Improvement of the fiber-bonding capacity of unbleached recycled pulp by the Laccase/Mediator Treatment

PAULINA MOCCHIUTTI, MIGUEL ZANUTTINI, KRISTIINA KRUUS, AND ANNA SUURNÄKKI

ABSTRACT: The authors studied the oxidative and delignifying effects of the *Trametes hirsuta* laccase-HBT (1-hydroxybenzotriazole) system (LMS) on the papermaking properties of an unbleached recycled softwood kraft pulp. The effects of pulp washing with a non-ionic surfactant (Tween 20) as a way to recover the enzyme and the effects of a final alkaline treatment were also analyzed. LMS treatment was applied to the fibrous fraction of a kappa-92 recycled pulp. The kappa-number reduction, the formation of radicals, and the increase in absorption coefficient that resulted verified that the LMS was active on this kind of pulp. Compared with the control treatment, the LMS treatment reduced the kappa number of the pulp by 8% and increased fivefold the number of radicals as analyzed by electron spin resonance. The LMS treatment also increased the light-absorption coefficient by 19%, suggesting that the fibers were oxidized. Alkaline treatment subsequent to the LMS treatment did not produce additional delignification and slightly decreased the light-absorption coefficient. The authors also found that LMS treatment or LMS treatment followed by alkaline treatment increased the fiber tensile and compressive strengths. It can be concluded that enzymatic delignification, oxidized residual lignin, or both contributed to improving the bonding capacity of the recycled fibers.

Application: Enzymatic delignification or lignin oxidation could be useful for increasing the strength of high-kappa recycled unbleached kraft papers.

Unbleached recycled pulps are extensively used for the production of test liner, corrugating-medium papers, or boards, all used for packaging. These pulps have lower strength compared with their corresponding virgin pulps, mainly due to the hornification process that takes place during the drying stage in the papermaking operation [1].

Another reason for the loss of bonding capacity is the deactivation of the fibrous surface during drying because of redistribution or migration of rosin and fatty acids to the surface, reducing its hydrophilicity [1]. Laine and Stenius [2] found that the surface of unbleached kraft pulps is strongly enriched by extractives. The drying process also affects fiber morphology. Page et al. [3] showed the significance of curl and microcompression in pulp fibers due to drying.

Refining is usually used to improve the papermaking properties of recycled fibers, but generation of fines and reduction in pulp freeness are drawbacks of this operation. Another alternative is to use the oxidative action of ozone to improve pulp physical properties. Zanuttini et al. [4] used ozone on OCC (old corrugated container), liner, and corrugating-medium pulps. Unfortunately, the freeness of the pulps was also affected [4], and the degree of polymerization of carbohydrates could potentially be reduced by the ozone treatment.

A selective chemical action on lignin can be achieved by using laccases in the presence of mediators. It has been shown that the laccase-mediator system (LMS) delignifies [5,6] and eliminates methyl groups from the phenylpropane lignin structures of low-kappa-number kraft pulps [6]. It has also been shown that LMS delignifies high-kappa-number pulps when followed by alkaline extraction [7].

Lund and Felby [8] showed that the long-living lignin radicals generated by the LMS treatment react even during paper formation, improving the wet strength of unbleached kraft pulps. On the basis of the presence of this kind of radical as determined by electron spin resonance, Grönqvist et al. [9] showed that the laccase is active on unbleached and bleached thermomechanical pulps.

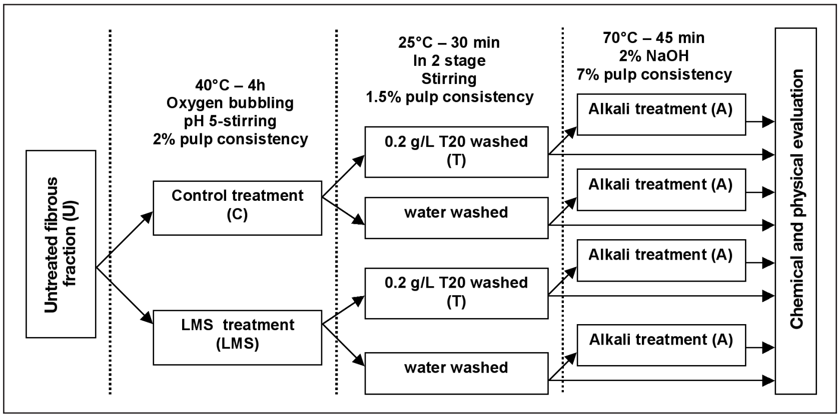
Wong et al. [10] analyzed the effects of the HBT mediator in the presence of oxygen and the effects of the laccase-HBT system before refining. They found that, at the same density, a virgin softwood kraft pulp of kappa 70 showed an increase in tensile index of 6–7 units after LMS treatment compared with the control treatment. Unfortunately, the control used was the pulp treated with the mediator only, which is known to modify the paper density. Under the same conditions as the LMS treatment, researchers would have needed controls with neither enzyme nor mediator added to evaluate the enzymatic treatment effects.

Recovery of enzymes could reduce the cost of the enzymatic treatments. Jackson et al. [11] used a dilute solution of NaOH in combination with a non-ionic surfactant to recover part of the enzymatic activity of cellulases and xylanases after pulp treatment. However, they did not analyze the effects of the surfactant on papermaking properties.

Alkaline treatment has been repeatedly investigated as a means of improving the properties of recycled pulp [12,13].

Use of this treatment subsequent to an LMS treatment could produce pulp delignification because the oxidized structures generated by the enzymatic treatment are susceptible to cleavage by the alkaline treatment [14]. For this reason, it is apparent that alkaline treatment after LMS could be more effective in improving the bonding capacity of recycled fibers compared with a simple alkaline treatment.

In this paper, we present the effects of the laccase-HBT system on the papermaking properties of the fibrous fraction of a recycled unbleached softwood kraft pulp. A subsequent washing with a non-ionic surfactant and the effects of a final alkaline treatment are also considered.



1. Pulp treatment scheme. The untreated fibrous fraction (U) was laccase-HBT treated (LMS) or control treated (C). Then samples were Tween 20 washed (T) or water washed. Finally, samples were alkaline treated (A) or directly evaluated. The treatment conditions are shown in the figure.

EXPERIMENTAL

Raw Materials

The fibrous fraction of a recycled unbleached softwood kraft pulp was used for the study. The raw material was a liner paper (kappa number: 91.6) supplied by Papel Misionero S.A. (Argentina). The paper was soaked for 20 h, disintegrated in a standard laboratory disintegrator for 12 minutes at 1.5% pulp consistency (freeness: 715 ml CSF), and refined using a PFI mill (TAPPI T248 cm-85) at 4,000 revolutions and a 1.8 N/mm load (final freeness: 625 ml CSF). The pulp obtained was screened using a Bauer-McNett classifier according to SCAN-M6:69, and only the R30 fraction was collected. Handsheets were formed according to SCAN-C 26:76 with the exception of the grammage, which was 470 g/m^2. The handsheets were dried under restraint and under standard conditions (23ºC–50% relative humidity) according to TAPPI 205-02. Afterward, the handsheets were additionally dried at 105±2ºC for 1.5 h and stored in polyethylene bags until use. Sheets were soaked overnight and disintegrated for 5 min. To remove the metallic ions, this fraction was treated twice with HCl 0.1N at 4% pulp consistency for 45 minutes and then washed with distilled water until the pH was between 4.0 and 5.0. The pulp was stored at 4ºC until use. This pulp is referred to as "untreated fibers" (U). Its tensile strength was 21.9 Nm/g, and its zero-span tensile strength was 133.0 Nm/g. The kappa number of this fraction was 72.6. This value was lower than the kappa number of the original whole pulp (91.6), indicating that the fines contribute significantly to the total lignin content.

Enzyme assay

Trametes hirsuta laccase was produced and partially purified as described by Poppius-Levlin et al. [15]. Laccase activity was 124.7 U/ml (specific activity: 11.6 U/mg protein) determined at 40ºC and pH 5.0 (acetic acid-sodium acetate buffer, 38 mM) using guaiacol as a substrate in a way similar to Guillén et al. [16]. The absorbance level of the oxidation product from guaiacol via laccase was measured at 465 nm (ε_{465}=12100 M^{1}cm^{-1}). One unit of laccase activity was defined as the amount of guaiacol oxidized by 1 ml of enzyme per minute.

Experimental design

Figure 1 shows the scheme that was followed in the different treatments. To analyze the effect of the laccase-HBT system (LMS) treatment, the effect of the non-ionic surfactant Tween 20 (T), and the effect of the alkaline treatment (A) on pulp physical properties, a 2^3 experimental design was carried out (**Table I**). A multifactor analysis of variance (ANOVA) was used to determine which factors have a statistically significant effect on pulp physical properties. Four replicas were performed for the LMS treatment, and the control treatment and two replicas were performed for the other treatments. For the ANOVA test, two replicas were randomly chosen from each treatment condition.

LMS treatment

The enzymatic treatment was carried out at 2% pulp consistency, 40±1ºC, and pH 5.0 (acetic acid - sodium acetate buffer, 38 mM) under constant stirring and oxygen bubbling for a period of 4 h. The laccase (20.8 U/g dried pulp, 1.8 mg protein/g dried pulp) was added to a solution of HBT mediator (1-hydroxybenzotryazole monohydrate from ICN Biomedicals) at pH 5.0 and immediately mixed with the pulp suspension. The

Factor	Levels for each factor
Laccase - HBT treatment	0 U/g dried pulp – 0% HBT (on dried pulp)
	20.8 U/g dried pulp [a] - 3% HBT (on dried pulp)
Tween 20 washing (g Tween 20 / L)	0 g/L
	0.2 g/L
Alkaline treatment	Without treatment
	2% NaOH (on dried pulp)

[a] Laccase activity using guaiacol as substrate

I. 2^3 experimental design for analyzing the effects of the following factors: laccase-HBT treatment; Tween 20 washing; and alkaline treatment on the fibrous fraction of recycled unbleached softwood kraft fibers.

charge of HBT was 3% on dried pulp. Every 30 minutes, a volume of the suspension was assayed for laccase activity.

Control treatment (C)

The pulp was treated in the same manner as the LMS treatment, but adding neither enzyme nor mediator, and then washed with Tween 20 solution or with water as explained below.

Tween 20 washing (T)

After a four-hour reaction, the pulp was filtered, dispersed, and washed twice with 0.2 g/L Tween 20 (Anedra) solution at 1.5% consistency and under stirring for 30 minutes. After the Tween 20 washing, the pulp was washed several times with distilled water.

Alkaline treatment (A)

The alkaline treatment was performed at 7% pulp consistency, 70ºC, for 45 minutes and using 2% NaOH on dried pulp. The pulp was washed with distilled water until pH 7.0 and stored at 4ºC.

Pulp and paper analyses

The pulp was converted to the calcium form, and the water retention value (WRV-Ca) was determined as in previously published work [17]. Handsheets of 60 g/m^2 grammage were prepared from the treated pulps according to SCAN Methods (SCAN C26:76) . Researchers determined the kappa number (TAPPI T236 om-99), the tensile index (TAPPI T404 cm-92), the short-span compression test (SCT, TAPPI T826 pm-92), and the light-scattering coefficient *s* (SCAN-C 27:76) using the R68 filter (effective wavelength 681 nm). Furthermore, the specific light absorption coefficient *k* at 681 nm according to the Kubelka-Munk theory was determined.

Measurement of radical formation

Electron spin resonance (ESR) measurements were made on both the LMS-treated pulp and the control pulp using a Bruker ER-200 Spectrometer. Pieces of 90-mg vacuum-dried handsheets were cut and stored in a freezer until the spectra were run. The measurements were made at room temperature using a microwave frequency of 9380.96 MHz under the following conditions: central field, 3340 Gauss; field sweep, 100 Gauss; modulation frequency, 100 kHz; modulation amplitude, 5 Gauss; and microwave power, 1 mW. The relative radical concentration was determined by the following equation (1):

$$\frac{n_{spin}(LMS)}{n_{spin}(Control)} = \frac{A(LMS)}{A(Control)} \cdot \frac{\Delta H^2(LMS)}{\Delta H^2(Control)} \cdot \frac{G(Control)}{G(LMS)} \cdot \frac{Wd(Control)}{Wd(LMS)} \tag{1}$$

where

A= amplitude of the peaks at the field where the resonance takes place,

Wd = weight of the dried pulp (g) ,

ΔH= width of the peaks at the field where the resonance takes place,

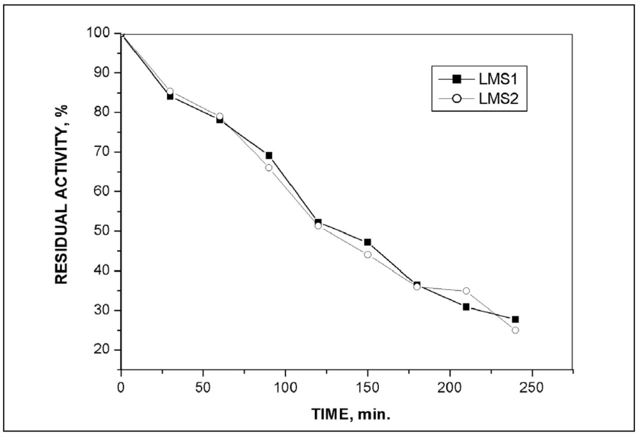
G = equipment gain.

RESULTS AND DISCUSSION

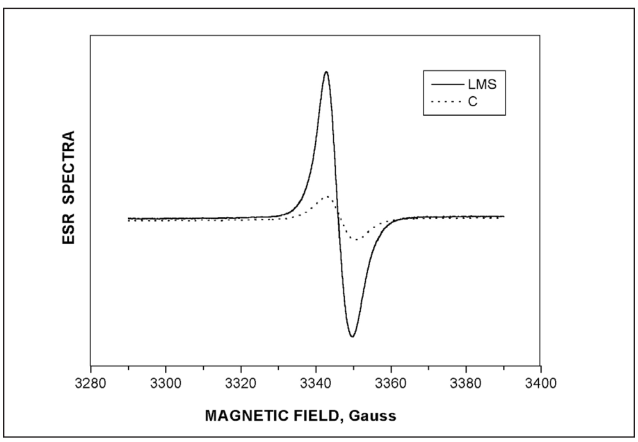
The enzymatic activity in the reactor was tracked during the LMS treatment. **Figure 2** shows that this activity decreased as a function of time. After a four-hour treatment, the activity had decreased by 70% from the initial level (125 U/ml enzymatic extract). This behavior could be partially ascribed to the inhibitory effect of the HBT. Bourbonnais et al. [18] found that laccase from *T. versicolor* is less stable with HBT than with other mediators. Nevertheless, the HBT gave the most extensive delignification [18].

The radicals produced by laccases were verified by ESR (**Fig. 3**). According to equation (1), the number of long-living radicals after the LMS treatment was five times greater than with the control treatment. The spectrum of the LMS-treated pulp does not show the hyperfine splitting expected from a nitroxy radical. This radical is generated on the HBT by the laccase and is probably either less stable than the lignin radicals or not detected because of its low concentration.

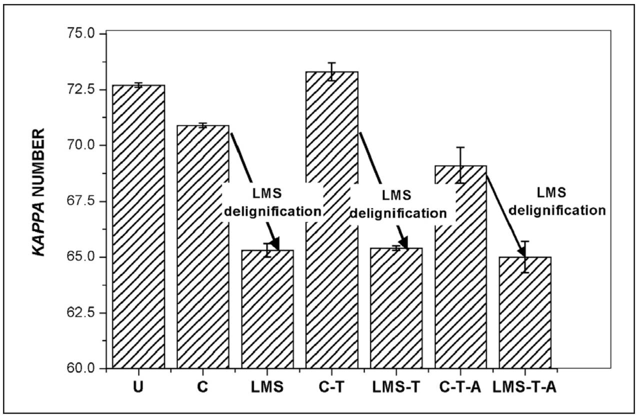
Figure 4 shows the kappa-number results. The control treatment (C) (4 h under constant stirring and oxygen bubbling) decreased the kappa number of the untreated fibrous fraction (U) by 2.5%. This effect was due not only to the pres-



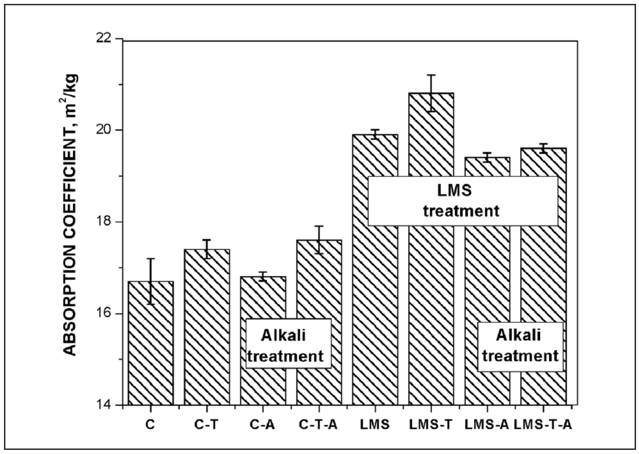
2. Residual enzymatic activity in the liquid medium during the LMS treatment. LMS 1 and LMS 2 are the replicas of the laccase/mediator system treatment.



3. ESR spectra for the LMS-treated pulp (LMS) and the control pulp (C).



4. Kappa-number results as a function of the different treatments. (U): untreated fibrous fraction; (C): control treatment; (LMS): laccase-HBT treatment; (T): Tween 20 washing; (A): alkaline treatment. The kappa number of the original pulp (fibers and fines) was 91.6. The error bars show the range obtained from two replicas of the method.



5. Light-absorption coefficient (k) as a function of the different treatments. (C): control treatment; (LMS): laccase-HBT treatment; (T): Tween 20 washing; (A): alkaline treatment. The error bars show the standard deviations obtained from two replicas of the treatments.

ence of oxygen, but also to stirring which could remove small lignin fragments from the pulp. Compared to this control pulp (C), the LMS treatment decreased the kappa number by 8%, indicating that the laccase was active on this kind of pulp. The alkaline treatment reduced the kappa number by 6%; however, alkali treatment after the LMS treatments did not produce additional delignification.

The increment in k-coefficient produced by the LMS treatment (**Fig. 5**) can be ascribed to the increment in the content of chromophores like quinones, aldehydes, and carboxylic acids formed by oxidation reactions. The alkaline treatment slightly decreased the *k*-coefficient. The ANOVA test for the *k*-coefficient (**Table II**) shows that the three principal factors and also the pairwise interactions had a statistically significant effect at the 95.0% confidence level.

The increment in the number of long-living radicals, the kappa-number reduction, and the increment in the light-absorption coefficient indicate that LMS was active on unbleached recycled fibers, producing delignification, residual lignin oxidation, or both.

Figure 6 shows that the WRV of the control pulp compared with the untreated pulp had increased from 1.16 to 1.32 (g water/g dried pulp), indicating that stirring and oxygen bubbling improve the swelling of the fibrous fraction. The alkaline treatments increased it even more. As other authors have found [19] for recycled pulps, swelling increases under alkaline conditions. However, the LMS treatment did not modify the WRV. On the other hand, the Tween 20 treatment decreased the WRV, probably because the surfactant remained adsorbed on the fibrous fraction.

Tensile strength was strongly modified by the treatments investigated. The ANOVA test (Table II) for the tensile index shows that the three principal factors and also the pairwise interactions had a statistically significant effect at the 95.0% confidence level.

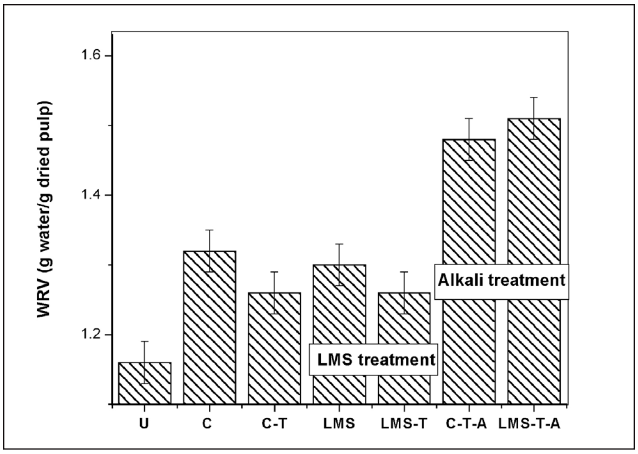
Figure 7 shows the tensile index as a function of light-scattering coefficient. Tensile strengths were higher and scattering coefficients lower for the LMS-treated pulps than for their respective control pulps. Tween 20 washing decreased the tensile index and increased the scattering coefficient, as could be expected from the WRV reduction previously mentioned. However, when Tween 20 washing was followed by alkaline treatment, the harmful effects of the surfactant disappeared. It is possible that the surfactant was partially saponified and removed by the alkaline treatment.

Figure 8 shows compressive strength (SCT) as a function of tensile strength. These properties are similar in behavior; the higher the tensile strength, the higher

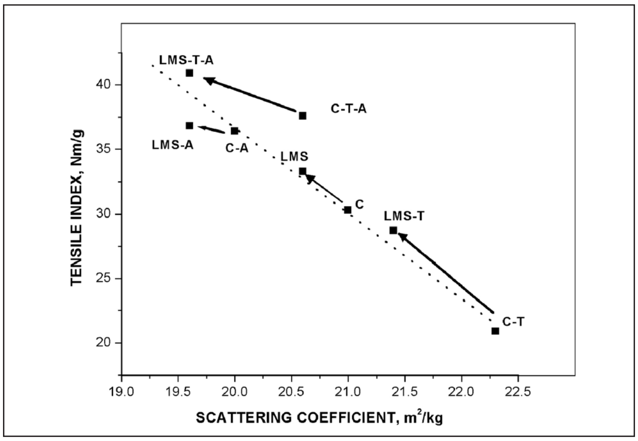
Factor	Df [a]	ANOVA for the *k*-coefficient		ANOVA for tensile strength	
		Sum of Squares	p-value	Sum of Squares	p-value
PRINCIPAL EFFECTS					
A: LMS treatment	1	36.663	0.0000	51.84	0.0000
B: Tween 20 washing	1	0.893025	0.0047	18.49	0.0001
C: Alkali treatment	1	1.01003	0.0034	372.49	0.0000
INTERACTIONS					
AB	1	0.4096	0.0306	11.56	0.0004
AC	1	2.25	0.0003	16.0	0.0001
BC	1	0.0144	0.6362	100.0	0.0000
ABC	1	0.000225	0.9525	1.0	0.1223
RESIDUAL	8	0.4768		2.68	
TOTAL	15	41.7171		574.06	

[a] Degrees of freedom

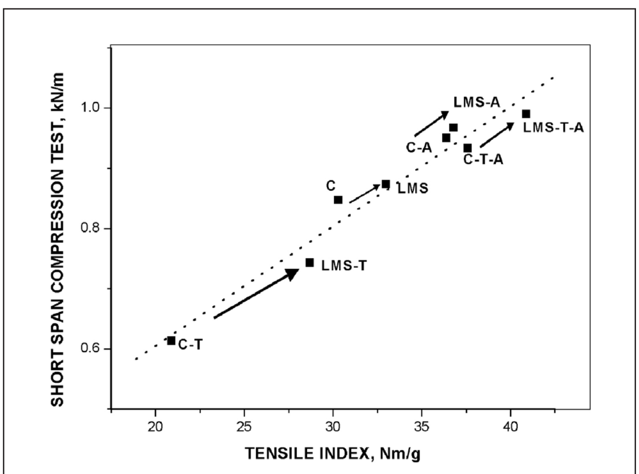
II. Analysis of variance for k-coefficient and tensile strength. For the k-coefficient, five p-values are less than 0.05, and for tensile strength, six p-values are less than 0.05. These factors have a statistically significant effect at the 95.0% confidence level.



6. Water-retention-value results for the R30 pulp fraction (centrifugation conditions: 30 min at 1750 g). (U): untreated fibrous fraction; (C): control treatment; (LMS): laccase-HBT treatment; (T): Tween 20 washing; (A): alkaline treatment. The error bars show the standard deviation obtained from three replicas of the method.



7. Tensile-index results as a function of light-scattering coefficient. (C): control treatment; (LMS): laccase-HBT treatment; (T): Tween 20 washing; (A): alkaline treatment. The tensile index of the untreated fibers (U) was 21.9 Nm/g. The arrows show the effects of the LMS treatment.



8. Short-span compression test results as a function of tensile strength. (C): control treatment; (LMS): laccase-HBT treatment; (T): Tween 20 washing; (A): alkaline treatment. The arrows show the effects of the LMS treatment.

9. Tensile-test sample (grammage 60 g/m², width 15 mm) of the paper made from the fibrous fraction of the unbleached recycled kraft pulp. The rupture zone of the paper shows that many intact fibers were pulled out during the rupture.

the compressive strength. The ANOVA test for SCT showed that the principal factor, LMS treatment, had a statistically significant effect at the 90.0% confidence level.

The Page theory [20] can be useful for analyzing the relative contributions of bonding strength and intrinsic fiber strength to tensile strength. **Figure 9** shows that, in the tensile rupture zone of the paper, many intact fibers have been pulled out during the rupture. With low sheet consolidation, the rupture of the paper under tensile stress can be ascribed to a failure of interfiber bonding, with fiber strength having a lower and probably secondary significance. According to the first premise of Page's theory [20], it is possible to estimate the ratio between the number of broken fibers and the total number of fibers crossing the rupture zone of the paper as a function of tensile strength:

$$\frac{n_f}{(n_f + n_p)} = \frac{9T}{8Z} , \qquad (2)$$

where n_f = number of fibers crossing the rupture zone that take the load at failure and then break,
n_p = number of fibers crossing the rupture zone that pull out intact because of earlier bond breakage,
Z = zero-span tensile strength (Nm/g)
T = tensile strength of the strip (Nm/g)

For the fibers used in this work (zero-span tensile index 133 Nm/g and tensile index between 20 and 40 Nm/g), between 66% and 83% of the fibers will be pulled out during rupture. In this way, it can be said that the increments in tensile strength found in this work are related to the increments in interfiber bonding capacity. As shown in Fig. 8, compressive strength exhibited similar behavior to tensile strength.

CONCLUSIONS

The increments in the number of long-living radicals and in the absorption coefficient and the reduction in kappa number provided verification that the laccase-mediator system was active on unbleached recycled softwood kraft pulps.

The kappa number was reduced by 8% by the LMS treatment. However, subsequent alkaline treatment did not produce an additional effect on lignin content. The *k*-coefficient was increased by 19%, indicating that the fibers were oxidized by the LMS treatment. Subsequent alkaline treatment slightly decreased the *k*-coefficient.

Tween 20 washing had a clear negative effect on swelling and tensile index. This effect was eliminated with a subsequent alkaline treatment, indicating that only in this case can this surfactant be useful for recovering the enzyme.

Tensile and compressive strengths were increased by LMS treatment or by LMS treatment followed by alkaline treatment, indicating that this system could be useful for improving the bonding capacity of the fibrous fraction of a recycled softwood kraft pulp. **TJ**

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INSIGHTS FROM THE AUTHORS


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We consider it important to study alternative methods to the traditional refining to improve the papermaking properties of recycled lignified kraft pulps. The use of specific enzymes (such as laccase) that selectively react onto lignin could be an alternative.

The effects of the laccase/mediator system on lignified pulps have not been as extensively studied as those on bleached pulps. More specifically, in the case of lignified kraft pulp, the high content of lignin (10% to 15 %) as well as its favorable greater content of hemicellulose, which can be of up to 13.0 %, can bring about a singular behavior.

The biggest challenge of this work was that we found it difficult to discriminate between the effects of the LMS enzymatic treatment and the alkali treatment, and the effects of the LMS enzymatic treatment and the surfactant washing. We discovered that the enzymatic delignification or lignin oxidation could be useful to increase the strength of high kappa recycled unbleached kraft papers. On the other hand, we discovered that the free radicals remain active after several hours. The most surprising finding was that the surfactant Tween 20 clearly negatively affected the swelling and the tensile index of recycled unbleached softwood kraft pulps.

In the future, it could be possible to replace a part of the refining process by the laccase/mediator treatment in order to improve the papermaking properties. The next step would be to find new mediators of lower cost than the 1-hydroxybenzotriazole.

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