THE EFFECTS OF HYDROLYTIC ENZYME TREATMENTS ON THREE BRITISH COLUMBIAN INTERIOR FIR KRAFT PULPS DIFFERING IN THEIR INITIAL FIBER COARSENESS

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ABSTRACT

The effects of carbohydrate-degrading enzymes on three softwood kraft pulps, differing primarily in their initial fiber coarseness, were assessed. The pulps were treated with three different enzyme preparations (a crude cellulase, an endoglucanase, and a xylanase) to assess the potential of the different enzymes to alter handsheet properties and to evaluate their effects on fiber coarseness. All enzymatic treatments increased handsheet densities irrespective of the furnish used. The most significant modifications in handsheet properties were evident after treatment with either the crude cellulase or the endoglucanase. Although increased densification occurred with all the pulps, the degree of fiber coarseness of the original pulp influenced the magnitude of response to the different enzymatic treatments. While the tensile index of the coarser pulp was improved by treatment with the crude cellulase, a similar trend was not evident with the pulps of lower coarseness. In contrast, the tensile strength of all pulps, irrespective of the inherent fiber coarseness, was improved by the endoglucanase treatments. The tear strength decreased after treatments with both the crude cellulase and endoglucanase. Xylanase treatments did not significantly alter the handsheet properties of any of the pulps, regardless of the nature of the starting furnish.

Keywords: Fiber modification, enzyme, cellulase, endoglucanase, xylanase, tensile index, tensile strength, carbohydrate solubilization, paper properties, paper strength, kraft pulp, interior fir.

INTRODUCTION

Extracellular microbial enzymes have the potential to be powerful tools for modifying cellulose, hemicellulose, and lignin, the main constituent lignocellulosics. As a result, several enzyme applications for processing pulp and paper have been actively pursued over the last few decades. Although most areas are still

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evolving, some applications have already become, or are approaching, commercial use. To date, the biggest success is the use of xylanases for bleach boosting, with several mills routinely using xylanase prebleaching (Viikari et al. 1994; Tolan et al. 1995). Other applications include, reduced beating times (Noé et al. 1986; Yamaguchi and Yaguchi 1996), elevated drainage rates of recycled fiber (Pommier et al. 1989, 1990), enhanced interfiber bonding (Mansfield et al. 1996), improved deinking of secondary fiber (Welt and Dinus 1996), and enhanced bleaching using oxidative enzymes (Paice et al. 1995).

In the last few years, various groups have attempted to enhance the physical properties of handsheets produced from pulps with "undesirable" inherent characteristics, through selective enzymatic modifications (Mansfield et al. 1996, 1999; Edgar et al. 1998; Mansfield and Saddler 1999). It is widely recognized that fiber properties such as strength, length, and coarseness, as well as the degree of fiber to fiber bonding, all strongly influence the strength and quality of the paper products (Seth 1990a, b). Previously we had shown that the coarseness of coastal Douglas-fir kraft pulp fibers could be reduced slightly by mixed cellulase/hemicellulase treatments, leading to denser and smoother handsheets (Mansfield et al. 1996). However, these modifications were usually achieved at the expense of yield loss and reduced sheet and fiber strength. Further studies indicated that one way of circumventing these two detrimental effects was to use pure endoglucanase and xylanase preparations (Edgar et al. 1998). This earlier work with Douglas-fir was initiated because the coarse, stiff, and inflexible nature of Douglas-fir fibers yields paper products that are relatively rough and weak (Seth and Page 1988; Seth 1990a, b). However, true fir species are known for their finer fibers, which provide good conformability and interfiber bonding capacities, yielding paper products with exceptional strength. The current study was undertaken to assess whether the specificity and selectivity of the enzymes could be used to further enhance the sheet properties of kraft pulps derived from British Columbian interior fir species, and to ascertain how furnishes of different inherent coarseness respond to enzymatic treatments.

METHODS AND MATERIALS

Pulps

Three kraft pulps derived from British Columbian interior fir were obtained from the Pulp and Paper Research Institute of Canada (PAPRICAN), British Columbia, Canada. Fiber coarseness and fiber length distribution of the pulps were measured using a Kajaani FS-200 instrument (Kajaani, Finland) (TAPPI Test Method T 271 pm-91). The lignin and carbohydrate content of the three pulps was determined using triplicate H_2SO_4 (72%) hydrolysates. Each hydrolysate was filtered using a sintered-glass filter of medium coarseness for the gravimetric determination of acid-insoluble lignin (TAPPI Test Method T 222 om-88), and its absorbance at 205 nm was measured for the quantification of acid-soluble lignin (TAPPI Useful Method 250). The hydrolysates were subjected to secondary acid hydrolysis to convert the oligomeric carbohydrates to their monomeric components, and the sugar composition was determined by subsequent high performance anion-exchange chromatography (HPAEC) on a CarboPac PA-1 column using a Dionex DX-500 HPLC system (Dionex, Sunnyvale, Calif.).

Enzymes

Novozyme SP 342 (crude cellulase/xylanase) and SP 613 (endoglucanase), derived from *Humicola insolens,* were obtained from Novo Nordisk, Denmark. Ecozyme (xylanase) was obtained from Thomas Swan and Co., England. The endoglucanase (CMCase), xylanase, mannanase, and filter paper activities of these commercial enzyme preparations were measured on carboxymethylcellulose (1% CMC, Sigma), xylan (1% birchwood xylan, Sigma), galactomannan (1% locust bean, Sigma) and filter paper (No. 1 Whatman) respectively, using methods described previously (Wood and Bhat 1988; Bailey et al. 1992;

TABLE 1. *Activities and protein content of three commercial enzyme preparations.*

Enzyme	Protein (mg/mL)	CMCase (IU/mL)	Xylanase (IU/mL)	Mannanase (IU/mL)	Filter paper (IU/mL)
SP 342	44.1	30	1,614	0.1	2
SP 613	27.8	18	2	0	0
Ecozyme	6.12	0	6,471	0	0

Gübitz et al. 1996). Total protein in solution was determined using the bicinchoninic acid protein assay (Stoscheck 1990).

Enzymatic treatment of pulp

The enzyme concentrations added to the pulp treatments were based on CMCase or xylanase activity (IU) per gram of oven-dry fiber. The enzyme loadings were chosen such that the individual endoglucanase (SP 613) and xylanase (Ecozyme) activities were comparable to the respective activities of the crude cellulase enzyme (SP 342), which exhibited both cellulolytic and hemicellulolytic activities. All enzyme treatments were incubated for 1 h at 50°C under continuous agitation at 175 rpm, at 3% pulp consistency in 50 mM phosphate buffer (pH 7.0). Reactions were terminated by boiling for 30 min to inactivate the enzyme. Control pulp treatments were run in parallel under similar conditions except that no enzyme was added. Following the enzyme treatments, the pulps were collected for handsheet making and analysis, while the carbohydrate composition of the reaction filtrates was determined by HPAEC. The net solubilization during the individual enzyme treatments was determined by subtracting both the carbohydrate solubilized during the control treatments and those present in the enzyme preparations.

Paper testing

Handsheets were prepared according to TAPPI T 205 sp-95, with the white water recycled to ensure that the fines were included in the resultant handsheet. For the determination of handsheet density (Precision Micrometer, Testing Machines Inc.), burst index (Mullen Tester, B. F. Perkins & Sons, Holyoke, Mass.), tensile index (Model 4202 Universal Testing Instruments, Instron, Canton, Mass.), zero-span breaking length (TroubleShooter, Pulmac Instruments Int., Montpelier, Vt.), and tear index (Series 400 Monitor/Tear, Testing Machines Inc., Amityville, N.Y.) tests were conducted according to TAPPI Test Methods T 547 om-88, T 403 om-91, T 494 om-88, T 231 cm-96 and T 414 om-88, respectively.

TABLE 2. *Fiber coarseness (mg/m) of three interior fir softwood kraft pulps.*

PULP A	PULP B	PULP C
0.229	0.208	0.195

RESULTS

Enzyme activities

Prior to evaluating the enzymes for their potential to modify the pulp characteristics, the three enzyme preparations were assessed for their ability to hydrolyze standard substrates (Table 1). Although the SP 342 preparation was marketed as a commercial cellulase, it still contained high levels of xylanase activity. The SP 613, an endoglucanase preparation, also contained low, but significant levels of xylanase activity, while the commercial xylanase (Ecozyme) demonstrated a high degree of purity and specificity for its substrate.

Pulp characteristics

Initially the fiber coarseness, as measured by Kajaani FS-200, of the three interior fir pulps was determined (Table 2). Pulp A exhibited the highest fiber coarseness, followed by pulps B and then C. The weighted fiber length distribution of the different furnishes was also different (Fig. 1), with pulp C containing a slightly higher proportion of the long fiber length fractions (3.0 mm–7 mm) followed by pulps B and then A, while pulp A contained slightly more of the shorter fibers (0.1 mm–3 mm). The average weighted fiber length was 2.8 mm, 2.9 mm, and 3.0 mm for pulps A, B, and C, respectively. An analysis of the carbohydrate and lignin content of each

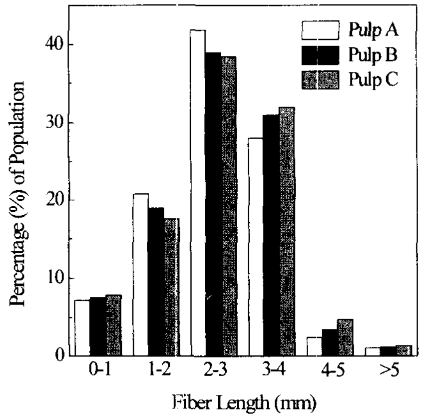

FIG. 1. Weighted fiber length distribution of three interior fir kraft pulps.

TABLE 3. *Carbohydrate and lignin content (% dry weight) of three interior fir softwood kraft pulps.*

Compositional Component	Pulp A	Pulp B	Pulp C
Arabinose	0.9	0.9	0.9
Galactose	0.6	0.5	0.5
Xylose	7.4	7.6	7.6
Mannose	8.1	7.5	7.7
Glucose	78.2	78.6	76.9
Total sugars	95.2	95.1	93.6
Acid insoluble lignin	4.2	4.2	4.7
Acid soluble lignin	0.41	0.40	0.42
Total lignin	4.61	4.60	5.12

pulp indicated that the three furnishes did not show any significant differences in their compositional constituents (Table 3).

Degree of hydrolysis of the pulps

The amount and types of sugar solubilized from the three different pulps were representative of the respective activities of the enzymes (Fig. 2). As expected, the xylanase released mainly xylose, while the crude cellulase released both xylose and glucose together with some mannose. The xylanase and the crude cellulase also released low levels of arabinose. The endoglucanase released mainly glucose, some xylose, and low levels of mannose although no mannanase activity was detected in the preparation. The liberation of mannose may be a result of nonspecific enzymatic hydrolysis of pulp glucomannans or solubilization caused by the very close association of cellulose and glucomannan in softwood pulps.

Handsheet testing of the enzymatically treated pulps

A comparison of the various control handsheets (Fig. 3) indicated that, as expected, the increases in sheet density corresponded to the decreases in fiber coarseness. Similarly, the in-plane strength of the control handsheets, as measured by both tensile and burst indices, increased with decreasing fiber coarseness. In contrast, the intrinsic fiber strength and tear index decreased with reduced fiber coarseness.

The initial enzymatic treatments of pulps A, B, and C, with the crude cellulase and the endoglucanase, were loaded at 5 CMCase units per gram of oven-dried fiber. At this CMCase dosage, the crude cellulase also contained ~270 xylanase units per gram of oven-dry fiber. Therefore, to ensure that the xylanase activity of Ecozyme was comparable to that of the SP 342, the Ecozyme preparation was also loaded at 270 units per gram of oven-dry fiber. The handsheet densities of all three pulps increased after treatments with both the crude cellulase and the endoglucanase (Fig. 3). In contrast, the xylanase treatments increased the

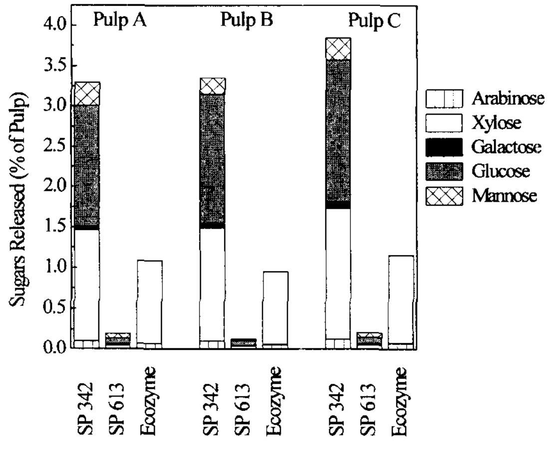

FIG. 2. Sugars solubilized from pulps A, B, and C after enzymatic treatments. SP 342 = Crude Cellulase; SP 613 = Endoglucanase; Ecozyme = Xylanase.

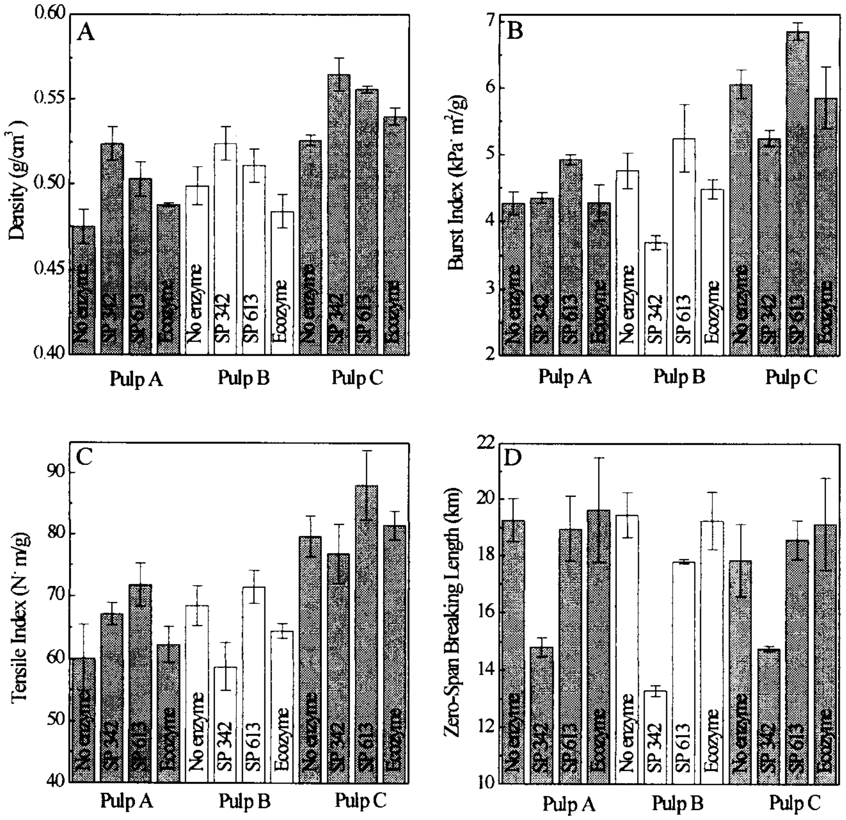

FIG. 3. Handsheet density (A), burst index (B), tensile index (C) and zero-span breaking length (D) of pulps A, B, and C after control and enzymatic treatments. SP 342 = Crude Cellulase; SP 613 = Endoglucanase; Ecozyme = Xylanase.

density of pulps A and C, while that of pulp B was slightly reduced.

The three pulps responded differently to the enzyme treatments in terms of their strength properties. The burst and tensile indices of pulps B and C decreased after the treatments with the crude cellulase while those of pulp A (highest coarseness) increased, with the tensile index increasing by approximately 12%. Endoglucanase treatments increased the burst and tensile strengths of all three pulp furnishes. However, the most beneficial modifications occurred with pulp A, as indicated by a ~19% increase in tensile strength. Similarly, the tensile indices of pulp B and pulp C were increased by approximately 5% and 11%, respectively.

Both the zero-span breaking length and the tear index of all of the pulps were greatly reduced after treatments with the crude cellulase. Although the endoglucanase treatments did not significantly alter the zero-span breaking length of pulps A and C, it did reduce that of pulp B, while the tear indices of all pulps were reduced by as much as 30%. Generally, the effect of Ecozyme on the fiber strength properties of all the pulp samples was less clear than that of both the cellulolytic preparations. Although neither the zero-span breaking length nor the tear index of pulp C was affected, the tear index values for pulp B increased slightly, while that of pulp A decreased.

Dose dependent treatment of pulps A and C

Pulps A and C were chosen for further enzyme dose dependent studies since these pulps

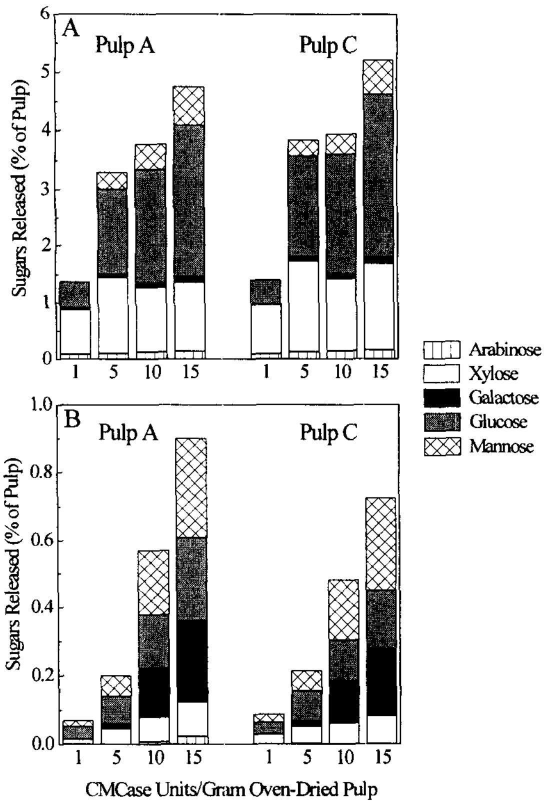

FIG. 4. Sugars solubilized from pulps A and C after dose dependent enzymatic treatments. SP 342 = Crude Cellulase; SP 613 = Endoglucanase; Ecozyme = Xylanase.

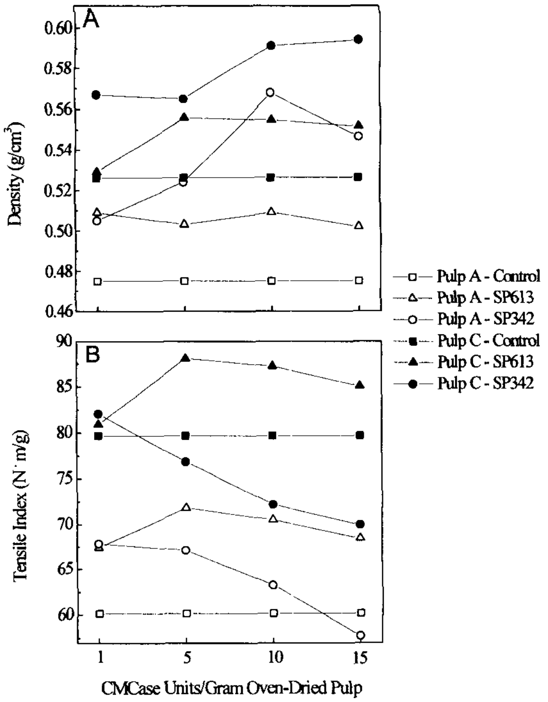

FIG. 5 Changes in handsheet density (A) and tensile index (B) of pulps A and C after control and enzyme treatments at 1, 5, 10, and 15 CMCase units/g of oven-dried pulp.

responded differently to the treatments with the crude cellulase, and they exhibited the greatest differences in both fiber coarseness (Table 2) and fiber length (Fig. 1). Similarly, both the crude cellulase and endoglucanase preparations were chosen for further studies since they were able to effect different changes to the burst and tensile indices of the pulps of different coarseness (Fig. 3).

As expected, the levels of carbohydrate dissolution increased with increasing levels of enzyme concentration (Fig. 4). Treatment with the crude cellulase resulted in greater hydrolysis of both pulps than was obtained with treatments with the endoglucanase at comparable enzyme loadings. The handsheet densities of pulps A and C increased after treatments with both the crude cellulase and the endoglucanase (Fig. 5). However, the degree of densification was greater for both pulps when treated with the crude cellulase, and both enzymes increased the density of pulp A more than that of pulp C.

Although there was an initial increase in tensile strength of pulp A after treatment with the crude cellulase at the 1, 5, and 10 CMCase units/g pulp loadings, at a loading of 15 CMCase units/g pulp, the resulting tensile strength was comparable to that of the control sheets (Fig. 5). A similar trend was not detected during the treatments of pulp C, which demonstrated compromised tensile strengths at the low loading of 5 CMCase units/g fiber. In contrast, the tensile strengths of both pulps increased after treatments with the endoglucanase preparations at all enzyme loadings, with the highest increase evident at a loading of 5 CMCase units/g of pulp. The most significant increase in tensile strength occurred with pulp

A, which after a treatment of 5 CMCase units/g pulp, resulted in an increase of 19% over the corresponding control (Fig. 5).

The intrinsic fiber strength of both pulps, as measured by zero-span breaking length, decreased after enzymatic treatments in a dose-related fashion. However, the reductions in fiber strength after treatments with the endoglucanase preparation were considerably less than those obtained after treatment with the crude cellulase. Tear index was also detrimentally affected by these enzyme applications (data not shown). It was apparent that the damage effected by the crude cellulase and the endoglucanase to the individual fiber strength was more pronounced on the finer-fibered pulp, as indicated by the greater reductions in tear and zero-span strengths of pulp C.

DISCUSSION

Fiber coarseness plays a very important role in both paper formation and strength development (Seth and Page 1988; Seth 1990a, b). For example, the coarser the furnish, the fewer the number of fibers present in a sheet at a given grammage. High coarseness is also often associated with high inherent fiber rigidity, which limits the number and degree of interfiber contacts. Therefore, these fibers are not held tightly in the fibrous network and subsequently result in porous paper with inferior strength, as compared to paper produced from fibers of lower coarseness. In the work summarized here, we wanted to assess whether various polysaccharide-degrading enzyme preparations would act differentially on pulps derived from the same species (interior fir), but differing in fiber coarseness. Following the control treatments, a comparison of the pulps clearly indicated that handsheets made from the finer-fibered pulp had superior paper strength as measured by both burst and tensile indices (Fig. 3). This is related to the greater fiber conformability, yielding a higher degree of interfiber bonding.

Both the tensile and burst strengths of paper are influenced by fiber length, with strength increasing with increasing fiber length (Seth 1990a). As the average weighted fiber lengths determined by Kajaani FS-200 analysis was 2.8 mm, 2.9 mm, and 3.0 mm, respectively for pulps A, B, and C, it is probable that the higher fiber length may also have contributed to the observed higher burst and tensile strengths, as the strengths of the control pulps decreased as the fiber length decreased. However, it has been shown that, in well bonded sheets derived from unbleached, never-dried softwood kraft pulp, an increase in fiber length will not benefit the sheet tensile properties once a fiber length of 2.95 mm is reached (Seth 1990a). Given the range of fiber lengths of pulps A, B, and C, it is reasonable to assume that fiber coarseness was the main determinant for the observed differences in the tensile and burst strengths of the three control pulps. In contrast, the coarser-fibered pulps demonstrated greater zero-span breaking strength, a characteristic indicative of individual fiber strength and reflective of thicker cell walls. Since intrinsic fiber strength significantly influences the tear strength of well bound softwood sheets (Seth and Page 1988), the tear strength of sheets made from pulp A was greater than those produced from either pulp B or C.

The three pulps responded differently to the treatments by the different enzyme treatments, with the crude cellulase (SP 342) and the endoglucanase (SP 613) resulting in the most significant modifications to the handsheet properties. Xylanase treatments of the different pulp furnishes did not result in any substantial alteration in either fiber or handsheet properties.

The handsheet density of all three pulps increased markedly after treatments with either the crude cellulase or the endoglucanase. The greatest increase was obtained with the crude cellulase preparation, which contained significant amounts of both endoglucanase and xylanase activities, suggesting that synergy among the multiple enzyme activities within the crude enzyme preparation may have contributed to more extensive fiber modifications.

The increases in apparent density after the enzymatic treatments suggested that, at least within the coarseness range of 0.229–0.195 mg/m, fiber collapsibility could be induced by enzymatic treatments. At the lower enzyme loadings with the crude cellulase sheet densification of the coarser fibered pulp was also reflected in the improvements in both tensile and burst indices. However, similar treatments of the lower coarseness pulps (B and C) with the crude cellulase resulted in reductions in these handsheet parameters, although concomitant increases in densification were observed. It appears that only the coarser furnish could withstand treatments with a complete cellulase complex and that the combined action of the multiple enzyme activities was more damaging to the finer fibers than to the coarser fibers. It is probable that the higher specific surface area exhibited by the finer fibers, as found in pulp C, resulted in a greater degree of specific surface modification.

After treatments with the crude cellulase preparation, regardless of the initial fiber coarseness, the tensile strength decreased with increasing sheet densification (Fig. 3). However, comparable endoglucanase treatments showed an increase in the tensile strength with a concurrent increase in apparent density (Figs. 3 and 5). These results implied that the limited hydrolysis attained by the endoglucanase treatments was enough to effect enhanced interfiber bonding, consequently improving handsheet strength, irrespective of the initial fiber coarseness of the pulp furnish. Previously, the combined action of endoglucanases with cellobiohydrolases or xylanases has been shown to be more damaging to pulp fibers than was the action of endoglucanase alone (Kibblewhite and Clark 1996). Different endoglucanases have also been shown to affect sheet strength properties differently (Pere et al. 1995). However, the employment of this endoglucanase preparation significantly restricted both the reduction in yield, and individual fiber strength and tear strength, while elevating the degree of interfiber bonding resulting in increased tensile and burst indices.

In general, these results demonstrate that enzymatic treatments can be used to improve certain qualities of handsheets produced from softwood kraft pulps. The behavior of the various pulps differing primarily in inherent fiber coarseness after treatment with a carbohydrate-degrading enzyme preparation suggests that fiber coarseness may play a role in determining how different pulps respond to various enzyme treatments. The most beneficial effects attained with minimal yield loss were obtained as a result of endoglucanase treatments, which improved both the tensile and burst indices of all pulps, irrespective of the initial fiber coarseness.

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