# Enzymatic Treatment of Mechanical Pulp Fibers for Improving Papermaking Properties

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Three enzyme preparations (crude cellulase, laccase, and proteinase) were evaluated for their potential to improve the papermaking properties of mechanical pulp. After treating a long fibre-rich fraction of the pulp with enzyme, the fibres were recombined with untreated fines for handsheet making and testing. None of the enzymes altered the retention of fines or the consolidation of the furnish mix during handsheet formation. All three enzymes increased tensile stiffness index, which is a measure of the initial resistance of the handsheets to strain. Only the laccase preparation, an enzyme that modifies pulp lignin, consistently increased fibre bonding to enhance other strength properties of the handsheets.

## Introduction

Enzyme applications have been proposed for pulp and paper manufacture to enhance pulp bleaching, pulp refining, deinking, cellulose purification, deposit control, and papermaking (Wong and Mansfield, 1999). For mechanical pulping, wood fibers are separated and refined using mechanical actions that are energy intensive and lead to some damage of the fibers. Consequently, alternative approaches are sought to minimize these deleterious effects.

The cellulase component cellobiohydrolase I, from *Trichoderma reesei*, has been reported to enhance mechanical pulp refining by reducing energy consumption, apparently as the result of selective action on crystalline cellulose (Pere et al., 1996). However, when a crude cellulase preparation was evaluated in our fiber processing pilot plant, more energy was required to achieve a target freeness (drainage property of the pulp, which is an indicator of pulp refining) during secondary refining (Richardson et al., 1998a). This undesirable effect was apparently due to a higher loss of pulp fines after the inter-stage treatment of the pulp with enzyme. Subsequent work in our laboratories showed that cellulase treatment of a long fiber-rich fraction of a mechanical pulp increased its retention of untreated fines during papermaking, thus improving the tensile and burst strength of the resultant sheets (Wong and Richardson, 1999). However, this effect was observed in a relatively pure long fiber fraction isolated using laboratory scale equipment.

Carbohydrate-degrading enzymes are only one of the classes of enzymes produced by microorganisms for the degradation of wood. Other microbial enzymes known to modify non-carbohydrate components, including lignin and minor wall constituents, could also be expected to alter pulping and papermaking. Lignin-degrading or lignin-modifying enzymes, such as laccase, lignin peroxidase, manganese peroxidase, and particularly the laccase−mediator system, have already shown promise for applications in pulp delignification and bleaching (Paice et al., 1995). It has also been suggested that proteinases, which attack the protein concentrated in the primary wall of wood fibers, may enhance fiber separation during mechanical processing (Pokora and Johnson, 1992; Salmén and Petterson, 1995; Mansfield et al., 1999), even though this protein is a minor structural component of wood (Bao et al., 1992). Similarly, other components of the primary wall, such as pectins and xyloglucans (Aspinall, 1980), may warrant consideration. Furthermore, a recent report indicated that the removal of lipid extractives from mechanical pulp fibers could improve fiber bonding to increase tensile strength (Buchert et al., 1998).

Degradative or modifying enzymes for many wood components are already commercially available. The present study examined the potential of using crude cellulase, laccase, or proteinase to treat the long fibers of mechanical pulp to improve their papermaking properties. The treatments targeted the long fibers because these fibers are generally more resistant to mechanical refining. For the present study, the long fibers were isolated using commercial scale equipment, and their papermaking properties were evaluated after recombination with untreated fines from a mechanical pulp. The fines are mixed back into the pulp furnish because they make substantial contributions to paper strength.

## Materials and Methods

**Pulp Samples.** PRMP (pressurized refiner mechanical pulp) samples were produced in the PAPRO fiber processing pilot plant (Peacock et al., 1993) from radiata pine top log chips obtained from the Kaingaroa Forest of New Zealand. Before laboratory screening, pulp latency was removed by agitation at 90 °C and 1.25% stock consistency for 10 min.

The fines were removed from primary stage PRMP pulps by screening the pulp three times at 0.5% consistency in a Dorr Oliver DSM bow screen with 0.10 mm slots. This process was carried out on two primary stage pulps, one was obtained with 1310 kW·h·odt$^{-1}$ of refining energy and used to evaluate cellulase treatments, while the other was obtained with 1400 kW·h·odt$^{-1}$ of refining

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energy and used to evaluate laccase and proteinase treatments. The composition of these two PRMP screen rejects, or long fiber fractions essentially free of fines (Wong and Richardson, 1999), was similar with approximately 33.1% acid insoluble lignin, 0.3% acid soluble lignin, and 1.5% arabinosyl, 2.0% galactosyl, 44.8% glucosyl, 6.6% xylosyl and 10.0% mannosyl residues.

Untreated fines were obtained from second stage PRMP pulps (2190−2780 kW·h·odt$^{-1}$) by collecting the white water from two screening operations, leaving it to stand and settle, and concentrating the fines by decantation. Since bacterial growth prevents the storage of the fines, different batches were prepared for the evaluation of each enzyme for the treatment of screen rejects. A typical batch of fines (>99% Bauer-McNett P200 fines, Tappi Standard Method T233 os-75) was composed of 36.5% acid insoluble lignin, 0.6% acid soluble lignin, and 1.8% arabinosyl, 3.7% galactosyl, 34.8% glucosyl, 4.7% xylosyl and 6.2% mannosyl residues (Richardson et al., 1998a).

**Enzyme Samples.** A commercial cellulase, Novozyme SP 342, was obtained from Novo Nordisk (Denmark). Its activity on carboxymethyl-cellulose, xylan, and mannan at pH 7 and 50 °C was 11.7, 65.2, and 2.3 nkat·mg$^{-1}$ of enzyme, respectively (Richardson et al., 1998a). This enzyme was applied at a low and high dose that corresponded to 0.05 and 0.54 $\mu$kat·g$^{-1}$ of pulp (based on activity on carboxymethyl-cellulose), respectively.

An experimental laccase (ML-101) was provided by Tomas Hansen (Novo Nordisk). Its activity (115 nkat·mg$^{-1}$, pH 4.5, 50 °C) was determined with 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) using a method modified from Wolfenden and Willson (1982). This enzyme was found to be essentially free of cellulase, mannanase, and xylanase activities (<0.14 nkat·mg$^{-1}$) using the DNS-based method of Bailey et al. (1992). It was applied at a low and high dose that corresponded to 3.2 and 12.6 $\mu$kat·g$^{-1}$ of pulp, respectively.

A commercial proteinase, Alcalase DX, was obtained from Novo Nordisk. Its activity was determined to be 170 nNPU·mg$^{-1}$ using Protazyme AK tablets of azurine-cross-linked casein (Megazyme, Australia) at pH 7.0 and 40 °C, where 1 NPU is a neutral protease unit that is equivalent to the release of 1 $\mu$mol·min$^{-1}$ of tyrosine from soluble casein. The enzyme was applied at a low and high dose that corresponded to 6.8 and 17.0 $\mu$kat·g$^{-1}$ of pulp, respectively.

**Enzymatic Treatments.** Prior to cellulase or proteinase treatments, slurries containing PRMP screen rejects were adjusted to pH 7 with NaOH. The enzyme was added to the screen rejects in plastic bags with the dilution water required to achieve a pulp consistency of 5%. The slurries were incubated for 2 h, at 50 °C for the cellulase treatments and 40 °C for the proteinase treatments.

For the laccase treatments, fiber slurries were initially adjusted to pH 4.5 with $H_2SO_4$. The enzyme and the Na-acetate buffer (final concentration 50 mM, pH 4.5) were added to the slurry with the dilution water needed to bring the slurry to 5% consistency. Each mixture was transferred to a 2 L stainless steel reactor, which was rotated at 50 °C for 5 h. When a chemical mediator (1% 1-hydroxybenzotriazole) was added to the low dose of laccase to give the laccase−mediator system, the reactor was pressurized to 1 MPa (10 bar) with $O_2$ during the treatment.

Following enzymatic and control treatments, the fibers were washed at 1% consistency in hot tap water for 1 h and recovered by filtration. Control screen rejects for each experiment were produced in parallel with the treated samples, using the same procedure but without enzyme addition. Therefore, the control for the treatment with the laccase−mediator system involved adding the mediator and pressurized oxygen.

**Table 1. Net Solubilization of Carbohydrate from PRMP Screen Rejects[a] during Enzymatic Treatments**

| | | carbohydrate solubilized (mg·g$^{-1}$ pulp, sugars in anhydro form) | | | | |
|---|---|---|---|---|---|---|
| enzyme | dose | Ara | Gal | Glu | Xyl | Man |
| cellulase | low | 0.1 | 0.3 | 0.8 | 1.0 | 0.9 |
| | high | 0.3 | 0.6 | 6.9 | 1.6 | 1.9 |
| laccase | low | trace | 0.2 | 0.1 | 0.3 | 0.4 |
| | high | trace | 0 | 0 | 0.1 | 0.3 |
| | LMS[b] | trace | 0.1 | 0 | 0.1 | 0.2 |
| proteinase | low | 0.1 | 0.1 | 0.2 | 0 | 0.1 |
| | high | 0.2 | 0.1 | 0.4 | 0.2 | 0.2 |

[a] Note that two batches of screen rejects were used, one for the cellulase treatments and the other for the laccase and proteinase treatments. [b] LMS = laccase−mediator system (solubilization of methoxyl units was 0.04 mg·g$^{-1}$ pulp).

**Pulp Testing.** The characteristics of the control and enzyme-treated fibers were compared after they were mixed with increasing amounts of untreated fines. Standard APPITA (Australian and New Zealand Pulp and Paper Association) methods were used to determine the freeness of each furnish mix and to prepare and evaluate handsheets with nominal grammage of 60 g·m$^{-2}$. The oven-dry basis weight was used to calculate the physical properties of handsheets.

**Filtrate Analysis.** The carbohydrate composition of the treatment filtrates, expressed in the anhydro form, was determined by high performance anion-exchange chromatography after secondary acid hydrolysis (121 °C, 103 kPa, 60 min) was carried out to convert the oligomeric carbohydrates to their monomeric components. Methanol was determined in filtrates using gas chromatography on a 30 m × 0.53 mm i.d. × 1 $\mu$m film thickness BP-Wax capillary column (Alltech Associates, Australia).

## Results and Discussion

The present study used two different batches of PRMP screen rejects (long-fiber-rich fractions) and three batches of untreated fines. Since the same batch of fibers and fines were used for the evaluation of each enzyme, the results of enzymatic treatments could be compared to the corresponding control treatment. However, comparisons between the different enzymes could not be considered completely conclusive.

**Carbohydrates Solubilized.** As could be expected, cellulase treatments solubilized small amounts of carbohydrates from PRMP screen rejects, while treatments with laccase, laccase−mediator system, or proteinase solubilized negligible amounts (Table 1). The amounts solubilized by the high cellulase dose (1.7% of total carbohydrate available) was greater than that previously observed when a Bauer-McNett R30 long fiber fraction, which contained less short fibers, was treated with the same dose of cellulase (0.5% of total carbohydrate available) (Wong and Richardson, 1999). For both fiber samples, the carbohydrate solubilized by the commercial cellulase was found to be predominantly composed of glucosyl residues.

**Pulp Properties.** During the making of handsheets, a small proportion of the fiber furnish was lost through the handsheet wire. The material lost can be assumed to be composed of fines, and this assumption was supported by the observation that less of the furnish mix

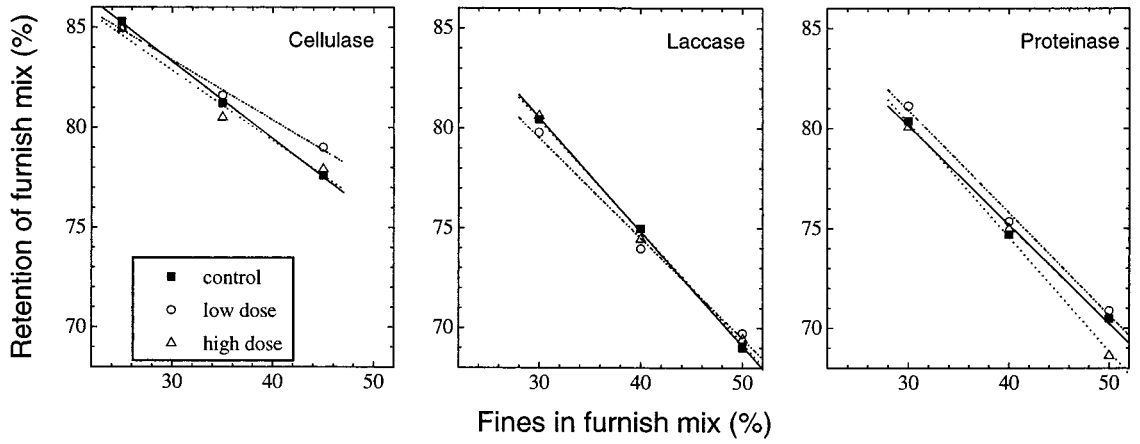


**Figure 1.** The retention of furnish mixes, made with enzymatically treated fibers and untreated fines, during handsheet making.

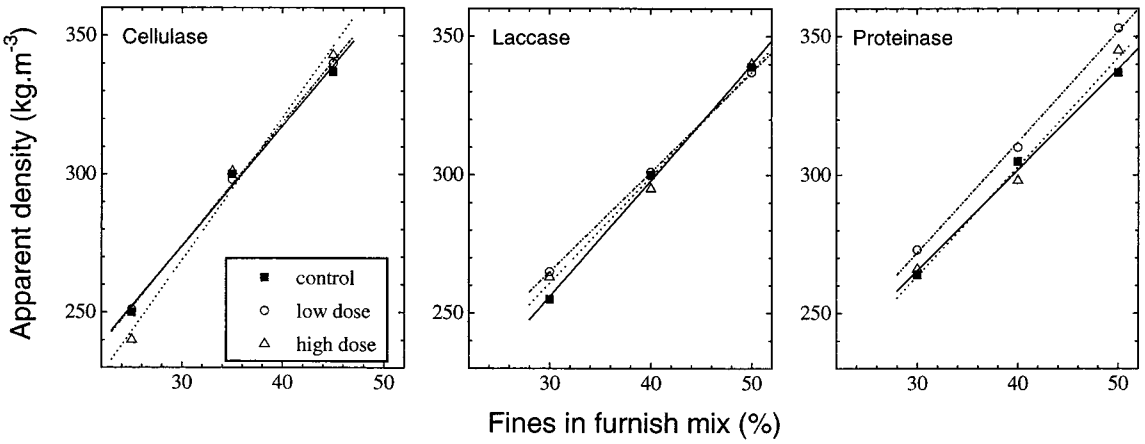


**Figure 2.** The apparent density of handsheets made with furnish mixes containing enzymatically treated fibers and untreated fines.

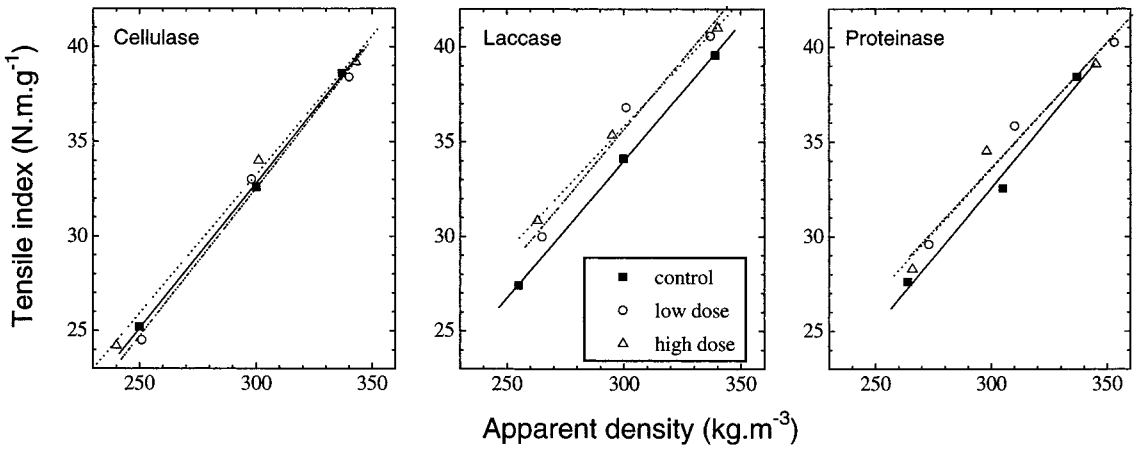


**Figure 3.** The tensile index at a given apparent density for handsheets made with furnish mixes containing enzymatically treated fibers and untreated fines.

was retained when supplementation with fines was increased (Figure 1). Previous observations indicated that when a R30 long fiber fraction from mechanical pulp is treated with cellulase and mixed with untreated fines, there is an increase in the retention of fines during subsequent handsheet making (Wong and Richardson, 1999). Such an increase in retention values was not seen in any of the enzymatic treatments carried out on PRMP screen rejects in the present study (Figure 1), probably because this fiber sample had more short fibers than the R30 fraction. There was also no substantial change in the freeness of the furnish mixes as a result of the cellulase and laccase treatments (data not shown), but there may have been an increase of freeness of ~10 mL CSF after the proteinase treatments.

**Handsheet Properties.** Since there was little difference in retention values, handsheets prepared with enzymatically treated fibers and their corresponding controls could be assumed to contain similar amounts of fines. The consolidation of the furnish mixes was not altered by treatments with crude cellulase, laccase alone, or proteinase, yielding handsheets with similar apparent

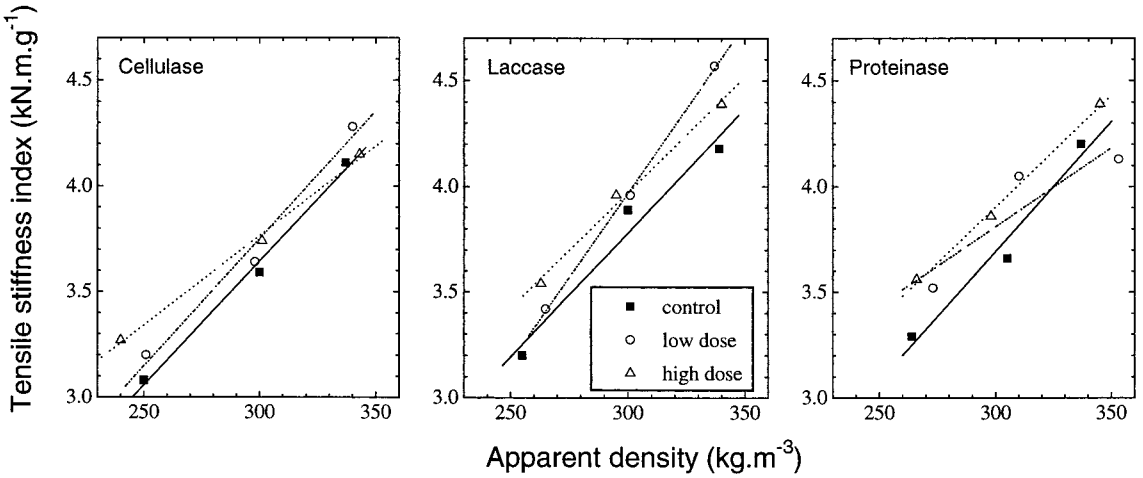


**Figure 4.** The tensile stiffness index at a given apparent density for handsheets made with furnish mixes containing enzymatically treated fibers and untreated fines.

densities (Figure 2). For these reasons, any change to physical properties at a given sheet density would suggest a change in the strength of bonding among the fibers and fines.

The strength properties evaluated included burst, tear, tensile, tensile energy absorption, and tensile stiffness indices. There were few consistent and substantial changes to these properties after treatment with crude cellulase, laccase alone, or proteinase. Only treatments with laccase alone consistently increased tensile index at a given sheet density (Figure 3), and there were corresponding increases in the burst indices (data not shown). In contrast, tensile stiffness indices at a given sheet density seemed to be increased by all three enzymatic treatments (Figure 4). Increases in tensile stiffness indices were also observed when the fines were treated with cellulase and mixed with kraft fibers before handsheet making (Richardson et al., 1998b). The consistency with which increases in the tensile stiffness index, a measure of the initial resistance of the sheet to tensile strain, have been observed makes it important to evaluate the contribution of the protein and formulation chemicals in commercial enzyme preparations.

PRMP screen rejects were used in the present study because they were more representative of long fiber fractions that could be obtained from mill processing than the R30 long fiber fraction obtained using the Bauer-McNett fiber classifier. The initial work using the R30 long fiber fraction suggested that cellulase treatments could increase tensile index by increasing the retention of fines (Wong and Richardson, 1999). There was no evidence for these effects when the PRMP rejects were treated with the same crude cellulase, and this could be due to the enzymes acting preferentially on the short rather than the long fibers, as has been previously observed (Richardson et al., 1998a).

Of the three enzymes discussed above, only laccase alone improved the papermaking properties of the PRMP screen rejects. Laccase is an oxidative enzyme that is expected to modify lignin without depolymerizing lignin or removing lignin from pulp. In the presence of oxygen and a chemical mediator, it is presently thought that laccase makes lignin more alkaline extractable and/or degradable, perhaps without lignin depolymerization immediately after treatment with the laccase−mediator system (Bourbonnais et al., 1995). Since the laccase−mediator system has been shown to enhance kraft pulp bleaching, it was also evaluated on mechanical pulp in

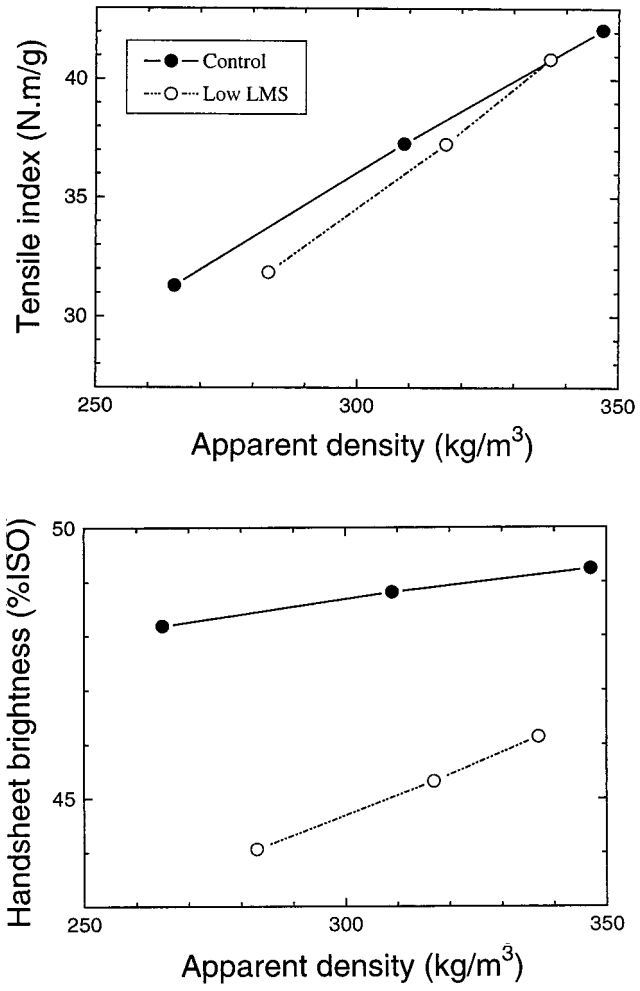


**Figure 5.** The tensile index and brightness at a given apparent density for handsheets made with furnish mixes containing fibers treated with the laccase−mediator system and untreated fines.

our laboratories. The results indicated that the presence of the mediator does not yield any improvement in the papermaking properties of the mechanical pulp fibers, while decreasing pulp brightness substantially (Figure 5). Furthermore, the mediator alone was found to affect the physical properties of handsheets, as has previously been observed in high kappa kraft pulps (Wong et al.,

1999). Since laccase alone did not alter pulp brightness substantially and it has previously been reported to improve auto-adhesion of fibers in medium density fiberboard (Felby et al., 1997), reduce energy consumption during mechanical pulping by ∼5% (Wong and Mansfield, 1999), increase tensile strength of sheets derived of mechanical pulp (Buchert et al., 1998), and preserve tensile strength through calendering (Wong and Mansfield, 1999), further evaluation of laccase treatments for mechanical pulps is warranted.

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