

# VARIATION IN THE RESPONSE OF THREE DIFFERENT *PINUS RADIATA* KRAFT PULPS TO XYLANASE TREATMENTS

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## ABSTRACT

Two xylanase preparations (Pulpzyme HC and Xylanase E) were assessed for their ability to enhance the refining properties of three different *Pinus radiata* kraft pulps. Both preparations selectively solubilized a significant proportion of the available xylan; however, xylanase E proved to be more aggressive, regardless of the pulp type. The selective removal of pulp xylan improved pulp beatability by increasing the apparent densities of the resultant handsheets over their corresponding controls. There were, however, variations in the response of the different pulp types, with an unbleached kappa 70 pulp showing the greatest improvement in sheet densification, as compared to an isothermal-cooked (kappa 33) and a fully bleached pulp. In general, xylanase treatments improved tear strength at a given density without significant loss in tensile strength and intrinsic fiber strength. These results suggest that xylanase treatments may be a means of enhancing the collapsibility/flexibility of certain kraft fibers while maintaining intrinsic strength.

*Keywords:* Pulp strength, enzyme, xylanase, xylan, kraft pulp, fiber flexibility, refining, paper properties, drainage.

## INTRODUCTION

Compared to some northern softwood species, radiata pine (*Pinus radiata*) yields kraft pulp that is more difficult to refine for the development of papermaking properties (McKenzie and White 1997). An improvement to the refining properties of radiata pine kraft pulp would therefore be beneficial to its competitive position in industrial markets. Our research efforts continue to focus on assessing commercially available enzyme preparations for their ability to enhance fiber/papermaking characteristics of both kraft and mechanical

pulps derived from radiata pine. This research philosophy utilizes microbial enzymes to selectively modify cell-wall components and may thus facilitate desirable transformations in innate fiber characteristics. For example, the low degree of fiber collapsibility/flexibility inherent in kraft pulp derived from coarse fibers can be improved by enzymatic treatments (Kibblewhite and Clark 1996; Wong et al. 1999; Mansfield et al. 1999; Mansfield and Saddler 1999), perhaps by enhancing relative bonded area and interfiber bonding. Similarly, the energy load required for the refining of

mechanical pulps may be reduced by selective enzymatic treatments (Pere et al. 1996).

The application of cellulases to pulp fibers has usually resulted in some beneficial changes in paper properties (i.e., sheet densification), however, at the expense of both strength and yield losses. In order to circumvent undesirable strength losses, researchers have focused on using monocomponent cellulases (endoglucanase and cellobiohydrolase) (Pere et al. 1995; Stork et al. 1995; Kibblewhite and Clark 1996). Although endoglucanase treatments maintained the improvements in sheet densification and curtailed some of the yield loss, their application still resulted in loss of intrinsic fiber strength. In contrast, cellobiohydrolase treatments reduced the extent of yield loss, while fiber strength properties and bonding potential were not significantly modified.

Polysaccharide-degrading enzymes of the hemicellulolytic nature (xylanases and mannanases) have conventionally been employed in the pulp and paper industry as a means to improve the bleachability of kraft fibers and thus reduce the amount of bleaching chemicals required to achieve desired brightness (Wong et al. 1997). More recently, a substantial amount of work has investigated the use of xylanases to alter the response of radiata pine kraft pulp to refining (Wong et al. 1999; Dickson et al. 1999). This approach has previously been explored by Noé et al. (1986) and Bhardwaj et al. (1996), and our present results indicate that the selective removal of xylan from unbleached kraft pulp yields a furnish that is more easily refined. Both sheet density and tensile strength of the pulp developed quicker with PFI refining after less than 0.5% of the pulp (approximately 5% of the available xylan) was enzymatically solubilized.

Our initial investigations on xylanase-aided fiber modification examined an unbleached, medium coarseness kraft pulp derived from radiata pine. These positive results have led to the research presented in the current report. The specific objective was to assess the response of three different types of kraft pulp to

treatment with two different xylanase preparations, by measuring the modifications in handsheet properties after subsequent laboratory refining.

## MATERIALS AND METHODS

### *Pulp*

Three kraft pulp samples derived from radiata pine (*P. radiata*) were compared in this study:

- ITC: An unbleached, high coarseness pulp was obtained from Tasman Pulp and Paper (Kawerau New Zealand) in mid-1996. It was produced by an isothermal cooking process, and no laboratory washing was carried out before the experiments.
- K70: An unbleached, kappa 70 pulp was prepared in the pulping laboratories at PAPRO using 2 liter stainless steel reactors with 200 g chips. The active alkali was 18.82% on the wood, effective alkali was 16% as Na<sub>2</sub>O, sulfidity was 30%, and liquor:wood ratio was 4:1. Bombs were heated to 170°C over 90 min and held at maximum temperature for 35 min. The pulp was passed through a mini-Bauer refiner and screened through 0.25-mm slots to reduce the amounts of shives.
- LCB: A fully bleached, low coarseness pulp was obtained from Tasman Pulp and Paper (Kawerau) in early 1997. The pulp was prepared from low density chips (398 kg/m<sup>3</sup>), and bleached with a (D/C)E<sub>0</sub>DnD sequence using 45% chlorine dioxide substitution.

The lignin and sugar compositions of the pulps (Table 1) were determined using replicated sulphuric acid hydrolysates (TAPPI Method T249 cm-85 1985). Each hydrolysate was filtered using a sintered-glass filter of medium coarseness for the gravimetric determination of Klason lignin (acid-insoluble lignin), and its absorbance at 205 nm was measured for the quantification of acid-soluble lignin (TAPPI Useful Method UM250 1991). The monosaccharide constituents were quantified by anion-exchange chromatography on a

TABLE 1. The percent composition of pulp samples.

Pulp	Pulp coarseness (mg/m)	Kappa number	Klason lignin	Acid soluble lignin	Monosaccharide residue (anhydro-form)					Total accounted
					Ara	Gal	Glu	Xyl	Man	
ITC	0.254	32.7	4.0	0.4	0.8	0.3	82.0	4.9	5.3	97.7
K70	0.268	69.6	10.6	0.4	1.3	0.7	74.3	6.7	5.5	99.5
LCB	0.199	—	—	0.3	1.0	0.7	75.8	6.5	5.0	89.3

CarboPac PA-1 column using a Dionex HPLC system (Dionex, Sunnyvale, California) and fucose as an internal standard. Fiber coarseness was measured using a Kajaani FS-200 instrument (Table 1).

### Enzymes

A xylanase (Xylanase E) purified from the fungus *Trichoderma reesei* was provided by Genencor International (USA), while Pulpzyme HC is a commercial xylanase available from Novo Nordisk (Denmark). Their xylanase activities were 216 and 149 nkat/mg solution (Wong et al. 1999), respectively, according to the method of Bailey et al. (1992). No contaminating hydrolytic activities were detected on locust bean gum galactomannan, while activities on carboxymethyl-cellulose were <0.26 and <0.14 nkat/mg solution, respectively.

### Enzyme treatments

Enzymatic treatment of the pulps was carried out in plastic bags. The pH of the pulp slurry was initially adjusted with sulphuric acid in order to stabilize the pH at the desired level (pH 5 for the Xylanase E and pH 7 for Pulpzyme HC). The enzyme was added with the dilution water needed to bring the pre-heated pulp to the target consistency of 5%. The pulp was then mixed thoroughly using a hand-held mixer and incubated for 2 h at 50°C. After the enzyme treatment, an aliquot of the filtrate was collected for analysis. The pulp sample was adjusted to pH 12 with sodium hydroxide and held at room temperature for 15 min, and then washed with water until a neutral pH was attained. Control pulps for each enzyme treatment were treated in parallel

under the same conditions except that no enzyme was added. Both enzyme preparations were loaded at a dose of 1.8  $\mu$ kat/g oven-dried pulp.

### Pulp testing

Handsheets were prepared and pulp physical evaluations made in accordance with AP-PITA standard procedures. The load applied during pulp refining with the PFI mill was 3.4 N/mm, and pulp consistency was 10%. All data from physical testing are reported on an oven-dried (o.d.) basis.

### Filtrate analysis

The carbohydrate composition of the filtrate, expressed in the anhydro-form, was determined by high performance anion-exchange chromatography after secondary acid hydrolysis was carried out to convert the oligomeric carbohydrates to their monomeric components (4% H<sub>2</sub>SO<sub>4</sub>, 121°C, 103 kPa, 60 min). Net solubilization was calculated by subtracting the carbohydrates solubilized during the control treatment and those present in the enzyme preparation itself.

## RESULTS

### Carbohydrate solubilization

Both xylanase preparations (Xylanase E and Pulpzyme HC) employed in this study were highly selective for the removal of the arabinoxylan constituent of the pulp, with little or no solubilization of the galactan, mannan, or glucan components. When comparing the absolute amount of carbohydrate solubilized (mg/g pulp) by these xylanases, it would appear that each pulp responded differently (Ta-

TABLE 2. Net arabinoxylan solubilization by xylanase treatment of the different kraft pulps.

Enzyme	Pulp	Arabinoxylan solubilized (mg/g pulp, anhydro-form) <sup>#</sup>	Percent of available substrate hydrolyzed <sup>§</sup>
Pulpzyme HC	LCB	5.5	7.5
	ITC	6.1	10.5
	K70	7.4	9.4
Xylanase E	ITC	9.2	15.9
	K70	14.5	18.3

<sup>#</sup> Values represent sugar liberated into filtrates by enzyme treatment minus the corresponding controls.

<sup>§</sup> Percentage of the total available arabinoxylan (dry weight fiber) hydrolyzed into the reaction filtrates by enzymatic treatment.

ble 2). There was a marked difference in the absolute amount of xylan solubilization by the two xylanase preparations when they were compared at similar loadings (based on the standard xylanase assay, using birchwood xylan as the substrate). Xylanase E was more aggressive and demonstrated a greater ability to solubilize arabinoxylan than even a twofold higher charge (3.6  $\mu$ kat/g) of Pulpzyme HC (data not shown).

#### Handsheet properties

At a given level of refining, the handsheets made from xylanase-treated pulp generally showed an increase in apparent density over their corresponding controls (Fig. 1). However, there were variations in the responses of the different pulp types. The unbleached kappa 70 (K70) pulp showed the greatest improvement in sheet densification, with Xylanase E yielding the most significant increases. The response of the high coarseness isothermal pulp (ITC) also demonstrated a substantial increase in densification, while the response of the fully bleached, low coarseness pulp (LCB) was marginal at best.

*Fully bleached, low coarseness (LCB) kraft pulp.*—The LCB pulp was treated with Pulpzyme HC only. This treatment resulted in a slight increase in pulp freeness at a given apparent density over the corresponding control (Fig. 2A). In addition, there was a slight increase in the air permeability of the sheets (Fig. 2B). The optical properties, including

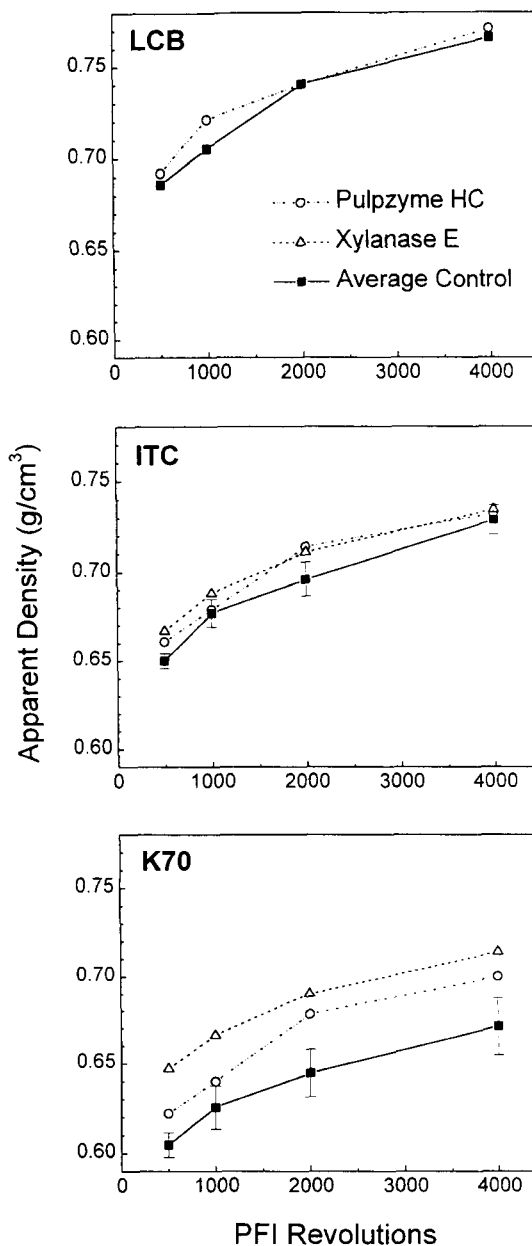


FIG. 1. Development of handsheet density after xylanase treatment of three kraft pulps. (A) LCB; (B) ITC; (C) K70.

opacity (Fig. 2C) and scattering coefficient (data not shown) of the LCB pulp, were virtually unaltered by treatment with the Pulpzyme HC xylanase.

The application of Pulpzyme HC prior to

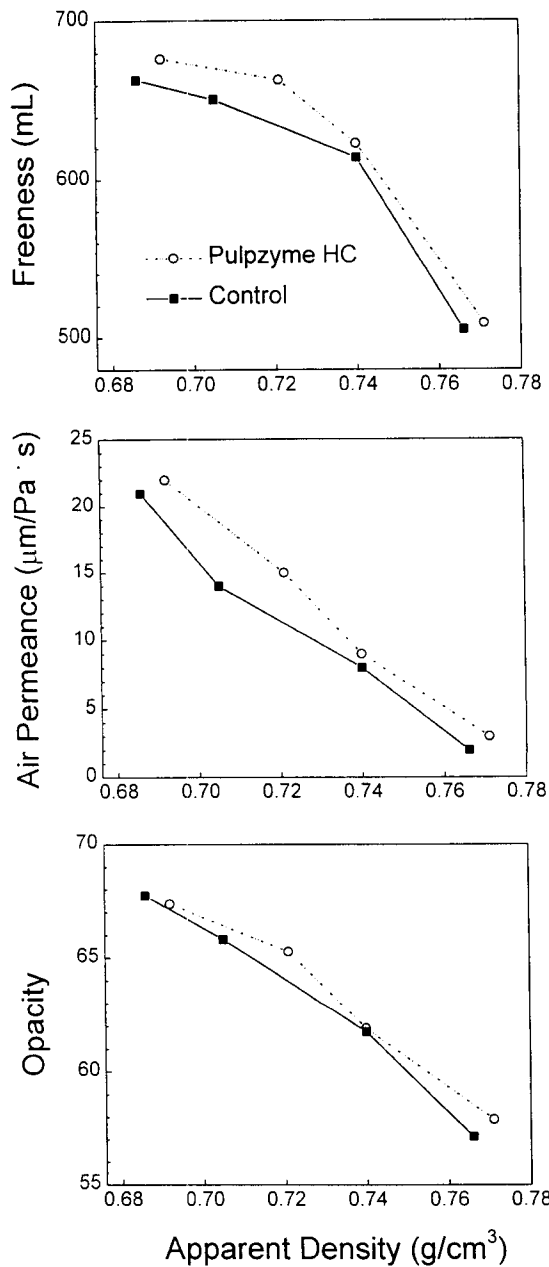


FIG. 2. Development of pulp properties after xylanase treatment of LCB kraft pulp. (A) Freeness; (B) Air permeance; (C) Opacity.

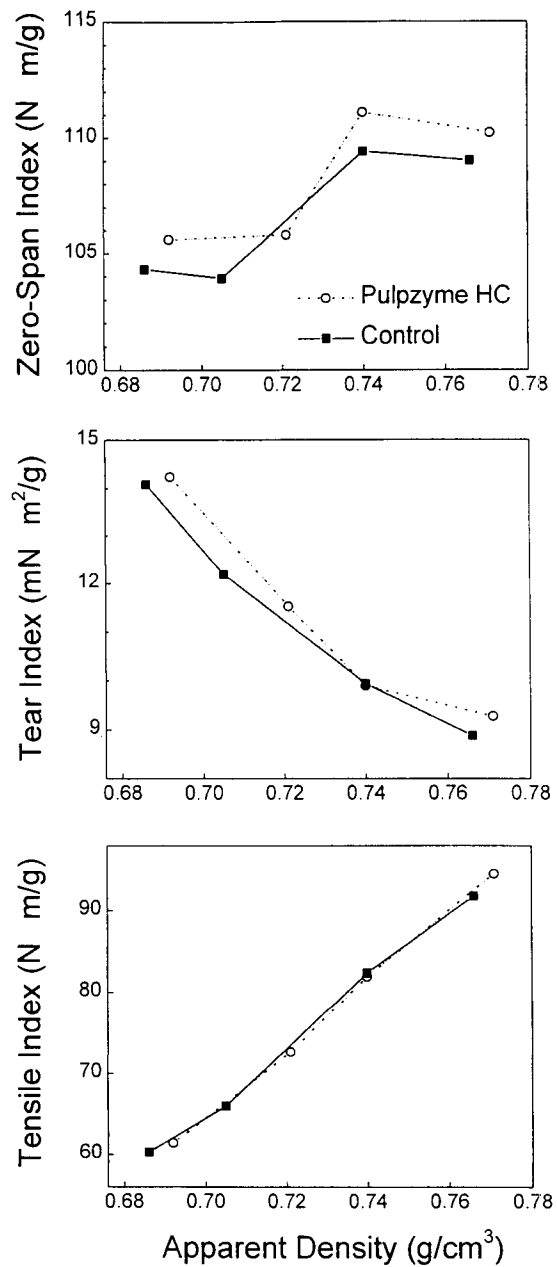


FIG. 3. Development of strength properties after xylanase treatment of LCB kraft pulp. (A) Wet zero-span tensile index; (B) Tear index; (C) Tensile index.

refining appears to improve the intrinsic fiber strength slightly, as measured by wet zero-span tensile index at a given level of refining (Fig. 3A). Tensile index (Fig. 3C) was not al-

tered by treatment with Pulpzyme HC; however, there appears to be a slight increase in the tear index at a given density (Fig. 3B). Consistent with the results on tensile index,

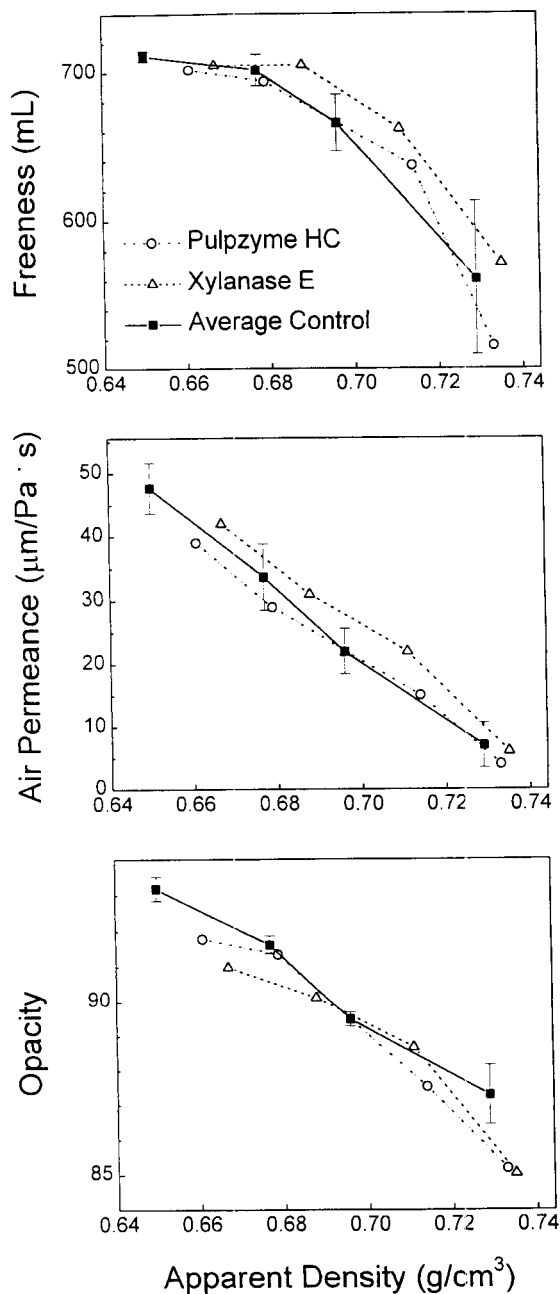


FIG. 4. Development of pulp properties after xylanase treatment of ITC kraft pulp. (A) Freeness; (B) Air permeance; (C) Opacity.

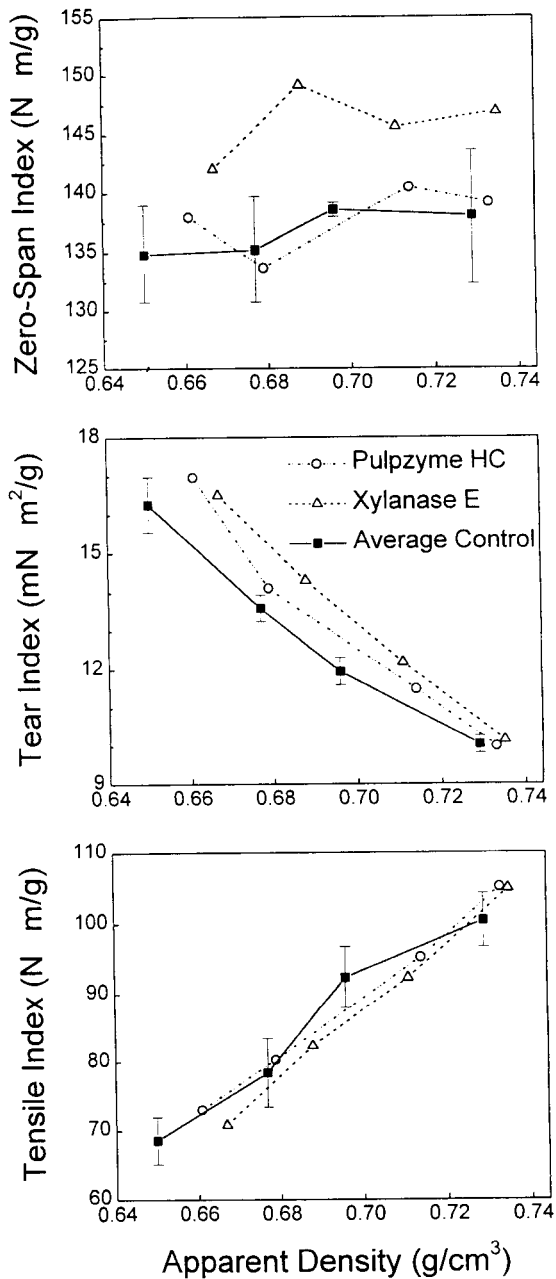


FIG. 5. Development of strength properties after xylanase treatment of ITC kraft pulp. (A) Wet zero-span tensile index; (B) Tear index; (C) Tensile index.

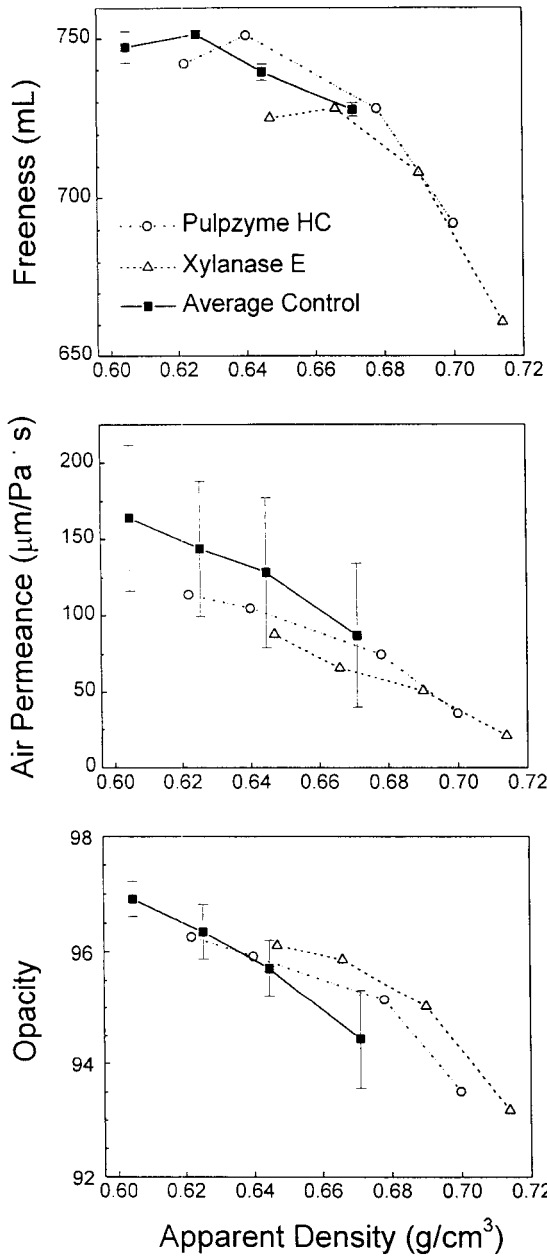


FIG. 6. Development of pulp properties after xylanase treatment of K70 kraft pulp. (A) Freeness; (B) Air permeance; (C) Opacity.

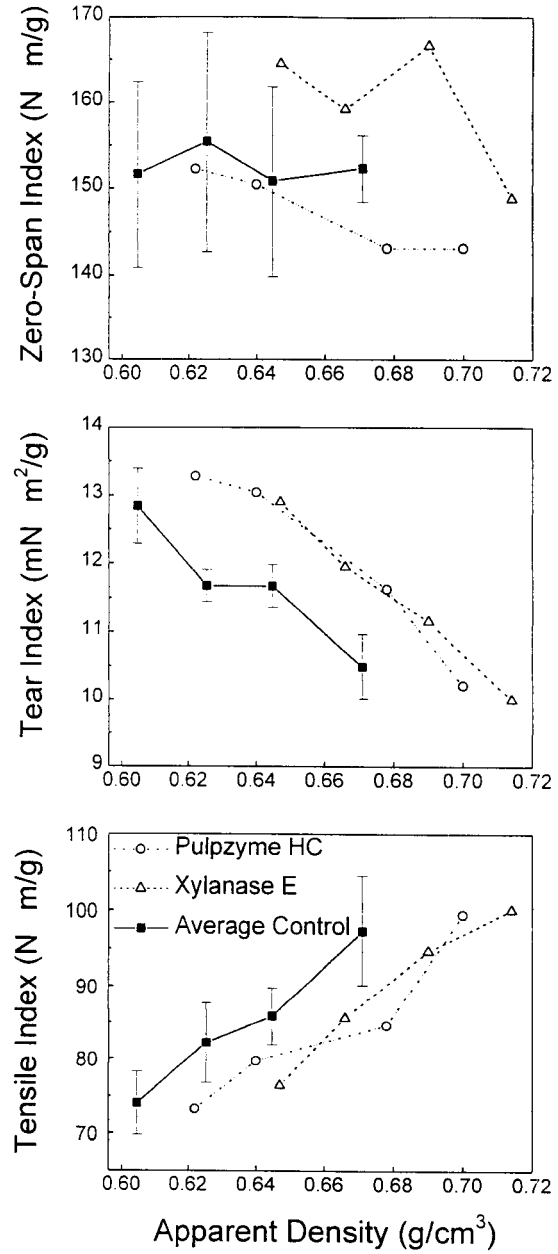


FIG. 7. Development of strength properties after xylanase treatment of K70 kraft pulp. (A) Wet zero-span tensile index; (B) Tear index; (C) Tensile index.

little change was observed with respect to TEA and tensile stiffness index after enzyme treatment, although a minor improvement in stretch was observed (data not shown).

*Unbleached, high coarseness, isothermally cooked (ITC) kraft pulp.*—There were differences in the response of the ITC pulp (Fig. 4) to xylanase treatment from that of the LCB

pulp. For example, the freeness at a given density was unchanged after treatment with Pulpzyme HC (Fig. 4A), while a slight increase seems to have occurred after treatment with Xylanase E.

The air permeance was relatively unchanged when compared to the controls at a given density, regardless of the type of xylanase applied (Fig. 4B). These results suggest that some of the larger fibers may have collapsed slightly, resulting in sheet densification, while some of the finer fibers or surface fibrillation may have been removed to increase freeness. There was little effect on sheet opacity at a given density (Fig. 4C), although the absorption coefficient was significantly reduced by the xylanase treatments (data not shown).

The effects that xylanase treatment had on intrinsic fiber strength (wet zero-span index) were inconsistent when considering the two different xylanase preparations. Pulpzyme HC treatments did not alter the wet zero-span index at a specific density (Fig. 5A), while xylanase E significantly improved the intrinsic strength of the ITC fibers. These strength increases of nearly 10 Nm/g were achieved at all levels of refining. The tear index at a given density of handsheets formed from ITC pulp was also significantly improved by xylanase treatment (Fig. 5B). The increase in tear index was again most prominent with Xylanase E. The resultant handsheets demonstrated tensile indices that fell within the range of the values obtained for average controls at a given density (Fig. 5C).

*Unbleached, kappa 70 (K70) kraft pulp.*—The freeness of the K70 pulp was substantially reduced with refining after xylanase treatment (Fig. 6A), particularly with Xylanase E. However, both the xylanase-treated and control fibers showed similar levels of freeness at given apparent densities. The air permeability was not significantly altered when compared to the control sheets at equivalent apparent densities (Fig. 6B). The optical properties of the K70 pulp were altered by xylanase treatments; both sheet opacity (Fig. 6C) and scattering coefficient

(data not shown) at a given density were increased when compared to the corresponding controls. Although the scattering coefficient was increased, it did not seem to be influenced by enzyme type.

Both the tear and tensile indices were greatly modified by enzyme treatment (Fig. 7B, 7C). There were significant improvements in tear index at a given density, while there was a reduction in the tensile index of the handsheets produced. Tensile stiffness index, TEA index, and stretch were all adversely affected by the xylanase treatment (data not shown), corresponding to the observed reduction in tensile index. Fiber strength (wet zero-span index) did not demonstrate consistent trends (Fig. 7A).

#### DISCUSSION

The importance of the hemicellulose component of pulp in papermaking has been well documented. Pulps that contain a higher concentration of hemicellulose, up to a certain threshold, exhibit greater strength. These conclusions were based on evidence that showed increases in the tensile and burst indices, but a decrease in tear strength, when certain hemicellulose fractions or related polysaccharides were added to pulp (Thompson et al. 1953; Dugal and Swanson 1972; Mobarak et al. 1973; El-Ashmawy et al. 1976). However, in addition to the quantity, chemical structure, and distribution of the hemicellulose, the degree of polymerization of this fraction plays an intricate role in determining the final paper strength (Eremeeva et al. 1995). Furthermore, those fibers that contain a higher proportion of glucomannan (hexosan) compared to xylan (pentosan), tend to show enhanced adhesive capabilities and result in greater paper strength (Cottrall 1950; Thompson et al. 1953). Results from our laboratories (Dickson et al. 1999) indicated that the partial removal of pulp xylan could enhance certain physical properties of unbleached radiata pine kraft pulp, a direct function of sheet densification. These beneficial modifications have



been shown to occur during refining in both a PFI mill and an Escher-Wyss conical refiner.

In the present study, two different xylanase preparations were assessed for their effectiveness in modifying three different kraft pulps derived from radiata pine. The two preparations were compared at similar loadings, according to their activity on birchwood xylan. Both preparations released substantial amounts of arabinoxylan, while the amounts of glucan and glucomannan solubilized from the pulps during treatments were essentially undetectable. The Xylanase E preparation was clearly more aggressive than the Pulpzyme HC preparation, regardless of the type of pulp. Xylanase E also showed a greater ability to induce sheet densification when compared to the Pulpzyme HC preparation. It would appear that the increased ability to solubilize arabinoxylan corresponds to elevated levels of sheet densification. Since an increase in fiber collapse was observed when fiber dimensions were measured after 14% of the pulp xylan was enzymatically removed from a medium coarseness kraft pulp (Kibblewhite and Clark 1996), fiber collapse appears to contribute to the observed increases in apparent beatability (sheet densification).

Although the idea of using xylanases to enhance pulp beating was introduced over a decade ago (Mora et al. 1986; Noé et al. 1986), the majority of the work concerning xylanase applications in the pulp and paper industry has since focused on its bleach-boosting capabilities (Wong et al. 1997). Few investigators have made an attempt to determine the changes to the physical characteristics of pulp following xylanase treatments. These previous reports have indicated that xylanase treatments cause little change to pulp strength (Roberts et al. 1990; Bhardwaj et al. 1996; Mansfield et al. 1996); however, none of the work evaluated these strength properties at multiple levels of refining. Our current work clearly indicates that the effect of xylanase treatments can improve certain strength properties of different types of radiata pine kraft

pulp fibers. However, the extent to which these beneficial modifications occur is not consistent between different types of pulps or different xylanase preparations. For example, a relatively large increase in zero-span tensile index was observed in the ITC pulp with Xylanase E only. Since Mohlin and Alfredsson (1990) have suggested that the zero-span tensile index is a good measure of fiber curl or deformation, xylanase treatment may be reducing fiber curl rather than increasing the intrinsic strength of the fibers.

At a given handsheet density, xylanase treatments prior to refining can substantially improve the tear index, regardless of which xylanase preparation has been utilized. The K70 pulp demonstrated greater improvement in tear index than the LCB and ITC pulps, however, at the expense of a loss in tensile strength. Since an increase in tear strength at a given tensile strength was previously shown when approximately 15% of the pulp xylan had been enzymatically removed from a medium coarseness kraft pulp (Kibblewhite and Clark 1996; Wong et al. 1999), the selective increase in tear appears to be a consistent effect of xylanase treatments.

The variability observed in the response of the different pulp fibers to xylanase treatments suggests that although the removal of surface xylan can result in beneficial improvements in handsheet properties, fiber type and morphology also influence the degree of modification. The factors determining the difference in the response of different pulps remain unclear. Although all of the kraft pulps used in the study were derived from radiata pine, the pulping procedures employed in their preparation were significantly different. The pulps therefore differed in xylan and lignin content. The present results support previous findings, which suggest that pulps that are unbleached and of higher coarseness are more responsive to treatments with different enzymes (Kibblewhite and Clark 1996). Current evidence indicates that xylanase treatments increase the inherent fiber flexibility and consequently contribute to

the increases in handsheet densification (Dickson et al. 1999).

#### CONCLUSIONS

Xylanases selectively remove a portion of the available pulp arabinoxylan, without solubilizing any of the other pulp carbohydrates, and yield beneficial modification of certain handsheet characteristics of three different radiata pine kraft pulps. These improved modifications include:

- an increase in sheet densification with PFI refining;
- slight improvements in wet zero-span tensile strength;
- improved tear index at a given density.

The degree of modification was not consistent among all three types of kraft pulp; being substantial in an unbleached kappa 70 pulp and marginal in a fully bleached low coarseness pulp. Xylanase E was also found to be more aggressive, solubilizing approximately 16–18% of the pulp arabinoxylan, and more effective in modifying radiata pine derived kraft fiber properties than the Pulpzyme HC xylanase. The level of fiber modification that can be achieved in the three radiata pine pulps by different loadings of xylanase remains to be determined.

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