Carbohydrate Polymers 84 (2011) 1384–1390

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Wet strength development in sisal cellulose fibers by effect of a laccase–TEMPO treatment

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ARTICLE INFO

Article history:
Received 29 October 2010
Received in revised form 18 January 2011
Accepted 24 January 2011
Available online 1 February 2011

Keywords:
Laccase
Sisal
TEMPO
Wet-strength

ABSTRACT

The aim of this study was to investigate the oxidation of sisal pulp fibers by laccase–TEMPO system which had never previously been examined. High cellulose content sisal fibers were treated with laccase from *Trametes villosa* and a varying amount of TEMPO in order to evaluate the potential of laccase–TEMPO system to improve the paper physical properties of pulp fibers. The effect of the oxidative treatment on the carboxyl group content and viscosity of pulp fibers was examined. The resulting papers were analyzed in terms of strength-related properties, water absorbency capabilities and fiber morphology. Oxidation of sisal pulp by laccase–TEMPO system resulted in a very important improvement of wet strength of the resulting papers, suggesting the formation of a substantial amount of aldehyde groups in cellulose chains providing interfiber bonding through hemiacetal linkages.

1. Introduction

Over the last few decades, laccase has played an increasingly important role in pulp and paper research by virtue of its broad oxidative capabilities and flexibility of use in the production of paper and its derivative (Widsten & Kandelbauer, 2008). Ever since the discovery of chemical mediators capable of extending enzymatic oxidation to nonphenolic compounds, research interests have focused mainly on the potential of laccase–mediator systems for aiding pulp bleaching (Barreca, Fabbrini, Galli, Gentili, & Ljunggren, 2003; Bourbonnais & Paice, 1990; Fillat, Colom, & Vidal, 2010). Recently, laccase has attracted considerable attention as a means for modifying fiber chemistry with a view to altering paper properties (particularly, strength-related properties). Thus, Felby, Pedersen, and Nielsen (2004) found laccase to increase auto-adhesion in wood fibers and the bonding mechanism to involve the enzymatic activation of lignin on fiber surfaces through the production of phenoxy radicals, which facilitated cross-linking of fibers during board making. Further studies showed combinations of laccase with a lignin-rich extractive or a mediator to substantially improve wet tensile strength in high-yield unbleached kraft pulp (Lund & Felby, 2000, 2001). The improvement was ascribed to polymerization of lignin in the handsheets, and also to enhanced production of phenoxy radicals increasing cross-linking between fibers and facilitating water-resistant interfiber bonding as a result. Radical-based activation has also been used to enable bonding of low-molecular weight compounds to lignin-rich cellulose fibers. The reactions involved introduce new functional groups and provide a means for altering the physico-chemical properties of fibers as desired (Chandra, Wolfaardt, & Ragauskas, 2003; Elegir, Kindl, Sadocco, & Orlandi, 2008; Grönqvist et al., 2006; Lund, Felby, & Bjerrum, 1998). For example, laccase-catalysed grafting of phenolic acids to kraft pulp fibers was found to increase tensile and burst strength in the resulting paper (Chandra, Lehtonen, & Ragauskas, 2004; Chandra & Raguaskas, 2002); the improvement was ascribed to the ability of carboxyl groups to promote interfiber bonding and fiber swelling (Barzyk, Page, & Ragauskas, 1997; Scallan, 1983). Laccase-based approaches to improving paper properties have been preferentially applied to lignin-rich pulp and aimed specifically at lignin modification.

The selective catalytic oxidation of primary hydroxyl groups in carbohydrates by stable, water-soluble nitroxyl radicals such as 2,2,6,6-tetramethyl-1-piperidinyloxy, TEMPO, has opened up new prospects for the modification of cellulose materials (Nooy, Besemer, & Bekkum, 1995). A number of studies have shown TEMPO-mediated oxidation to provide an efficient method for introducing carboxyl and aldehyde functional groups into cellulose in aqueous media at room temperature (Gert, Torgashov, Zubets, & Kaputskii, 2005; Saito & Isogai, 2004; Tahiri & Vignon, 2000). The reaction is commonly carried out in the presence of the co-

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doi:10.1016/j.carbpol.2011.01.046

oxidizer system NaOCl/NaBr, which affords the regeneration of TEMPO and hence its use as a catalyst. The application of TEMPO-mediated oxidation to cellulose pulp has been widely investigated over the last decade. Kitaoka, Isogai, and Onabe (1999) studied the chemical modification of bleached hardwood kraft pulp fibers with this method and found it to result in an increase in carboxyl content from 60 to 470 mmol/kg. Dang, Zhang, and Ragauskas (2007) demonstrated the potential of the TEMPO–KBr–NaClO system for oxidizing ECF bleached softwood kraft pulp fibers; the system provided a 480% increase in carboxyl groups, a 62.9% greater water retention value (WRV) and a 13.8% increase in tensile index. In recent work, TEMPO-mediated oxidation was successfully used to improve interfiber bonding, and hence strength-related properties, in thermomechanical pulp (Le Roux, Daneault, & Chabot, 2006; Lianshan, Kweinam, Daneault, & Brouillette, 2008). Saito and Isogai (Kitaoka et al., 1999; Saito & Isogai, 2005, 2006) found the wet tensile strength of sheets prepared from TEMPO-oxidized cellulose fibers to be considerably improved. Development of this property was ascribed to the formation of substantial amounts of aldehyde groups on the surfaces of cellulose fibers as intermediate structures during the course of the TEMPO-mediated oxidation; once formed, the aldehyde groups seemingly established covalent interfiber bonds through hemiacetal linkages with sterically close hydroxyl groups in cellulose. This hypothesis was supported by the facts that no improvement in wet tensile strength was obtained when handsheets were prepared from TEMPO-oxidized pulp reduced with $NaBH_4$ or oxidized with NaOCl (Saito, Yanagisawa, & Isogai, 2005). The contribution of hemiacetal linkages to wet strength improvement in paper has also been proposed for aldehyde-containing wet strength resins (Chen, Hu, & Pelton, 2002; Dunlop-Jones, 1991).

In 1999, Viikari, Kruus, and Buchert (2000) reported on the ability of laccase to catalyse the regenerative oxidation of TEMPO in the presence of oxygen, and hence on its potential for replacing the environmentally harmful halide-containing compounds commonly used as co-oxidizer systems in the TEMPO-mediated oxidation of cellulose materials. Laccase–TEMPO mediated oxidation was proposed as a method for oxidizing various types of cellulose pulp for the production of paper or board with improved properties (particularly, technical properties, flexibility, WRV and strength-related properties). In addition to the environmental benefits associated with the use of an enzyme, this method provides the advantage of operating at a near-neutral pH – the traditional method uses pH 10–11. This reduces the occurrence of β-elimination reactions causing depolymerization of cellulose chains having glucuronic acid residues, thus a loss in mechanical properties of the oxidized pulp (Isogai & Kato, 1998).

The purpose of this work study was to study the laccase–TEMPO mediated oxidation of low-lignin-content sisal pulp, which had never previously been examined. To this end, the effect of the oxidative treatment on the carboxyl group content and viscosity of pulp fibers was examined. The resulting papers were analyzed in terms of strength-related properties, water absorbency capabilities and fiber morphology.

2. Experimental

2.1. Chemicals, enzyme and pulp

All chemicals were purchased from Aldrich and used as received. Laccase (EC. 1.10.3.2) from *Trametes villosa* was kindly supplied by Novo Nordisk Biochem, North Carolina and frozen until use. Sisal (*Agave sisalana*) pulp (1% lignin content) from a soda–anthraquinone cooking process was supplied by a Spanish manufacturer (CELESA). The pulp was conditioned at pH 4 with H_2SO_4, under stirring for 30 min at 2% consistency, followed by filtration and extensive washing with de-ionized water. This step was necessary to remove contaminants and metals, and also to bring the pulp to the pH required for the enzymatic treatment.

2.2. Enzyme assay

Laccase activity was determined by the oxidation of 2,2′-azino*bis*-(3-ethylbenzylthiozoline-6-sulfonate) (ABTS) (Bourbonnais, Leech, & Paice, 1998). One activity unit (U) was defined as the amount of laccase transforming 1 μmol/min ABTS to its cation radical (ε_{436} nm = 29,300 M^{-1} cm^{-1}) in 0.1 M sodium acetate buffer at pH 5 at 25 °C.

2.3. Pulp treatment

Laccase (3.4 ml, 60 U/1 o.d.g. pulp) and TEMPO (1, 2, 4 or 8% by mass) were added with stirring to a 5% consistency aqueous suspension of sisal pulp buffered at pH 5 with 50 mM acetate buffer. The resulting slurry was stirred at room temperature under oxygen bubbling for 18 h. At the end of each treatment, the effluent pH was checked and only slight changes (<0.2) were observed with respect to the initial value. Pulp samples treated under identical conditions in the absence of enzyme, TEMPO or both were used as controls. After treatment, each pulp was filtered and washed with de-ionized water until a colorless, neutral filtrate was obtained.

2.4. Analysis of pulp properties

Pulp viscosity (as intrinsic viscosity for a sample of cellulose dissolved in a dilute solution of cupriethylenediamine) was determined in accordance with ISO 5351/1. Borohydride viscosity, measured after treatment with 2% $NaBH_4$, at 5% consistency at room temperature for 30 min, was also determined (Roncero, Queral, Colom, & Vidal, 2003). Pulp brightness was analyzed according to ISO 3688. The bulk acid group content was determined by conductimetric titration as described elsewhere (Katz, Beatson, & Scallan, 1984). In brief, an amount of 1.50 g o.d. pulp was stirred in 300 ml of 0.10 M HCl for 1 h, followed by rinsing with de-ionized water in a finely fritted funnel. The sample was then resuspended in 250 ml of 1.00 mM NaCl, spiked with 1.5 of 0.10 M HCl and titrated against 0.05 M NaOH in 0.25 ml increments, with conductivity measurement after each addition. Titration data were plotted in conductivity vs. volume graphs in order to determine the milliequivalents of acid groups present in each gram of pulp. All reported results were the averages of two measurements which typically differed by less than 5%.

2.5. Paper testing

The treated pulp samples were disintegrated for 50,000 revolutions and then refined for 4500 revolutions according to TAPPI T 248 (Tappi Test Methods, 2002). Handsheets were formed according to TAPPI T 205 (Tappi Test Methods, 2002) and TAPPI conditioned (23 °C, 50% relative humidity) for 48 h before physical testing. Apparent density, dry tensile strength, tearing resistance, bursting strength, wet zero span tensile strength and wet tensile strength were determined according to TAPPI T 210, T 494, T 414, T 403, T 273 and T 456, respectively (Tappi Test Methods, 2002). Wet tensile index was measured in 25 mm wide specimen strips soaked in deionized water for 5 s.

Vertical wicking was performed on 10 cm × 1.5 cm strips of the treated handsheets. The strips weighed 0.10 ± 0.01 g and had densities of 0.60 ± 0.02 g/cm^3. A Petri dish filled with nanopure deionized water was used as reservoir. The bottom 1 cm of each strip was inserted into the reservoir and the timer immediately started. The top of the strip was fixed with a movable clamp. Each time the

Table 1
Bulk acid group content of control pulp, laccase-treated pulp, TEMPO-treated pulp and laccase/TEMPO-treated pulp (Lac/T), and physical properties of the resulting handsheets.

Sample ID	Bulk acid groups (meq/g)	Dry tensile index (N m/g ± 3%)	Wet tensile index (N m/g ± 3%)	Tear index (mN m^2/g ± 3%)	Burst index (kPa m^2/g ± 3%)
Control	83 ± 1	79.9	3.3	16.1	5.82
Laccase	91 ± 3	82.2	3.2	16.3	6.15
TEMPO	85 ± 1	79.0	3.0	16.0	6.04
Lac/TEMPO	108 ± 1	76.9	6.2	15.9	5.64

water climbed 1 cm on a strip, the time was recorded. After 5 min, the total distance of liquid absorbed was also recorded. Each sample was analyzed in duplicate.

Surface SEM pictures of the handsheets were taken on a Hitachi S-800 FE-SEM instrument. Samples were placed on the SEM sample holding stub by the conductive double side sticky carbon film and coated with Au/Pt alloy prior to analysis. Cross-sectional SEM images were obtained with a JEOL JSM-6400 microscope, the handsheet samples being coated with Au/Pd alloy before analysis.

3. Results and discussion

3.1. TEMPO-mediated oxidation of sisal pulp: effect on carboxyl groups in pulp fibers and on physical properties of the paper

A preliminary study was conducted by using 1% TEMPO as oxidant in order to assess its impact on the carboxyl group content of pulp fibers and strength-related properties of the resulting handsheets. The results are shown in Table 1. The laccase treatment slightly increased the content in acid groups relative to the control pulp as a result of lignin being oxidized by the enzyme (Konishi, Inoue, & Higuchi, 1974). The TEMPO-treated pulp had an acid content similar to that of the control pulp. The laccase–TEMPO treated pulp exhibited the highest yield in carboxyl groups (a 30% increase relative to the control pulp) as a result of the conversion of C6 primary hydroxyl groups in cellulose via an aldehyde group. Under the reaction conditions used, however, the laccase–TEMPO system increased the carboxyl group content to a much smaller extent than did others in the presence of NaOCl/NaBr (Kitaoka et al., 1999; Mao, Law, Brouillette, & Daneault, 2008; Saito & Isogai, 2005). Several studies (Barzyk et al., 1997; Chandra et al., 2004; Mao, Law, Daneault, & Brouillette, 2008) have shown carboxyl groups to promote hydrogen bonding between fibers and improve dry-strength properties in paper as a result. Our tests showed the laccase treatment to bring about a slight increase in dry tensile and burst strength in the resulting paper relative to the control treatment. The laccase–TEMPO treatment resulted in a slight loss of all dry-strength properties despite the higher increased yield in acid groups obtained; however, the treatment had a significant beneficial effect on wet strength in the modified paper, which was 88% higher than in the control sample. Depolymerization by some active species such as hydroxyl radicals formed *in situ* in side reactions of the hydroxylamine structure with oxygen during the oxidative treatment (Shibata & Isogai, 2003) may have degraded strength-related properties in the pulp. The gain in wet tensile strength suggests the formation of interfiber covalent bonds in the handsheets through hemiacetal linkages between aldehyde groups on fiber surfaces formed as intermediates in the TEMPO-mediated oxidation and hydroxyl groups in adjacent fiber surfaces (Saito & Isogai, 2006). The modest increase in carboxyl group content provided by the laccase–TEMPO system under the conditions used here was probably due to the formation of hemiacetal linkages between aldehyde groups in the fibers and hydroxyl groups in cellulose sterically close to each other, which, owing to steric hindrance, are likely to have strongly resisted conversion into carboxyl groups in the next oxidation step (Praskalo et al., 2009).

3.2. Effect of the TEMPO dose on intrinsic viscosity, strength and carboxyl group content of oxidized pulp fibers

The next step in this study was to examine the effect of the TEMPO dose on various properties of oxidized fibers. As noted earlier, the oxidative treatment may cause depolymerization reactions involving radical species and leading to a diminished fiber strength. Changes in cellulose polymerization were assessed via intrinsic viscosity measurements. Because the cupriethylenediamine solution is highly alkaline, depolymerization through β-elimination reactions promoted by carbonyl groups may take place on oxidized fibers during viscosity measurements and provide underestimated viscosity values. This problem was avoided by measuring viscosity after the pulp was treated with sodium borohydride (borohydride viscosity) in order to inactivate carbonyl groups by reduction to hydroxyl groups (Cadena, Vidal, & Torres, 2010; Roncero, Colom, & Vidal, 2002). The difference between the viscosity values determined with and without the reductive treatment provided an indication of the depolymerizing effect of carbonyl groups produced in the TEMPO-mediated oxidation.

As can be seen from Table 2, a loss of borohydride viscosity of up to 22% with respect to the control value was observed as the TEMPO load was increased from 1 to 8%; this reflects the occurrence of degradation reactions in cellulose chains during the oxidative treatment, especially at high TEMPO doses. When no prior reductive treatment was applied, control and laccase-treated pulp had viscosity values similar to those obtained after borohydride treatment; by contrast, TEMPO-treated pulp exhibited a dramatic drop in viscosity (up to 64%) compared to the reduced samples, the difference increasing with increase in TEMPO dose from 1% to 8%. This suggests that substantial amounts of carbonyl groups are generated during a laccase–TEMPO treatment (particularly at high mediator doses).

Pulp degradation can also be assessed via the number of scissions in the cellulose chain (CS), which is defined mathematically as (Bouchard, Morelli, & Berry, 2000)

$$\mathrm{CS} = \frac{\mathrm{DP_O} - \mathrm{DP}}{\mathrm{DP}} \qquad (1)$$

where $\mathrm{DP_O}$ is the degree of depolymerization of the initial pulp and DP that after the oxidative treatment. The degree of depolymerization is calculated from the intrinsic viscosity value $[\eta]$, using the equation of Evans and Wallis (1987) (SCAN-CM 15:88):

$$\mathrm{DP}^{0.85} = 1.1 \times [\eta] \qquad (2)$$

In pulp subjected to no borohydride post-treatment, CS is the combination of scissions caused by the oxidative treatment ($\mathrm{CS_{GT}}$)

Table 2
Borohydride viscosity and standard viscosity values of control pulp, laccase-treated pulp and pulps treated with laccase and different amounts of TEMPO.

Sample ID	Borohydride viscosity (ml/g)	Viscosity (ml/g)
Control	736 ± 20	736 ± 33
Laccase	736 ± 13	727 ± 7
Lac/T 1%	710 ± 12	442 ± 19
Lac/T 2%	649 ± 23	348 ± 21
Lac/T 4%	626 ± 21	238 ± 3
Lac/T 8%	576 ± 30	207 ± 10

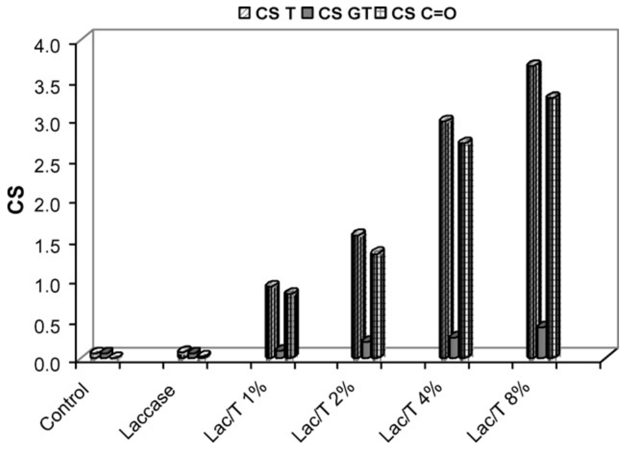

Fig. 1. CS_T, CS_{GT} and $CS_{C=O}$ of control pulp, laccase-treated pulp and pulps treated with laccase and different amounts of TEMPO.

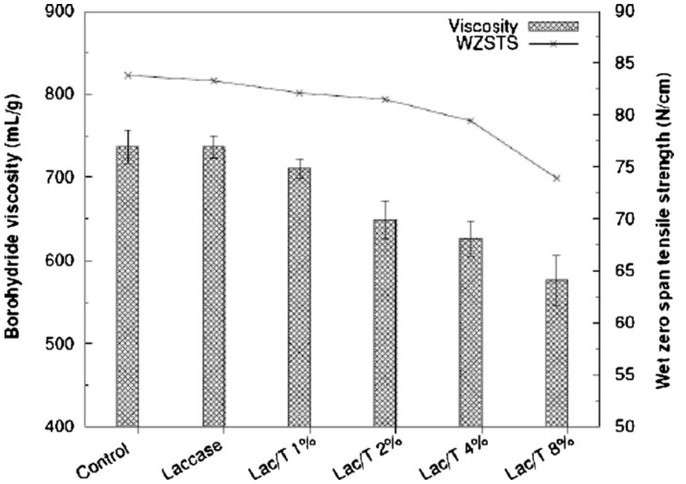

Fig. 2. Borohydride viscosity values of control pulp, laccase-treated pulp and pulps treated with laccase and different amounts of TEMPO, and wet zero span tensile strength of the resulting handsheets.

and the viscosity measurement procedure when carbonyl groups are present ($CS_{C=O}$):

$$CS_T = CS_{GT} + CS_{C=O} \tag{3}$$

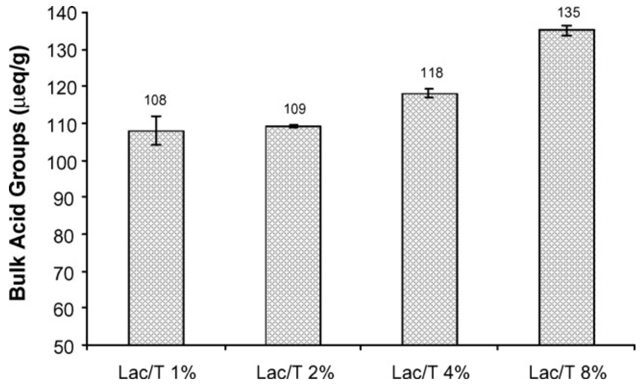

Fig. 3. Bulk acid group content of pulps that were treated with laccase and different amounts of TEMPO.

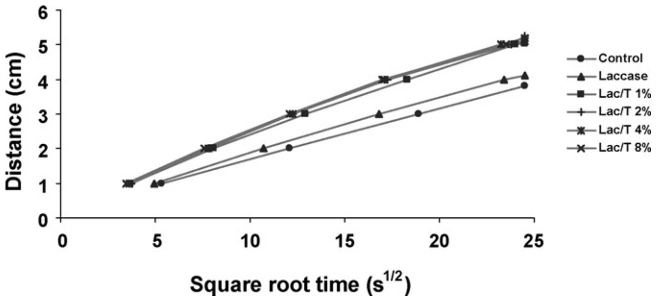

Fig. 4. Plot of distance wicked vs. square-root of time for samples treated with laccase and TEMPO.

On the other hand, the CS value obtained after the reductive treatment (CS_{TR}) represents depolymerization exclusively due to the oxidative treatment, with exclusion of the contribution of β-elimination reactions occurring around carbonyl groups:

$$CS_{TR} = CS_{GT} \tag{4}$$

Subtracting Eq. (4) from Eq. (3) gives the number of chain scissions due to the presence of carbonyl groups ($CS_{C=O}$):

$$CS_{C=O} = CS_T - CS_{TR} \tag{5}$$

The intrinsic viscosity values obtained in the presence and absence of a reductive treatment allowed us to calculate the number of scissions due to the oxidative treatment itself and to β-elimination reactions promoted by carbonyl groups. Fig. 1 shows the CS_T, CS_{GT} and $CS_{C=O}$ values for each pulp provided by the oxidative treatments. As can be seen, the control and laccase samples exhibited very low total chain scission values that were virtually the exclusive result of the oxidative treatment. Application of the laccase–TEMPO system at a TEMPO dose of 1%, 2%, 4% and 8% increased CS_T to 0.92, 1.54, 2.97 and 3.68, respectively. As can be seen, the number of chain scissions caused by the oxidative treatment increased as the TEMPO dose was increased; however, it

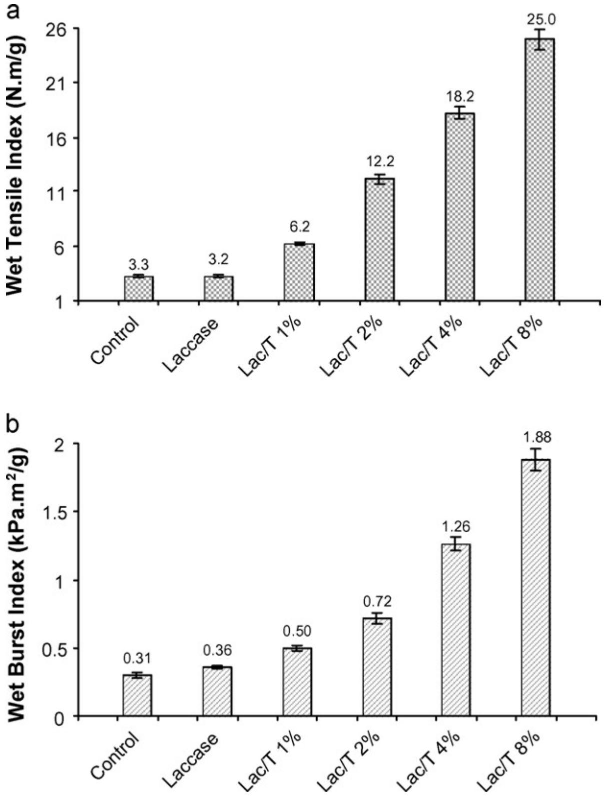

Fig. 5. Wet tensile (a) and wet burst (b) strengths of handsheets made from control pulp, laccase-treated pulp and pulps treated with laccase and different amounts of TEMPO.

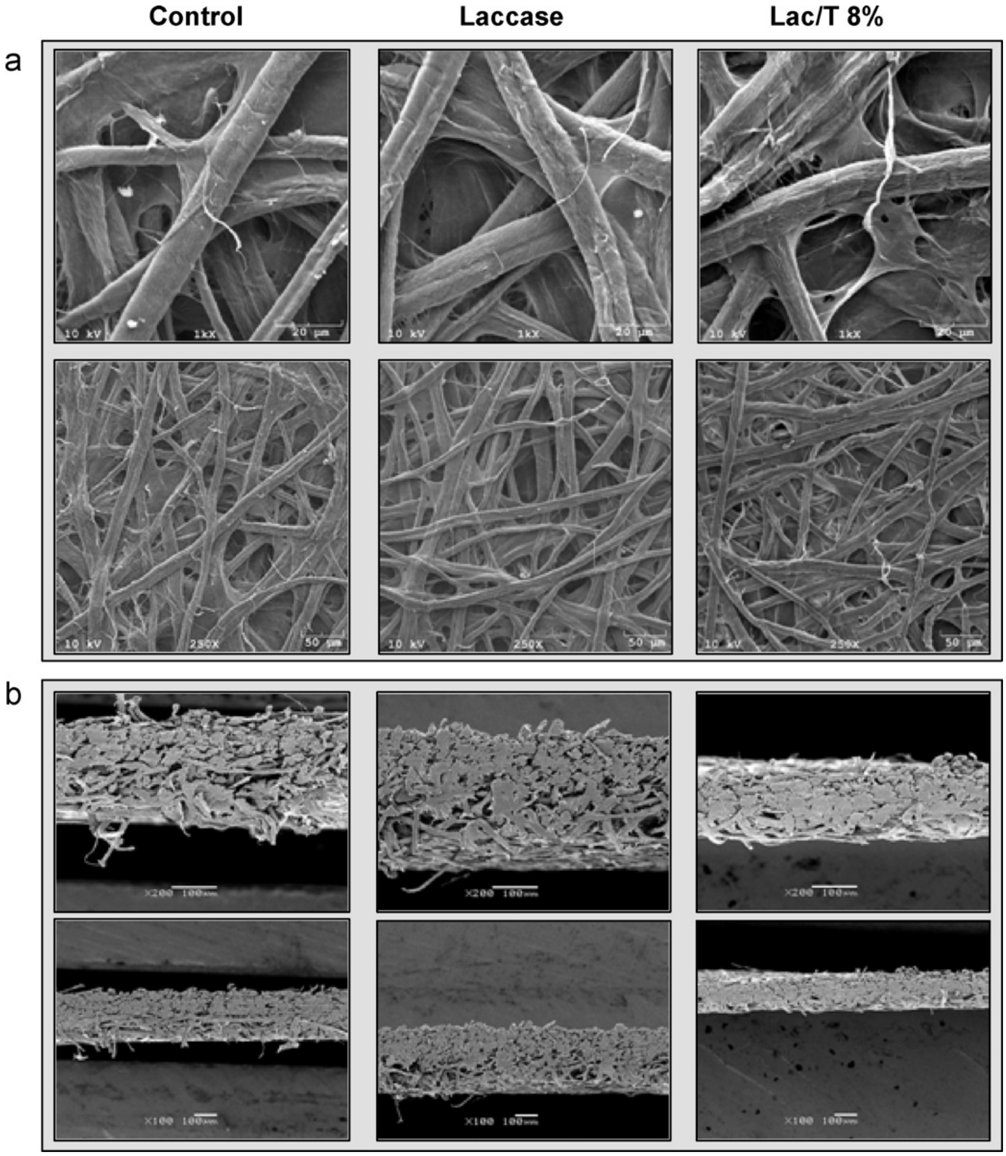

Fig. 6. Scanning electron microscope (SEM) images of surface (a) and cross-section (b) of handsheets made from control pulp, laccase-treated pulp and laccase/TEMPO 8%-treated pulp.

accounted for only ~10% of the total number of scissions (CS_T), most of which were due to carbonyl groups produced by the laccase–TEMPO system.

Modifications induced in lignin by the laccase–TEMPO system have not been investigated here; however, it is known from studies performed with lignin models (Barreca et al., 2003; Fabbrini, Galli, Gentili, & Macchitella, 2001) that TEMPO selectively interacts with benzyl alcohol (or ethers) groups of lignin oxidizing them to α-carbonyl derivatives. Pulp brightness was determined after each treatment in order to evaluate whether the introduction of new functional groups in fibers by the oxidative system led to a loss of this property. The results obtained (data not shown) indicate that no significant change of brightness was produced by laccase–TEMPO treatments with respect to control pulp.

The mean strength of fibers was estimated from the wet zero span tensile strength (WZSTS) of the handsheets. In theory, tensile tests on wetted samples with zero span between instrument jaws measures the work required to rupture individual fibers without altering interfiber bonding (Clark, Allison, & Kibblewhite, 1997).

Fig. 2 shows the borohydride viscosity values for the oxidized pulp samples together with the variation of WZSTS in the resulting handsheets. As can be seen, pulp viscosity was reduced by the oxidative treatments, and so was WZSTS. This confirms the correlation between cellulose depolymerization and a decreased fiber strength.

Fig. 3 shows the carboxyl group content of pulp fibers after treatment with TEMPO at a variable dose. The results indicate that the addition of 2% TEMPO failed to increase the carboxyl group content in relation to the initial dose. On the other hand, the addition of 4% and 8% TEMPO resulted in a 42% and 63% increase, respectively, in carboxyl groups with respect to the control value.

3.3. The effect of TEMPO charge on water absorbency capability of the resulting handsheets

An increased concentration of acid groups in pulp fibers is widely believed to increase fiber hydrophilicity and swelling (Dang, Elder, & Ragauskas, 2006; Scallan, 1983). In this work, we measured vertical wicking in order to confirm whether the increase in carboxyl groups derived from the laccase–TEMPO treatments enhanced the water absorbing capabilities of the handsheets. Wicking flow (*i.e.* spontaneous liquid flow in porous media) is strongly influenced by interfacial forces between the wetting fluid and the porous solid. Wicking flow in randomly oriented handsheets has been the subject of little study (Back, 1965; Hodgson & Berg, 1988a); Wicking flow in vertical tubes is governed by Eq. (6), proposed by Lucas and Washburn (Washburn, 1921):

$$\frac{dh}{dt} = \frac{\gamma r \cos\theta}{4\eta h} \frac{-r^2 \rho g}{8\eta} \tag{6}$$

where h denotes height of liquid rise, t time, γ surface tension, η viscosity, r pore radius, θ liquid contact angle, ρ liquid density, and g the gravitational constant.

Based on the integrated form of Eq. (6) in the paper by Hodgson and Berg (1988b), the square-root of time should be directly proportional to the distance travelled by the wetting front. This has been shown to be the case with random oriented handsheets (Hodgson & Berg, 1988b). Indeed, our distance vs. $t^{1/2}$ plots (Fig. 4) were virtually linear. As can be seen, the samples treated with laccase and TEMPO wicked at a faster rate than did the control samples, which indicates that the oxidative treatments increase pulp hydrophilicity. Although the higher TEMPO doses increased the carboxyl content, no further increase in wicking rate was observed in the samples treated with TEMPO at doses above 2%. As shown below, this result can be ascribed to an increased number of interfiber bonds leading to a more compact structure in the handsheets as the TEMPO dose was raised from 1% to 8%.

3.4. Effect of TEMPO dose on wet strength improvement in handsheets from oxidized pulp fibers

The preliminary paper tests performed in this study showed the use of 1% of TEMPO and laccase in the oxidative treatment of sisal pulp to have no beneficial effect on the dry-strength properties of the resulting paper sheets despite their increased carboxyl group content. On the other hand, the laccase–TEMPO treatment doubled wet strength, which suggests the formation of water-resistant interfiber bonds in the paper. Since increasing the TEMPO dose from 1% to 8% caused no significant further increase in carboxyl groups and detracted somewhat from viscosity and fiber strength, we believed it of interest to focus on the development of wet strength in the treated pulp samples, which was affected mainly by the presence of covalent interfiber bonds. Fig. 5 shows the wet tensile strength and wet burst strength – the latter was measured according to T 403 on water-soaked strips as for wet tensile strength measurements – for handsheets obtained from pulp treated with increasing doses of TEMPO. As can be seen, both properties were dramatically improved as the TEMPO dose was raised from 1% to 8%; thus, the wet tensile index and wet burst index were increased by up to 660% and 510%, respectively, relative to the control value. These results suggest the formation of increasingly large amounts of interfiber hemiacetal linkages during sheet-making as the TEMPO dose used in the oxidative treatment of pulp was increased. It is generally agreed that paper with a wet-to-dry (W/D) strength ratio (*i.e.* the ratio of wet tensile strength to dry tensile strength) of more than 15% should be considered wet-strength paper (Scott, 1996, chap. 9). W/D in our paper sheets increased from 4% in the control sample to 35% at the highest TEMPO dose. Wet strength development was achieved by using 2% TEMPO in the oxidative treatment, which provided W/D = 16%.

In previous studies, significantly improving wet strength with a TEMPO-mediated oxidation treatment of bleached pulp fibers was found to require the addition of aluminium sulfate or a cationic polymer in the handsheet-making process (Saito & Isogai, 2005, 2007). However, the degree of wet strength improvement obtained with these methods was not as high as in the present work, were the NaOCl/NaBr system was replaced with laccase and no further chemical agent was used to obtain the handsheets after pulp oxidation.

3.5. SEM analysis of handsheets

Fig. 6 shows surface and cross-sectional scanning electron micrographs for handsheets made from the control and laccase-treated pulp samples, as well as from pulp treated with the enzyme and 8% TEMPO. The surface images are consistent with a significant increase in fiber roughness and interfiber adhesion by effect of the laccase–TEMPO treatment. The effect of the oxidative treatment on interfiber bonding is more apparent in the cross-sectional images, which reveal strong agglutination of fibers leading to highly compacted handsheets. Based on these SEM images, the extent of fiber adhesion and sheet compaction is consistent with the increase in wet strength observed with the laccase–TEMPO treatment.

4. Conclusions

As shown for the first time in this work, a laccase–TEMPO treatment effectively improves wet strength in sisal pulp. The ability of the enzyme to catalyse the oxidation of TEMPO allows one to use a near-neutral pH to ensure the selective oxidation of cellulose fibers and avoid the need for environmentally harmful halide-containing chemicals. TEMPO-mediated oxidation causes the formation of aldehyde and carboxyl groups in proportions dependent on the particular reaction conditions. Under those used in this work, the laccase–TEMPO system resulted in a modest increase in carboxyl groups in cellulose fibers and, as suggested by the viscosity results and wet strength improvement obtained, the formation of a substantial amount of aldehyde groups that provide interfiber bonding through hemiacetal linkages. Further study is required to optimize the oxidation process and examine the effect of the operating conditions on the ratio and distribution of carboxyl and aldehyde groups in fibers.

Oxidation by the laccase–TEMPO system provides a novel, effective, environmentally friendly approach to the production of paper with excellent strength performance under high moisture conditions.

Acknowledgments

This work was supported by the Spanish MICINN project FUNCICEL (CTQ2009-12904) and a European Integrated Project from the Sixth Framework Program BIORENEW (NMP2-CT-2006-026456). E. Aracri is grateful to MICINN for award of an FPU research grant. The authors wish to thank CELESA (Spain) and Novo Laboratories, Inc. for kindly supplying the starting pulp and the enzyme, respectively.

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