

CHEMICAL CHARACTERIZATION OF PULP COMPONENTS IN UNBLEACHED SOFTWOOD KRAFT FIBERS RECYCLED WITH THE ASSISTANCE OF A LACCASE/HBT SYSTEM

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Oxidative treatments, without and with assistance of a Laccase-Mediator System (LMS), were characterized in relation to their effects on the chemical composition and strength properties of the fibrous fraction of an unbleached recycled softwood kraft pulp. The LMS, composed of a *Trametes hirsuta* laccase extract and 1-hydroxybenzotriazole (HBT), was applied on the fibrous fraction of a recycled pulp at low consistency under continuous stirring and oxygen bubbling. Control treatments adding neither the enzyme nor the mediator were also considered. The LMS treatment caused a partial reversion of the detrimental effects of hornification. A considerable increase in the amount of carbonyl groups on the lignin structure was observed as a result of the enzyme treatment. The amount of extractives in ethanol:toluene also increased after the enzymatic treatment, and the dioxane-soluble kraft lignin underwent a noticeable decrease in its apparent molecular mass. This latter effect was readily attributed to the hydrolysis of aryl-ether bonds that survived the severity employed in the pulping process. These observations were useful to explain why LMS-recycled fibers produce handsheets with 9.4% better tensile strength than the control pulps.

Keywords: Unbleached recycled kraft pulps, Laccase/mediator system, HBT, *Trametes hirsuta*, Chemical properties, Tensile index

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INTRODUCTION

The production and usage of paper have increased considerably over the past several years. The forecasted demand annual growth of paper and board until the year 2010 is 2.7% (Diesen 1998a). Therefore, it can be expected that environmental concerns regarding paper consumption will continue to grow. The increase in the use of wood resources as raw material, the environmental impact of industrial production, the need of more suitable landfill sites and the amount of waste to be disposed of by incineration are some of them.

The use of recycled fibers is growing rapidly. The global shares of recycled fibers in the furnish composition is expected to grow to 48% by 2010 (Diesen 1998b). For paper grades like corrugating material and cartoon board, the shares are expected to be as high as 61% for the year 2010.

Recycled fibers have inferior papermaking properties in relation to the corresponding virgin fibers. The fiber detriment is ascribed to the hornification process (Nazhad and Paszner 1994; Nazhad *et al.* 1995) but other reasons have also been indicated: (a) the inactivation or decrease in hydrophilicity of the fibrous surface during drying, particularly due to the redistribution or migration of resinic and/or fatty acids to the surface (Nazhad and Paszner, 1994) and (b) changes in fiber morphology such as curling and microcompression (Page and Tydeman 1962).

Several authors have studied the effects of recycling on bleached pulps (Howard and Bichard, 1992; Bhat *et al.* 1991). However, recycling of unbleached pulps has been scarcely studied.

Oxidative treatments have been used for improving papermaking properties. Zanuttini *et al.* (2007) found that the ozone treatment of recycled liner pulps improved the interfibrillar bonding, probably due to the reversion of the inactive external surface. Nevertheless, the pulps freeness was also negatively affected.

One alternative for improving the papermaking properties of unbleached pulps could be the modification or partial removing of lignin by enzymatic treatment. Previous reports have shown the efficiency of the Laccase-Mediator System (LMS) for the wet-strength improvement of unbleached kraft pulp (Lund and Felby 2001), as well as kraft pulp delignification when followed by alkaline extraction (Chakar and Ragauskas 2000), and lignin demethylation in kraft pulp bleaching (Paice *et al.* 1995; Sealey and Ragauskas 1998). Wong *et al.* (1999) applied the laccase-HBT system on a virgin softwood kraft pulp of κ 70 prior to refining. They found that, at the same density, the tensile index was increased by 6-7 units when compared to the pulp treated with the mediator alone.

Mocchiutti *et al.* (2005) found that the LMS, in combination with alkali treatment, could be useful to improve the bonding capacity of recycled softwood kraft pulps. The tensile index of the LMS-treated pulp was 11% higher than that of pulps treated exclusively with alkali. However, these authors did not characterize the effects of the LMS treatment on the chemical composition of the recycled kraft pulps.

In this paper, oxidative treatments, without and with the assistance of a laccase/HBT system, were characterized in relation to their effects on the chemical composition and strength properties of the fibrous fraction of an unbleached recycled softwood kraft pulp. We hoped to identify whether changes in strength properties could be correlated with changes in the pulp chemistry, including data on residual lignin, carbohydrates and extractable materials.

EXPERIMENTAL

Materials and Methods

A softwood kraft liner paper (κ 91.6) supplied by “Papel Misionero S.A.” (Argentina) was soaked for 20 h, disintegrated in a standard laboratory disintegrator (freeness: 715 mL CSF), and refined in a PFI mill (TAPPI T248 cm-85) at 4000 revolutions using a $1.8 \text{ N}\cdot\text{mm}^{-1}$ load (final freeness: 625 mL CSF). The pulp obtained was classified using a Bauer-McNett classifier (SCAN-M6:69) and only the R30 fraction was collected. This pulp fraction was separated in two parts. One part was washed with

sodium azide 0.02% (m.v⁻¹) and stored until use (one cycle pulp, identified as K pulp). Handsheets were formed from the other part (SCAN-C 26:76) with a grammage of 470 g.m⁻². These handsheets were dried under tension and under standard conditions (23°C, 50% relative humidity) and subsequently oven dried at 105 ± 2°C for 1.5 h for further storage in polyethylene bags until use.

Recycled pulps were prepared by soaking the handsheets overnight with water, followed by disintegration for 5 minutes. Metallic ions were removed by treating the fibers twice with HCl (pH 2.0) during 45 minutes. The pulp was then washed with distilled water until the pH was between 4.0 and 5.0 and stored at 4°C until use (two cycles pulp, identified as R pulp).

Treatment with the Laccase/Mediator System

Laccases from *Trametes hirsuta* were produced and partially purified at the VTT Biotechnology laboratories (Espoo, Finland) as described by Poppius-Levlin *et al.* (1999). The laccase activity of 124.7 U.mL⁻¹ (specific activity: 11.6 U/mg protein) was determined at 40°C and pH 5.0 (acetic acid-sodium acetate buffer, 38 mM) using guaiacol as substrate (Guillén *et al.* 1992). One unit of laccase activity was defined as the amount of guaiacol oxidized by 1 mL of enzyme per minute.

The enzymatic treatment was carried out for 4 h at 2% pulp consistency, 40 ± 1°C and pH 5 (sodium-acetate buffer, 38 mmol.L⁻¹) under stirring and oxygen bubbling. The laccase extract (15.6 U.g⁻¹ dried pulp) was added to a solution of the HBT mediator (1-hydroxybenzotriazole, monohydrate from ICN Biomedicals) and immediately mixed with the pulp suspension. The charge of the HBT was 3% on dried pulp. After the reaction, the pulp was filtered, washed several times with distilled water and stored at 4°C until use (identified as LMR pulp). As a control treatment, the pulp was treated as described above adding neither the enzyme nor the mediator. This pulp was identified as OR pulp.

Evaluation of the tensile index of pulp handsheets

Handsheets with a grammage of 60 g.m⁻² were prepared from the K, R, OR, and LMR fibrous fractions (SCAN-C 26:76). After drying at standard conditions (SCAN-P 2:75), the tensile strength was evaluated according to the TAPPI T494 om-88 Standard Method.

Pulp chemical composition

Moisture and ash contents were determined according to TAPPI Standards T550 and T244, respectively.

Extractives were determined by treating a fraction of the dried pulp with the following solvent sequence: ether, dichloromethane, 95% ethanol (remainder water):toluene (1:2 v.v⁻¹) and 95% ethanol (remainder water) according to TAPPI Standard T204. The resulting pulp was extracted with boiling distilled water according to TAPPI Standard T264.

Holocelluloses were determined following the method proposed by Guerra (2002). The pulp samples were delignified by a series four treatments with an aqueous solution of sodium chlorite in acid medium at 75°C, filtered, dried and weighed.

Hemicelluloses were isolated from the holocellulose employing 5% of KOH (wt% in water) and then 24% of KOH at 25°C for 2 h under inert (nitrogen) atmosphere. The residual material corresponded to α -cellulose. The filtrate obtained from each alkaline extraction was precipitated with a solution of ethanol:acetic acid (1:1), generating the hemicelluloses A and B, respectively. These fractions were then filtered, dried and weighed.

The pulp Klason lignin content was determined according to Gomide and Demuner (1986). Extractive-free pulps were pre-hydrolyzed with 72% (m/m) sulfuric acid at 20°C, followed by dilution to a 3% (m/m) acid strength and autoclaving for 1h at 118°C. Acid-soluble lignin was determined according to the TAPPI Useful Method 250.

The Klason lignin hydrolysates were centrifuged, filtered through a 0.45 μm nylon membrane and examined by ion exchange chromatography using a Shimadzu System model LC10AD (Kyoto, Japan) equipped with a SIL10A autosampler, a RID10A refraction index detector, and an SPD-M10A-VP photodiode array detector. The analyses were made with an Aminex HPX-87H (Bio-Rad) column at 65°C, in the presence of a precolumn Cation-H (Bio-Rad). The mobile phase used was H_2SO_4 8 mM at a flow rate of 0.6 $\text{mL}\cdot\text{min}^{-1}$. Calibration curves were obtained for each of the following components: cellobiose, glucose, xylose, arabinose, acetic acid, 2-furoic acid, hydroxymethylfurfural (HMF) and furfural. Since both 2-furoic acid and HMF displayed similar retention times, their quantification was made at different UV wavelengths because the latter has a λ_{max} at 280 nm (ϵ_{max} of 20156 $\text{L}\cdot\text{cm}^{-1}\cdot\text{mol}^{-1}$) and an ϵ of 3650 $\text{L}\cdot\text{cm}^{-1}\cdot\text{mol}^{-1}$ at 252 nm, whereas the former has a λ_{max} at 252 nm (ϵ_{max} of 13014 $\text{L}\cdot\text{cm}^{-1}\cdot\text{mol}^{-1}$) and an ϵ of 398 $\text{L}\cdot\text{cm}^{-1}\cdot\text{mol}^{-1}$ at 280 nm (or only 2% of the HMF absorptivity at 280 nm). Hence, in order to account for the interference of HMF at 252 nm, the area integration of 2-furoic acid at this wavelength was adjusted by the following equation:

$$A_{2-Fur}^{252} = A_{Total}^{252} - \left(A_{HMF}^{280} \times \frac{\epsilon_{HMF}^{252}}{\epsilon_{HMF}^{280}} \right) \quad (1)$$

The amount of 2-furoic acid was used to quantify the occurrence of hexenuronic acids (HexA) in the kraft pulp samples. After the 2-furoic acid concentration was expressed in relation to the dry pulp mass (% m/m), the HexA content was generated by multiplying these values by a factor of 1.5624, which corresponds to the stoichiometric conversion of 2-furoic acid to HexA.

The amounts of HMF and furfural in pulp hydrolysates were also converted back to pulp polysaccharides (glucans and pentosans, respectively) to account for the dehydration of pulp carbohydrates during hydrolysis. The conversion factors employed for this purpose were 1.4286 and 1.5625 for HMF and furfural, respectively. Likewise for HexA, these calculations were useful to obtain the mass balance of the entire acid hydrolysis procedure.

Determination of the monosaccharide constituents of extractives-free kraft pulps was based on Blakeney et al. (1982). The samples (10 mg) were treated with sulfuric acid 72% (0.125 mL) at 25°C for 45 min. After that, the acid was diluted with 1.35 mL of water and heated for 1 h at 105°C. After cooling, the samples were neutralized with

ammonia and the internal standard (*myo*-inositol) was added. The reduction of monosaccharides was made with a solution of sodium borohydride in DMSO for 90 min at 40°C. Methyl imidazole followed by acetic anhydride were added to the reduced mixture and kept for 10 min at 25°C. Cold water was then added to the mixture and the acetylated monosaccharides were extracted with dichloromethane. The resulting alditol acetates (1 μ L in a 1:25 split ratio) were analyzed in a Shimadzu GC-14B gas chromatograph with detection by flame ionization (FID). Analysis were carried out in three replicates using a Supelco SP2380 capillary column (30 m \times 0.25 mm), programmed from 190°C (0.5 min) at 25°C/min to 260 °C (then hold) with helium as the carrier gas. FID response factors were calculated for arabinitol (1.379), xylitol (1.076), mannitol (1.044), galactitol (1.004) and glucitol (1.003) acetates in relation to the internal standard. These components were individually expressed as anhydrous sugars before the monosaccharide composition of the kraft pulps was determined.

The molecular mass (MM) distribution of pulp holocelluloses was obtained by gel permeation chromatography (GPC) of their tricarbanyl derivatives. The procedure used for carbanylation (Ramos, 2001) was an adaptation of the method previously described by Schroeder and Haigh (1979). The GPC analysis of the tricarbanyl derivatives was carried out using a Shimadzu LC10AD liquid chromatograph. The tricarbanylated samples were solubilized in tetrahydrofuran (THF, 2.0 mg.mL⁻¹) and filtered through a Teflon membrane with a pore size of 0.45 μ m. Analyses were carried out using a series of one TSK-L guard column and four TSK-GEL (G6000 HXL, G4000 HXL, G3000 HXL and G1000 HXL) columns at 45°C, with exclusion limits ranging from 4×10^7 to 1×10^3 MM units. THF was the elution solvent at a flow rate of 1 mL.min⁻¹ and the column eluent were monitored by UV/vis spectroscopy (Shimadzu SPD-M10A-VP) at 240 and 254 nm.

The calibration curve was generated from the elution profile of twenty polystyrene standards. A typical dispersion of 2-3% was observed in the bimodal calibration curve as a result of quadratic fitting. Universal calibration was performed using the Mark-Houwink coefficients reported by Valtasaari and Saarela (1975). The degree of polymerisation (DP) of cellulose was obtained by dividing the MM of the tricarbanyl derivatives by the corresponding MM of the per-carbamylated anhydroglucose (MM of 519 Da). Both the number average (MM_N) and the mass average (MM_M) molecular mass, from which the DP_N and DP_M of cellulose were derived, were determined as described previously (Yau *et al.* 1979).

Lignin extraction and characterization

Lignin extractions were performed in extractive-free pulp fibrous fraction. Pulp samples were Soxhlet extracted for 6 h with a 9:1 solution of dioxane:HCl 2 mol.L⁻¹. After removing the solvent, the lignin component was recovered in water and the pH was neutralized with sodium bicarbonate. The isolated lignins were filtered, suspended in water and freeze-dried for storage until use.

The phenolic hydroxyl groups (OH_{phe}) were determined by UV differential spectrophotometry according to Guerra (2002), with the lignin spectra being obtained under alkaline and acid conditions. The percentage of OH_{phe} was determined by measuring the absorbance at 250 nm after the subtraction of the relative absorbance of the

baseline spectrum (acidic condition). The aliphatic hydroxyl groups (OH_{ali}) were estimated based on their reaction with phthalic anhydride in pyridine in the presence of benzene (Zakis, 1994). The percentage of OH_{ali} was obtained by titrating the excess of phthalic anhydride using KOH.

The occurrence of acid groups in lignin was determined by neutralization with an excess of lithium hydroxide in ethanol (Zakis 1994), whereas the carbonyl groups were determined by lignin oximation with a mixture of hydroxylamine hydrochloride ($\text{NH}_2\text{OH}\cdot\text{HCl}$) and triethanolamine in dimethylsulfoxide (Zakis, 1994).

Lignin acetylation was carried out according to Dence and Lin (1992). Lignin samples were treated with a mixture of acetic anhydride:pyridine, extracted in chloroform and dried over P_2O_5 and KOH after solvent elimination. Complete acetylation was ensured through the disappearance of the O-H stretching band in the FTIR spectra of the lignin derivative (data not shown).

The apparent MM distribution of each acetylated lignin derivative was obtained by GPC. Samples were dissolved in THF, filtered through a Teflon membrane with a pore size of $0.45\ \mu\text{m}$ and analysed in duplicates at 40°C using a series of two TSK-GEL columns (2000HXL and 1000HXL). THF was used as the eluting solvent at a flow rate of $1\ \text{mL}\cdot\text{min}^{-1}$ and the column eluate was monitored by UV spectroscopy at 280 nm.

RESULTS AND DISCUSSION

The fibrous fraction (R30) of a recycled unbleached softwood kraft pulp was selected for this study because, when compared to the whole pulp, it allowed a better observation of changes in the interfibrillar bonding. Under this lower sheet consolidation, the rupture of the paper under tensile stress could be ascribed to the failure in the interfibrillar bonding, having the fiber strength a lower and/or secondary significance. According to the theory developed by Page (1969), the paper tensile strength can be used to estimate the ratio between the number of broken fibers and the number of intact fibers crossing the rupture zone of the paper sheet. For the fibers used in this work (zero span tensile index of $133\ \text{kNm}\cdot\text{kg}^{-1}$ and tensile index lower than $40\ \text{kNm}\cdot\text{kg}^{-1}$), this linear relationship indicates that more than 65% of the fibers would be pulled out during the rupture.

The tensile strength of the handsheets made from pulps dried at 105°C for 1.5 h (R pulp: fibrous fraction that had two cycles) was nearly 50% lower than that made with the K pulp (fibrous fraction that had one cycle). A decrease from 44.5 to $21.9\ \text{Nm}\cdot\text{g}^{-1}$ was observed as a result of drying under drastic conditions (Nazhad *et al.* 1995). In a direct attempt to minimize this problem, the recycled fibers (R pulp) were oxidized in the absence (OR = Oxygen Recycled pulp) and presence (LMR pulp) of a LMS (laccase-HBT) to evaluate the effect of the enzyme treatment on the pulp tensile strength. The tensile strength of OR pulp was increased to $30.8\ \text{Nm}\cdot\text{g}^{-1}$, whereas the corresponding value for the LMR pulp reached $33.7\ \text{Nm}\cdot\text{g}^{-1}$. This means that oxygen bubbling under continuous stirring increased the papermaking properties of the recycled kraft fibers and this favorable effect was further improved by 9.4% when the treatment was assisted by a laccase-HBT system. In both situations, it was not possible to regenerate the value

originally observed in the original pulp (K pulp) but the favorable effects of oxidation were clearly demonstrated. The chemical reasons that justify this considerable improvement in pulp properties are discussed below.

Effect on Pulp Chemical Composition

Table 1 demonstrates that the treated fibers had lower *kappa* numbers (65-73) than the original liner paper (91.6), which includes fibers and fines. In general, the recycling procedure, without (R pulp) or with the assistance of oxygen (OR pulp) and/or the laccase/HBT system (LMR pulp), did not significantly modify the amount of holocellulose in the kraft pulp. The slightly higher holocellulose content of the K pulp was probably attributed to the experimental error usually attached to these measurements.

Table 1. Chemical Composition of the Untreated (K), Recycled (R) and Partially Oxidized Kraft Pulps that were Treated with Oxygen (OR) or with the Laccase:HBT System (LMR).

Component (% on o.d. pulp)	Kraft pulp			
	One cycle		Two cycles	
	K	R	OR	LMR
Holocellulose	84.09 ± 0.41	82.78 ± 0.11	83.08 ± 0.23	83.61 ± 0.14
Hemicellulose A	5.49 ± 0.30	4.46 ± 0.06	6.94 ± 1.62	7.14 ± 1.79
Hemicellulose B	4.80 ± 1.40	8.52 ± 0.74	6.21 ± 2.82	6.40 ± 2.98
Hemicelluloses A + B	10.29	12.98	13.15	13.54
α-Cellulose	73.84 ± 2.56	73.31 ± 0.28	70.82 ± 0.59	73.23 ± 0.51
Ash	0.90	1.00	0.66	0.64
Kappa no.	nd	72.7 ± 0.1	70.9 ± 0.1	65.3 ± 0.3
Klason lignin	11.20 ± 0.15	11.14 ± 0.22	11.41 ± 0.92	11.18 ± 0.55
Acid-soluble lignin	1.74 ± 0.05	1.76 ± 0.21	2.74 ± 0.46	2.23 ± 0.05
Total lignin	12.94 ± 0.09	12.90 ± 0.39	14.15 ± 0.67	13.41 ± 0.57
Extractives in ether	0.13 ± 0.03	0.27 ± 0.15	0.13 ± 0.02	0.20 ± 0.03
Extractives in DCM	0.35 ± 0.05	0.31 ± 0.08	0.42 ± 0.05	0.32 ± 0.09
Extractives in ET	0.18 ± 0.05	0.91 ± 0.06	0.72 ± 0.01	1.84 ± 0.08
Extractives in ethanol 95%	0.45 ± 0.17	0.29 ± 0.15	0.71 ± 0.18	0.56 ± 0.02
Total extractives*	1.11 ± 0.33	1.78 ± 0.45	1.98 ± 0.29	4.15 ± 0.18
Extractables in hot water	0.53 ± 0.05	0.58 ± 0.04	0.36 ± 0.04	1.23 ± 0.02
Mass balance**	99.04 ± 0.27	98.46 ± 0.32	100.13 ± 0.87	101.31 ± 0.29
*DCM, dichloromethane; ET, ethanol:toluene, 2:1 (v/v)				
**Summation of the pulp content in holocellulose, total lignin, total extractives and ash				

Compared to the K pulp, the *kappa* number of the OR pulp was reduced by 1.8 units, whereas the laccase/HBT system (LMR pulp) imparted an even greater reduction of 7.3 units in this pulp property (Table 1). However, no effect was observed in the amount of acid-insoluble lignin (Klason lignin), and changes in total lignin content were solely attributed to a slight increment in acid-soluble lignin. Hence, it seems that the lower *kappa* number of both OR and LMR pulps was associated to the higher state of oxidation of their non-carbohydrate components.

Chakar and Ragauskas (2000) found that the LMS treatment caused delignification of a high kappa number pulp when the pulp was subsequently washed thoroughly with alkali. Since alkali extraction was not performed in this work, it is possible that the oxidized lignin fragments were still retained in the pulp fibers with an average MM high enough to compromise their extractability in the aqueous media.

Regardless of the presence or absence of laccases, the oxidative treatments (OR and LMR pulps) increased the total extractives content of the kraft fibers (K and R pulps) (Table 1). It seems that more extractable lignin fragments of low MM were generated by oxidation. The ethanol:toluene (1:2) mixture extracted more material from the LMR pulp than from any of the other pulps involved in this study (K, R, and OR pulps), suggesting that the LMS released a greater amount of oxidized phenolic compounds that were readily solubilized in the solvent mixture. The amount of extractable materials in ethanol 95% was also greater after both oxidative treatments but the LMS system (LMR pulp) produced less of these polar compounds than the oxidation with molecular oxygen acting alone (OR pulp). This was a preliminary indication that the laccase-assisted oxidative treatment was more specific than molecular oxygen, because compounds that are insoluble in ethanol:toluene, but extractable with ethanol 95%, are likely to be residues derived from the carbohydrate moiety of the kraft pulp. This assumption was also confirmed by the amount of extractable materials in hot water. While undetectable in the unoxidized kraft pulps, 1.23% of the LMR pulp was water extractable, a nearly four-times greater value than that of the OR pulp.

Carbohydrates Composition and Characterization

Comparing the data of Table 1 for both K and R pulp samples, the recycling process produced a significant increase in the amount of hemicellulose B in relation to the amount of hemicellulose A. This behavior could be due to the hornification of pulp fibers, whereby the chemical accessibility of hemicelluloses is considerably decreased, together with their susceptibility to alkaline extraction. However, the oxidative treatment, without (OR pulp) and with (LMR pulp) the assistance of laccases, seemed to cause a partial reversion of this hornification effect because the ratio between their hemicelluloses A and B was closer to that of the K pulp (fibrous fraction that had one cycle) used as a reference.

The pulps derived from both oxidation procedures (OR and LMR pulps) displayed a greater hemicellulose contents than those detected in both original (K) and recycled (R) kraft pulps (Table 1). Since oxidation was not expected to result in an increase in pulp hemicellulose content, it is possible that this observation is a consequence of changes in their chemical accessibility. By contrast, the α -cellulose content remained relatively constant in all kraft pulps except the one recycled with molecular oxygen (OR), which had slightly lower values compared to that of the other pulps.

Table 2 reveals the effect of recycling on the carbohydrate composition of the unbleached kraft pulp as determined by HPLC of Klason lignin hydrolysates. These results have shown that the oxidative treatments caused no significant change in the amount of pulp glucans (mostly cellulose). This same analytical procedure also revealed that both OR and LMR pulp hydrolysates had furfural contents higher than those of the unoxidized kraft pulps (K and R pulps), suggesting that the oxidative treatments

increased the chemical accessibility of hemicelluloses. However, the proper accounting for these side reactions confirmed that none of the treatments had any significant effect on the original pulp carbohydrate composition.

For a better carbohydrate analysis, each of the kraft pulps involved in this study was hydrolysed and converted to alditol acetates for GC analysis (Table 3). In general, pulp polysaccharides were always composed of the same five monosaccharide residues, arabinose, xylose, mannose, galactose, and glucose, but the ratio among them varied a little from one sample to another. Heteroxylans were readily identified as the most predominant pulp hemicellulose. On the other hand, the carbohydrate profile of the unoxidized R30 fibrous fraction (pulp K) was similar to that of the LMR pulp, whereas the molecular oxygen-treated had a relatively lower hemicellulose content. This seemed to be consistent with the hypothesis that the LMS increased the selectivity of the process, leading to lower losses of pulp carbohydrates as a result of recycling.

Table 2. Chemical Composition of the Various Kraft Pulps (K, R, OR and LMR) Acid Hydrolysates in Relation to their Original Dry Mass, as Determined by HPLC

Component (% on o.d. pulp)	Kraft pulp			
	K	R	OR	LMR
Glucose ^a	70.60 ± 2.48	68.92 ± 2.35	68.75 ± 2.75	68.16 ± 2.13
Other hemicellulose sugars ^b	14.26 ± 1.02	14.04 ± 0.87	13.05 ± 0.46	12.80 ± 1.10
Arabinose ^a	1.61 ± 0.36	1.57 ± 0.29	1.51 ± 0.18	1.56 ± 0.22
Hexuronic Acid ^a	0.015 ± 0.002	0.014 ± 0.002	0.041 ± 0.023	0.041 ± 0.009
Furfural ^c	0.542 ± 0.034	0.522 ± 0.066	1.356 ± 0.350	1.358 ± 0.179
Hydroxymethylfurfural ^d	0.129 ± 0.011	0.126 ± 0.008	0.123 ± 0.016	0.131 ± 0.014
Total ^a	86.54	84.09	82.98	81.35

^aExpressed as anhydrous sugars to represent the corresponding contribution to pulp polysaccharides (cellulose and hemicellulose)
^bIncludes sugars that are not resolved by the HPLC system: xylose, mannose and galactose
^cExpressed as pentosan (anhydropentose) equivalents
^dExpressed as hexosan (anhydrohexose) equivalents

Table 3. Chemical Composition of Pulp Polysaccharides, as Determined by GC of their Alditol Acetates

Component (%)	Kraft pulp		
	K	OR	LMR
Arabinose	3.00 ± 0.20	2.34 ± 0.21	2.83 ± 0.43
Xylose	16.50 ± 1.75	14.80 ± 0.47	17.80 ± 1.10
Mannose	9.21 ± 0.49	8.21 ± 0.25	9.30 ± 0.39
Galactose	2.20 ± 0.43	1.40 ± 0.29	1.33 ± 0.13
Glucose	69.09 ± 1.32	73.25 ± 0.64	68.74 ± 0.84

There was a noticeable disagreement between the percentages of total pulp hemicelluloses displayed in Tables 1 and 2. Hence, it seemed that the gravimetric procedure based on alkali extraction (hemicelluloses A and B) did not ensure the complete isolation of pulp hemicelluloses. The subsequent analysis of the isolated α -cellulose by HPLC revealed that it still contained c.a. 7-8% of the hemicellulose content originally present within the pulp fibers (data not shown). Therefore, the total hemicellulose content (summation of hemicelluloses A and B) derived from the gravimetric procedure was partially underestimated.

The average degree of polymerization (DP) of pulp holocelluloses was similar for the original fibrous fraction (K pulp, one cycle) and the recycled fibrous fractions (OR and LMR, two cycles). The K pulp holocellulose had a DP_w of 1083 anhydroglucose (AnGlc) units for a polydispersity (DP_w/DP_n) of 10.61, whereas the holocellulose extracted from OR and LMR had DP_w figures of 979 (DP_w/DP_n of 6.94) and 1028 (DP_w/DP_n of 10.89) AnGlc units, respectively. Oxidation with molecular oxygen (OR) decreased the polydispersity of pulp holocellulose and this is clearly shown in Figure 1, where there is a decrease in the width of the elution profile, particularly at the region attributed to low MM oligosaccharides. By contrast, treatment of the recycled fibers with LMS prevented part of the hornification effect, with the LMR-derived holocellulose displaying a GPC elution profile very similar to that of the untreated kraft pulp.

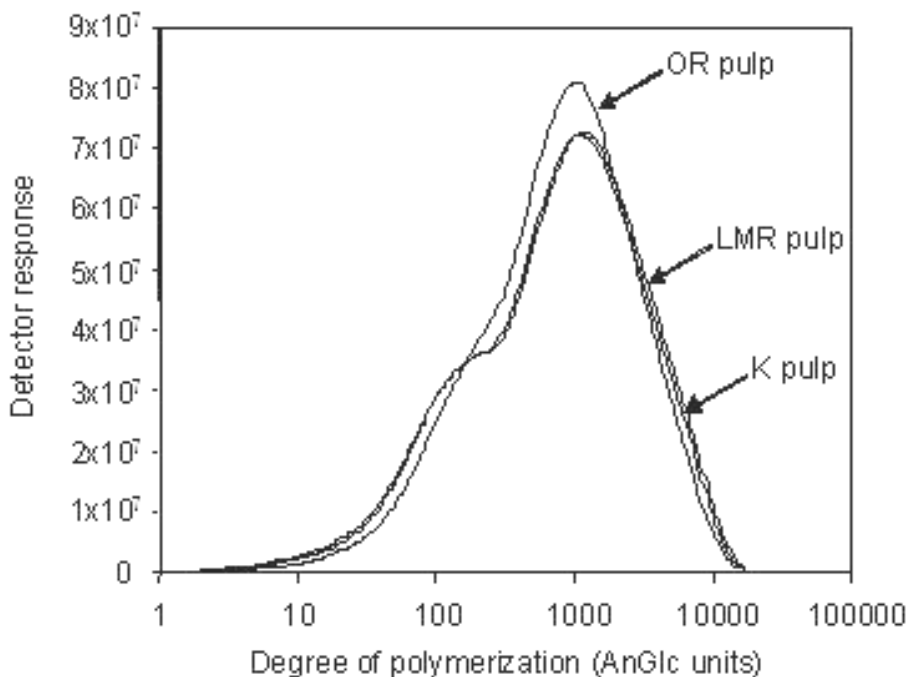


Fig. 1. Weight-average degree of polymerisation of holocellulose fractions derived from unrecycled (K) and recycled (OR and LMR) kraft pulps

The hexenuronic acids (HexA) were determined indirectly by the HPLC quantitation of 2-furoic acid at the retention time of 29.8 min and UV detection at 252 nm. For this purpose, the residual HMF absorptivity at this wavelength was subtracted from the detector readings as already described in the methods section. Both oxidative treatments had a measurable effect on the amount of HexA, but the detected levels were very low in relation to other pulp components.

Lignin Analysis

Lignin isolation was carried out by extracting untreated and partially oxidized kraft pulps with dioxane:HCl 2 mol.L⁻¹, 9:1 (v.v⁻¹). The average extraction yield varied between 6-7% of the original dry pulp mass.

GPC analyses were carried out after lignin acetylation. Figure 2 shows that the pulp drying followed by an oxygen treatment (OR pulp) produced an increment in the lignin apparent MM. It seems that lignin condensation reactions and/or an increase in associative intermolecular forces produced this slight displacement of the elution band. By contrast, the enzymatic treatment on the recycled fibrous fraction (LMR pulp) reduced this effect on the lignin structure, generating lower MM lignin fragments that were detected at higher elution volumes (around 26 min in Figure 2). Compared to the K pulp, pulp drying followed by the enzymatic treatment also decreased the amount of intermediate MM lignin fragments, leading to the accumulation of oligomers that seemed to correspond to a typical homologous series because their respective elution volumes suggested the loss of lignin fragments with almost equivalent average MM.

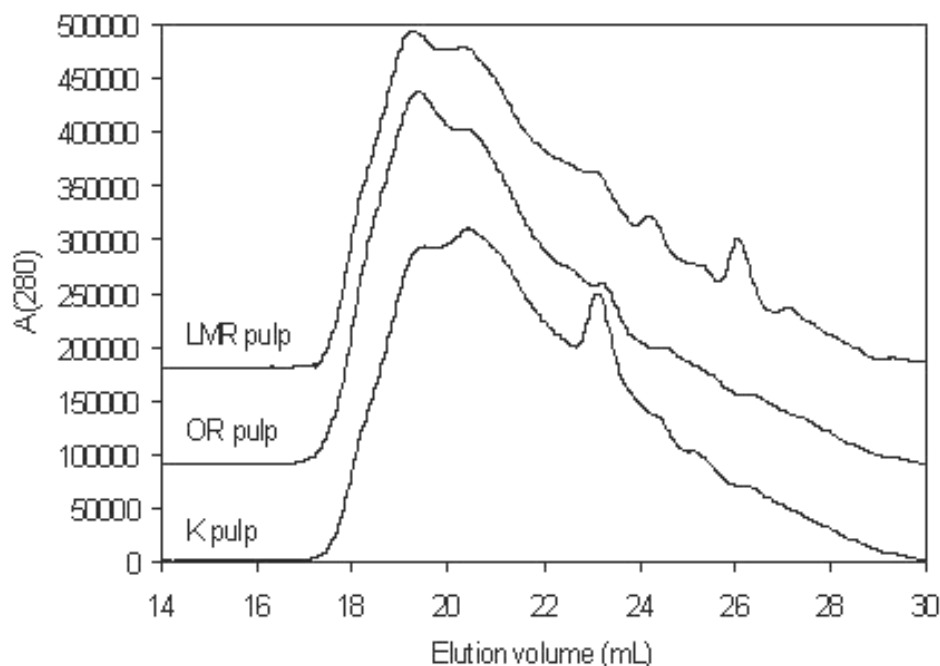


Fig. 2. Gel permeation chromatography of per-acetylated dioxane lignins isolated from unrecycled (K) and recycled (OR and LMR) kraft pulps

The oxygen treatment (OR pulp) also changed the amount of oxygenated functional groups in lignin (Table 4). Compared to the control (K pulp), there was an increase in OH_{phe} groups and a decrease in OH_{ali} groups, suggesting that oxidation was probably associated with the cleavage of arylether bonds and the modification of aliphatic side chains. The assistance of a laccase-HBT system changed this pattern considerably: a small, but noticeable increase on OH_{phe} was observed and there was a lower decrease in OH_{ali} groups.

Both oxidation procedures increased the amount of carbonyl and carboxyl (acid) groups in lignin. The LMS treatment increased the occurrence of these two functional groups by a factor of 8, whereas a much lower effect was observed as a result of oxidation with molecular oxygen (factors of 4.23 and 2.78 times, respectively). These modifications in the lignin structure, which are potentially beneficial for the bonding capacity of the fibers, revealed the selectivity of the laccase-HBT system for the phenolic structures found in lignin. This was also apparent from the FTIR analysis of the isolated lignins (data not shown). The C=O stretching vibration in LMR lignin was much broader and slightly displaced towards lower wavenumbers, revealing that the use of laccases increased the occurrence of conjugated carbonyl groups in the laccase-HBT recycled kraft fibers.

Table 4. Determination of Functional Groups in Dioxane Lignins Isolated from Kraft Pulp Samples (K, OR, and LMR)

Component (% of lignin mass)	Kraft pulp dioxane lignin		
	K	OR	LMR
OH _{phe}	0.305 ± 0.011	0.490 ± 0.099	0.315 ± 0.056
OH _{ali}	0.825 ± 0.014	0.526 ± 0.164	0.623 ± 0.033
Acid Groups	0.090 ± 0.013	0.250 ± 0.004	0.720 ± 0.014
Carbonyl Groups	0.220 ± 0.058	0.930 ± 0.179	1.790 ± 0.180

CONCLUSIONS

The Laccase/Mediator System (LMS), composed of laccases from *Trametes hirsuta* and HBT, produces changes in the chemical composition of unbleached recycled kraft pulps that contribute to the improvement of their bonding capacity. Compared to the control pulp, the results obtained from the LMS treated pulp led to the following conclusions: (a) the LMS treatment caused a partial reversion of the detrimental effects of fiber hornification; (b) pulp oxidation increased the amount of extractable materials in polar extraction solvents, and the assistance of the laccase/HBT system (LMS) increased this effect even more; (c) the apparent molecular mass of the kraft lignin decreased and the lignin acid groups and carbonyl groups increased as a result of the oxidative action of the LMS treatment.

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