based on our results for biobleached softwood sulphite pulp, the buffer solution seemed to produce a cleaning effect on fibre surfaces rather than being adsorbed onto them.

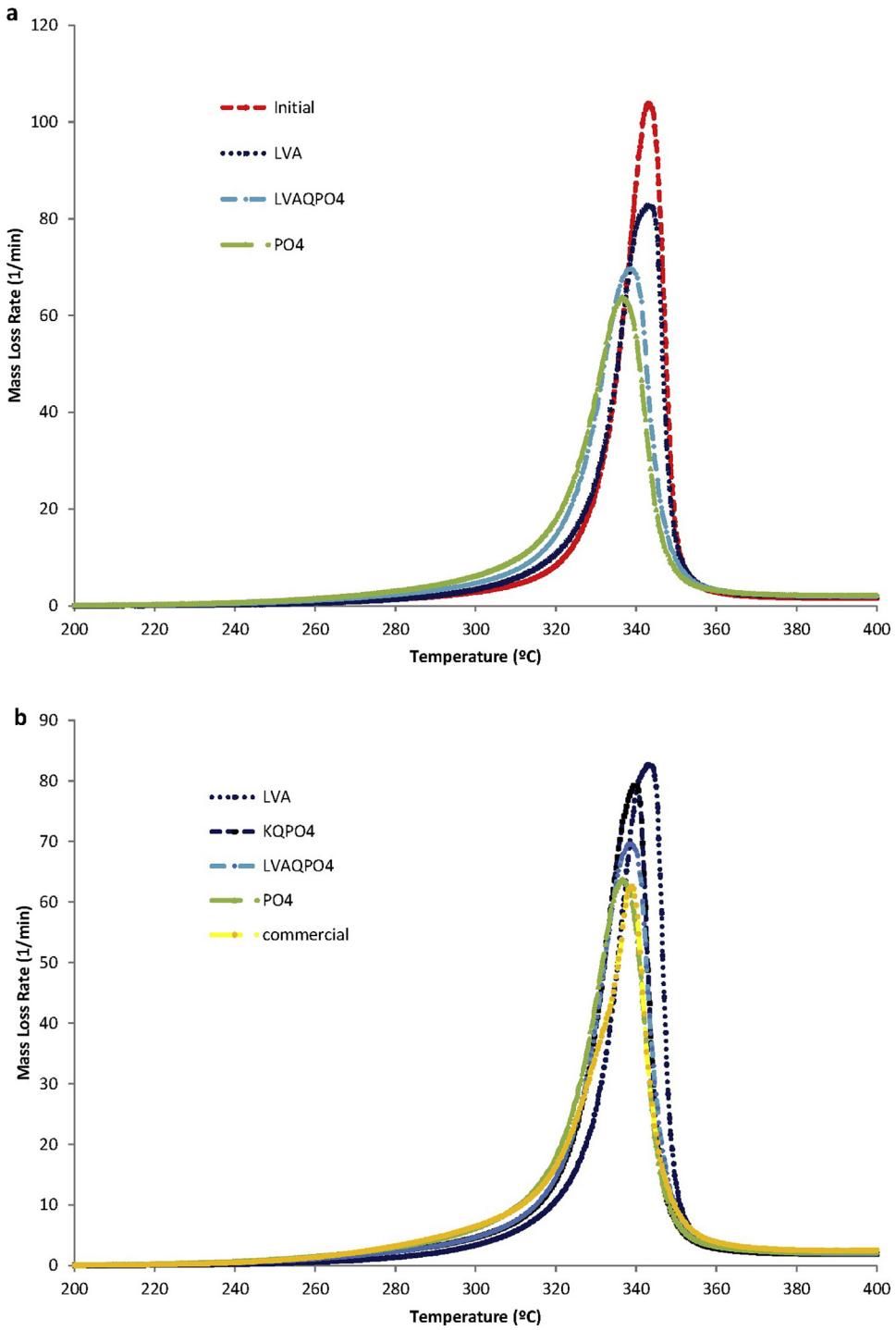

Fig. 3. DTG curves for pulp samples at different stages of the biobleaching process. Heating rate, 20 °C/min and the environment was in air. Initial (unbleached sulphite pulp), L_{VA} (enzymatic treatment), $L_{VA}QPO_4$ (enzymatic stage plus pressurized hydrogen peroxide stage), PO_4 (pressurized hydrogen peroxide stage or conventional treatment) and commercial bleached dissolving pulp. The mass loss rate was normalized to the initial mass of sample.

As shown in Fig. 4, the amount of cellulose degrading at a low temperature increased by effect of a laccase treatment in combination with a mediator in the following order: *p*-coumaric acid < violuric acid < syringaldehyde < hidroxybenzotriazole. In the same way, the DTG curves of pulp samples treated with the mediators SA, PCA and VA were similar to that for the initial pulp, but the mass loss rate resulting from the laccase–HBT treatment was considerably shifted to lower temperatures and its maximum much lower.

Combining TGA results and pulp optical properties revealed that dissolving pulps subjected to a LMS treatment presented a particular behaviour. In the case of natural mediators such as syringaldehyde (SA), laccase is known to promote condensation of mediator molecules onto fibre surfaces in eucalyptus (Valls, Vidal, & Roncero, 2014), flax (Fillat, Roncero, & Vidal, 2011) and kenaf pulp (Barneto et al., 2012); however, only non-wood pulp (flax and kenaf) has been found to undergo substantial grafting – as reflected in an increased kappa number. As noted earlier, the TGA curves

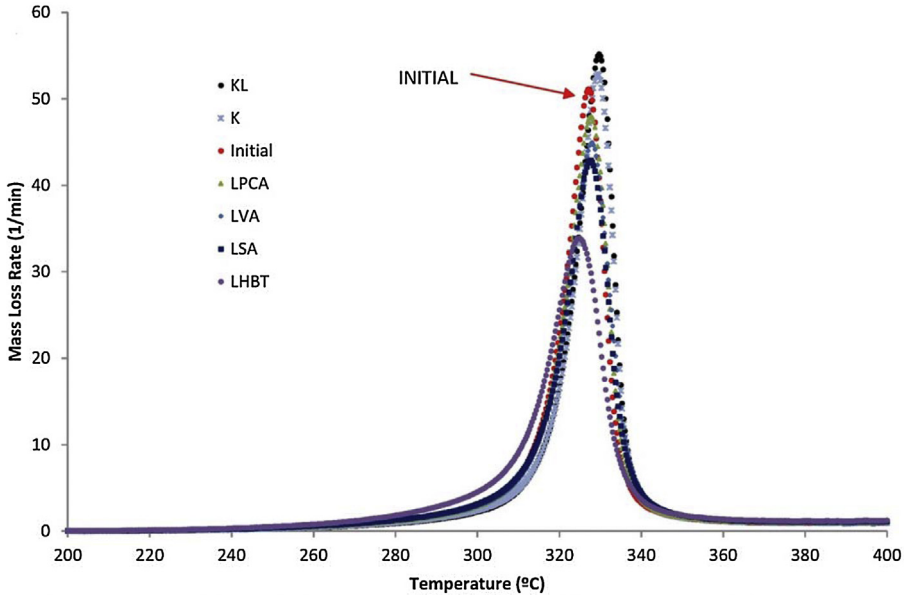

Fig. 4. Changes in the thermal degradation pathway for the initial pulp during biobleaching at 10 °C/min in an air atmosphere. The mass loss rate was normalized to the initial mass of sample.

excluded condensation and grafting reactions for the pulp samples treated with the natural mediators because the thermal degradation paths were similar to that for the initial pulp. In addition, the kappa number of treated sulphite pulp was slightly diminished. However, the increased chroma observed after the L stage suggested the formation of chromophores (quinones) by oxidation of residual lignin in the pulp (Aracri et al., 2009). Delignification after L was higher with the synthetic mediators than with their natural ones (Table 4). In fact, the reactivity and stability of N–O· radicals are seemingly better balanced than in phenoxy radicals, which may account for the better performance of the former radicals as mediators for laccase-based delignification (Andreu et al., 2013). Despite the reduction in lignin content observed with L_{VA} and L_{HBT}, these enzyme-treated pulp samples exhibited higher colour saturation (C^*) than the initial pulp but lower than that obtained with the natural mediators. Specifically, VA had the highest delignification and lowest gain in chroma (C^*) after the enzymatic stage. According to Barneto et al. (2011), the laccase–HBT system partially oxidizes the outer cellulose layer, changing hydroxyl groups to carbonyl groups, disordering the crystalline surface and increasing the paracrystalline cellulose content as a result. Fig. 4 testifies to these effects: the amount of cellulose that volatilizes at a low temperature increased with HBT. Moreover, these results were confirmed with the viscosity values where a minimum cellulose preservation of 90% was obtained with L_{HBT}P vs. the 97% obtained with L_{VA}P.

4. Conclusions

In our previous work, it was shown that the enzymatic sequence L_{VA}QPO provided biobleached sulphite pulps with a reduction in hydrogen peroxide dose about 70% and reaction time of 2 h in comparison to simple hydrogen peroxide treatment (PO). In the present study, the resulted biobleached sulphite pulps were thoroughly investigated since presented a potential use as dissolving pulp and its applications. The basic requirements that define dissolving pulps were successfully satisfied: low content of hemicellulose, satisfactory values of Fock's solubility, no significant cellulose degradation as shown by α-cellulose and HPLC results, high brightness value and good optical properties (C^* and L^* coordinates) and brightness stability against moist heat ageing. Furthermore, XRD and TGA showed that crystallinity and fibre surface were not altered by the application of LMS. In the second part, the biobleaching performance of natural mediators (SA and PCA) in combination with *Trametes villosa* laccase was discussed in relation to their fibre surface modification since treated pulps exhibited low brightness results and poor delignification in comparison to the synthetic mediators, VA and HBT. TGA confirmed that no grafting or condensation reactions onto sulphite fibres occurred in the presence of natural mediators and no significant changes in fibre surface in comparison to control treatment were observed.

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