

ABSTRACT

A softwood kraft pulp derived primarily from Douglas-fir was used in experiments with enzymes to improve strength properties. Industrial-scale fiber fractionation was combined with a cellulase treatment of the long fiber fraction. Handsheet properties were evaluated after the long fiber fraction had been recombined with the untreated short fiber fraction. Density and smoothness were improved in handsheets derived from the unfined pulps. Both the tensile index and burst index were increased by about 15% over their corresponding controls. Similar handsheet modifications could be achieved at lower enzyme dosages when the pulp was subjected to refining after the treated fibers were recombined with the untreated fibers.

Application: Fiber fractionation combined with enzymatic treatments of the long fiber fraction may offer a way to use of the coarse fibers of Douglas-fir in the manufacture of some fine paper products.

FIBER FRACTIONATION INVOLVES the mechanical separation of certain classes of fibers into a long fiber fraction and a finer fiber fraction (1). The finer fiber fraction contains the shorter, smaller fibers, and the long fiber fraction consists of longer, coarser fibers, bundles, and splinters. During fiber fractionation, the long fibers are separated and subjected to additional refining, which enhances the quality of the paper products.

Research in this area has been fueled by the demand for mechanical pulps that exhibit the superior surface properties required for high-grade printing papers (2). However, fractionation technologies are being employed or considered in other

Enhancing Douglas-fir pulp properties with a combination of enzyme treatments and fiber fractionation

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pulping applications as well. In the processing of secondary fibers, fractionation is used to yield different fiber feedstocks to produce different grades of paper (1, 3, 4-11). Fiber fractionation is also used in the production of linerboard and in the processing of corrugated containers and paperboards (12-15). In addition, fractionation is used in the production of grocery bags from kraft pulp (16).

In recent work, we tried to improve the quality of handsheets derived from Douglas-fir pulps by using hydrolytic enzymes (cellulase and xylanases) to modify the characteristics of the pulp fibers (17, 18). We succeeded in manipulating the extent of flexibility and collapsibility of kraft pulp fibers, which resulted in altered sheet properties. However, these modifications were attained at the expense of both fiber strength and paper strength (17).

To circumvent the loss in handsheet strength, we enhanced the selectivity of the enzymes by directing their attack at the coarser fibers in the pulp. We used laboratory fractionation (Bauer-McNett fractionation) to separate the long fibers prior to cellulase treatment. By using this approach, both with and without refining, we were able to substantially improve the paper properties of Douglas-fir handsheets (19). Now we wanted to determine if a similar improvement could be achieved with a market kraft pulp that had undergone industrial fractionation.

RESULTS AND DISCUSSION

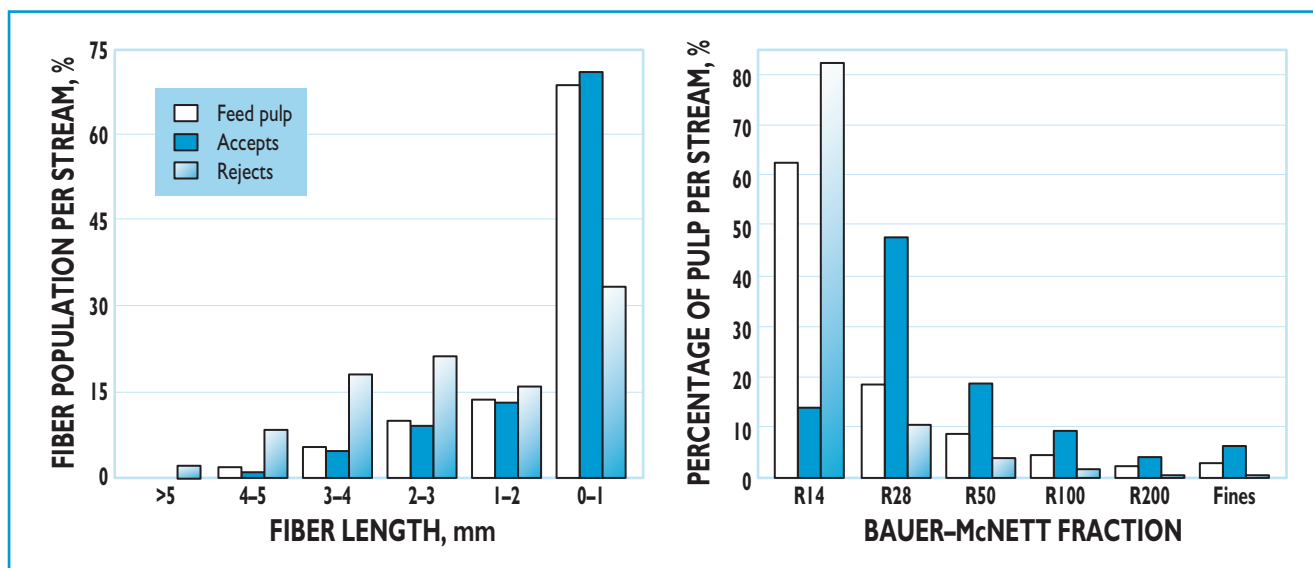
In our previous work (19), we were able to modify the quality of handsheets derived from a never-dried, unbleached, Douglas-fir kraft pulp by selectively treating the fractions of long fiber length with a commercial cellulase preparation. We separated the different fiber fractions using the Bauer-McNett fiber-length classifier, selectively treated the R14 fraction with a cellulase, and then recombined the treated R14 fraction with the other fractions in their original proportions.

Bauer-McNett separation is a clean separation process compared to industrial fractionation. The next question was, could similar improvements be reached with industrial fractionation?

Fiber separation

Initially, we subjected a dried, fully bleached, market kraft pulp to a single pass through a small industrial pressure screen. The accept stream contained slightly fewer of the long fibers (more than 1 mm long) and more of the shorter fibers and fines (less than 1 mm) than found in the feed pulp. The reject stream was enriched with long fibers, as verified by Kajaani analysis.

These results are reflected in the graph on the left in **Fig. 1**, which shows the breakdown by fiber length of the percent population for each of the three streams. Though the bar chart shows that the fractionation process had enriched the reject stream with long fibers, it appeared that this stream still carried a substantial quantity of small fibers.



1. Fiber population by fiber length per stream (left) and the fiber length distribution per stream (right) achieved through the industrial fractionation of a market kraft pulp high in Douglas-fir.

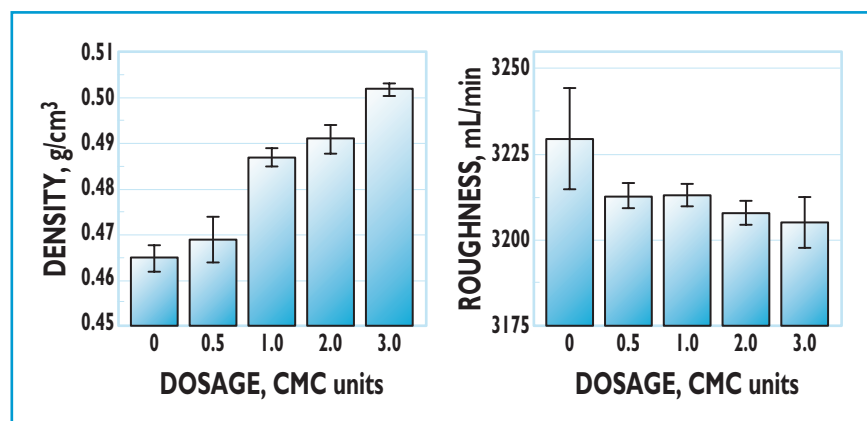
When the fiber length fractions were compared by weight using the Bauer-McNett classifier, however, it was clear that the portion of small fibers remaining in the rejects stream was substantially reduced. This result is shown in the graph on the right in Fig. 1. This plot also shows that 82% of the reject stream was composed of long fibers of the R14 fraction. By contrast, the accept stream was enriched in all fibers except the very long fibers of the R14 fraction.

The Bauer-McNett fractions of Fig. 1 are based on the percentages of fibers retained, R, on screens of different mesh sizes. These fractions are not the same as the accept and reject fractions obtained with the industrial pressure screen.

Cellulase treatment of the long fiber fraction (reject stream)

Previously we treated the R14 fraction of a never-dried, brownstock Douglas-fir pulp at various enzyme loadings to establish the best dosage for improving handsheet properties. These same dosages not could be applied now, primarily because of differences between the feedstock of the laboratory study and that used in this pilot plant work.

The feedstock used in the earlier study was unbleached (19), whereas

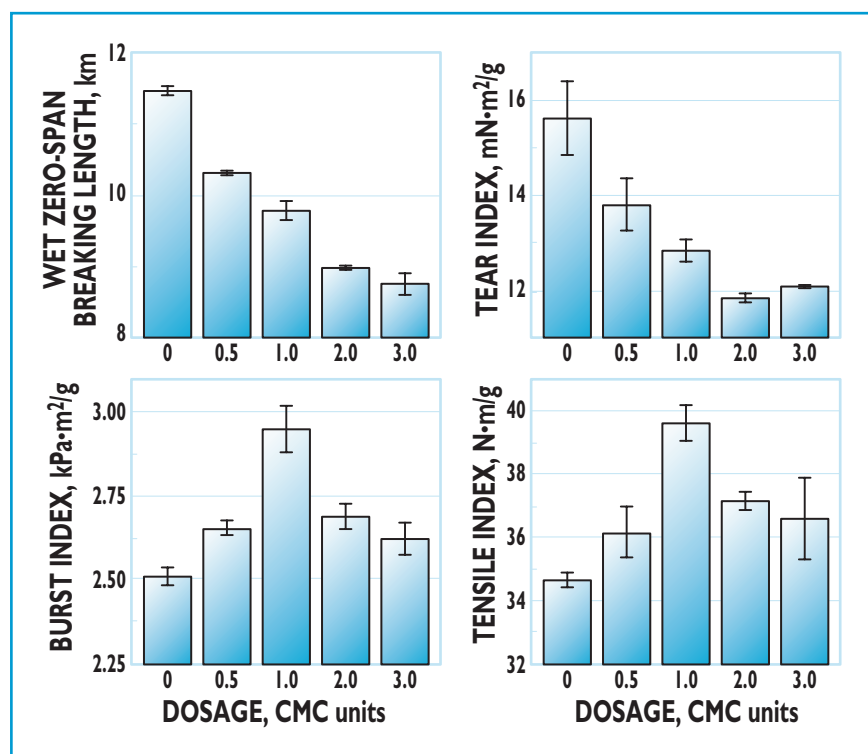


2. Density and roughness of handsheets formed after the fibers of the rejects stream have been treated with a cellulase and recombined with the untreated fibers of the accepts stream. (Error bar = standard deviation of three replicate experiments.)

the pulp we were now using was fully bleached. Bleached pulps are different from unbleached pulps in the way they react when treated with hydrolytic enzymes (20, 21). Another circumstance affecting the efficiency of the enzyme treatments is that the market pulp had been dried, whereas the brownstock used in the previous study was a never-dried pulp. Drying affects the pore-size distribution of fibers, potentially altering the interaction of the cellulase enzymes with the substrate (22) and therefore limiting the access of the enzymes to the fibers (23-26).

During the laboratory study, some improvement did occur when we treated a combination of the R14 and R28 fractions. However, these improvements were not as substantial as those obtained when we treated the R14 fraction alone. Furthermore, the maximal modifications obtained with the R14 and R28 fiber fractions together were reached at a much lower enzyme dosage than the dosage needed for the best improvements of the R14 fraction alone.

To determine how to achieve maximal effects with fractions obtained by industrial fractionation, we



3. Fiber and strength properties of handsheets formed after the rejects fraction has been treated with a cellulase and recombined with the untreated accepts fraction. (Error bar = standard deviation of three replicate experiments.)

	ENZYME DOSAGE, CMC units/g of o.d. pulp						
	0.25	0.5	0.75	1.0	2.0	3.0	4.0
Hydrolysis of rejects, %	0.42	0.55	0.77	0.87	1.31	1.71	1.83
Yield loss, %	0.17	0.22	0.30	0.34	0.52	0.68	0.73

1. Hydrolysis and yield losses for an industrially fractionated kraft pulp treated with a cellulase enzyme

ran a number of trials. We subjected the reject stream to various enzyme dosages, recombined the treated reject fibers with the accept fibers in their original ratios, and compared the handsheets made from them with handsheets made from control furnishes. The nondestructive handsheet properties were analyzed, and the results are presented in Fig. 2.

As the figure shows, substantial increases in handsheet density could be achieved, and these increases occurred in a dose-related fashion. This outcome was similar to the results obtained with the brownstock Dou-

glas-fir pulp in the laboratory study (19). The roughness values indicated that handsheet smoothness could also be improved. Generally, increases in enzyme dosage resulted in greater reductions in roughness.

To assess the degree of fiber degradation caused by the cellulase treatments, we determined the intrinsic fiber strength by wet zero-span measurements. As Fig. 3 shows, the fiber strength was considerably reduced, even at the low enzyme dosage of 0.5 CMC unit. At higher enzyme concentrations, the reductions were even greater.

For the tear index, which is directly influenced by intrinsic fiber strength (27-30), the enzyme treatments demonstrated a trend similar to that observed with the wet zero-span measurements, as shown in the graph for tear index in Fig. 3. In contrast to the fiber strength and tear index, both the burst and tensile indices were greater than those of the control pulp.

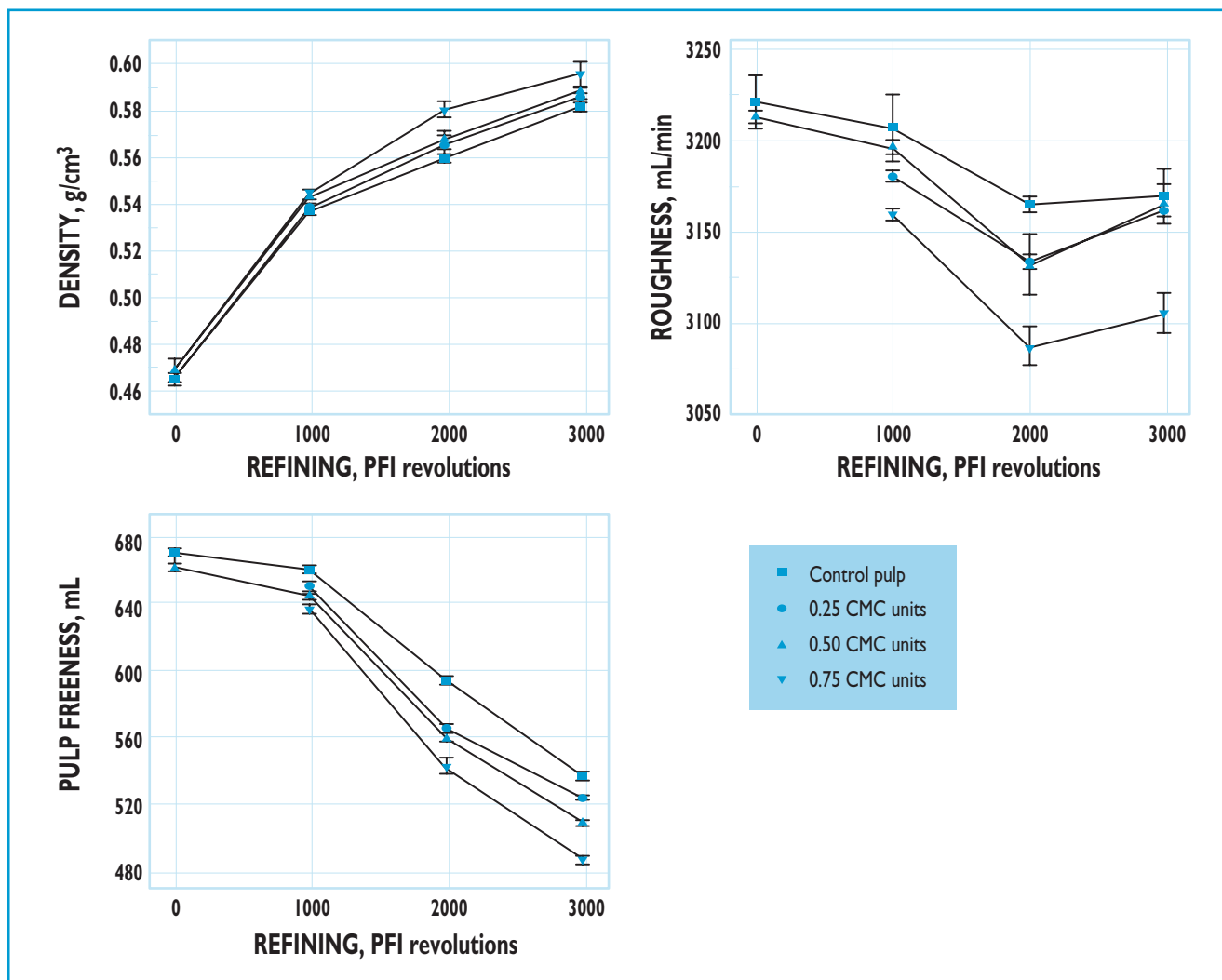
Maximal increases in burst index and tensile index were achieved at an enzyme dosage of 1 CMC unit per gram of oven-dried fiber. This dosage resulted in a 15% increase for burst index and a 14% increase for tensile index over the corresponding controls. These increases were not as substantial as those obtained in the treatment of the R14 fraction in the laboratory work. However, they do resemble the enhancements observed in the laboratory when the R14 and R28 fractions were treated together (19).

Cellulase treatment of the rejects followed by refining

At this point, we had established that handsheet properties could be modified by treating the reject stream of an industrially fractionated feedstock. Next we wanted to determine what effects it would have if the fibers were refined after the fractions were recombined.

To determine the effects of refining, we again exposed the reject stream to various enzyme dosages, recombined it with the accept stream, and then subjected the fibers to 1000, 2000, and 3000 revolutions on a PFI laboratory refiner. We chose to refine the accept and reject fractions together because this scheme had proven to be the best way to develop maximal handsheet strength in the earlier work in the laboratory (19).

For the refining experiments, cellulase loadings of 0.25, 0.5, and 0.75 CMC unit/g of o.d. pulp were applied to the rejects fraction. These treatments resulted in approximately



4. Sheet consolidation and pulp freeness after the rejects fraction has been treated with a cellulase, recombined with the untreated accepts fraction, and refined. (Error bar = standard deviation of three replicate experiments.)

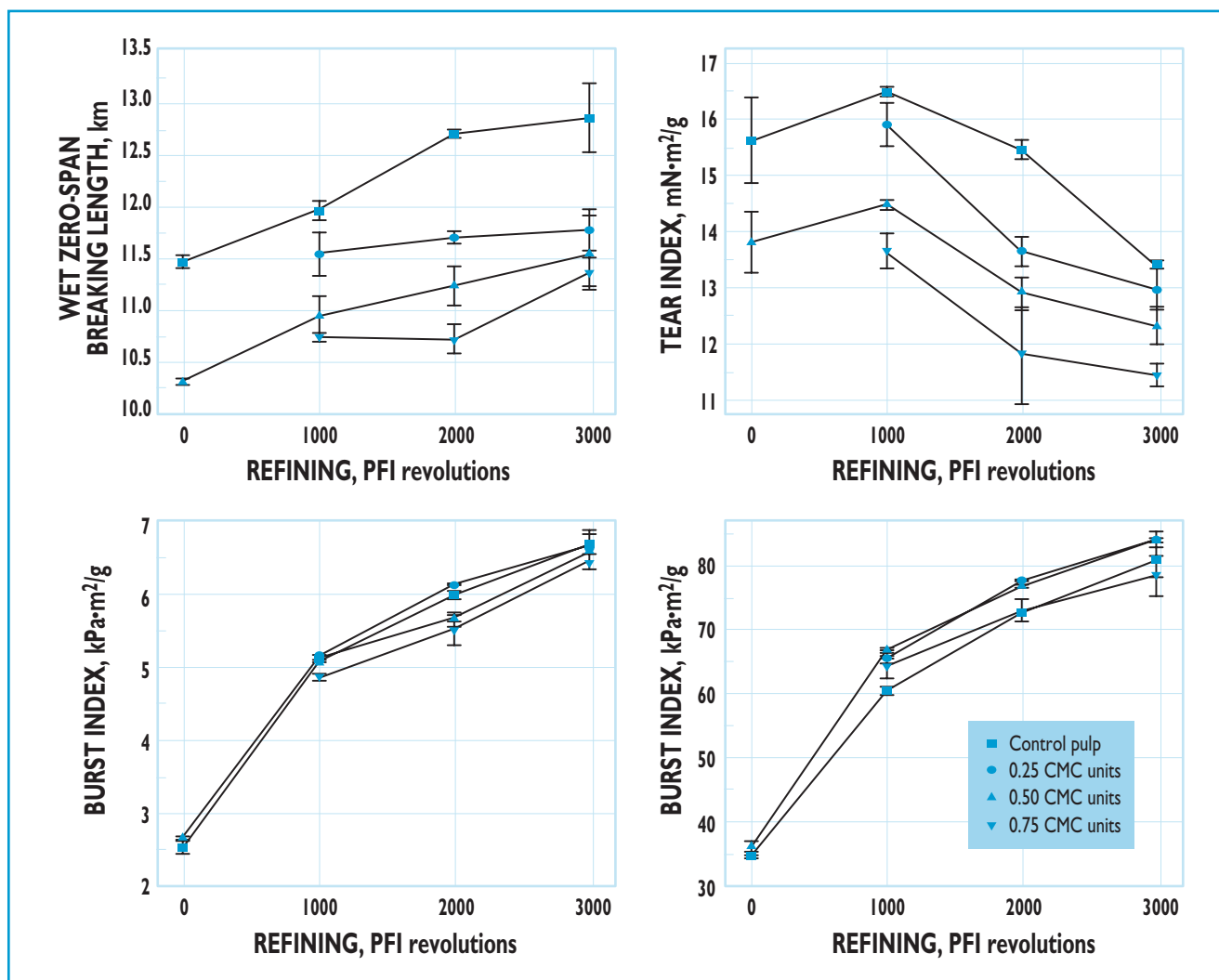
0.42%, 0.56%, and 0.77% hydrolysis of the rejects, respectively, as shown in Table I. These percentages were equivalent to feedstock yield losses of approximately 0.17%, 0.22%, and 0.30%, respectively.

These enzyme dosages increased handsheet densities and reduced sheet roughness in a dose-related fashion, as shown in Fig. 4. Although refining can reduce pulp freeness, cellulase pretreatment of the reject-stream fibers reduced the freeness of the pulp even further, as compared with the corresponding control pulps. The extent of the effect was again directly related to the enzyme dosage applied, as also shown in the figure.

As the results with the unrefined fibers showed (Fig. 3), cellulase treatment could significantly reduce the intrinsic fiber strength, even at low enzyme loadings. A combination of enzyme treatment and refining caused even greater reductions in intrinsic fiber strength. Therefore, we reduced the enzyme dosages. Even at these reduced dosages, however, the fiber strength was compromised. On the other hand, the refining process increased the strength of the control fibers slightly more at each level of refining. The fiber strength lost during the enzymatic treatments could not be recovered by refining, as the upper left graph in Fig. 5 shows. As the upper right graph illustrates, the

tear index was also reduced by enzymatic treatment.

The greatest increases in sheet density and the greatest reductions in sheet roughness were obtained with an enzyme charge of 0.75 CMC unit. At this dosage, however, all of the handsheet strength parameters were compromised, including the burst and tensile indices, as shown in the lower graphs in Fig. 5. Nevertheless, the charge of 0.25 CMC unit increased the burst strength slightly (about 4%) over the corresponding control. Still, at the higher dosages of 0.50 and 0.75 CMC unit, the burst strength of the treated furnish was lower than that of the control.



5. Strength properties of handsheets after the rejects fraction has been treated with a cellulase, recombined with the untreated accepts fraction, and refined. (Error bar = standard deviation of three replicate experiments.)

In contrast, the tensile strength was negatively affected only by the charge of 0.75 CMC unit after 3000 revolutions of refining, at which point the handsheets derived from the enzyme-treated fibers were not as strong as the control handsheets. At the lower enzyme dosages, the tensile strength of the paper increased, with the greatest improvement of about 10% occurring at an enzyme dose of 0.5 CMC unit and 1000 PFI revolutions. This enzyme charge of 0.5 CMC unit resulted in tensile strengths greater than that of the control pulp throughout the entire refining curve. Furthermore, after 3000 PFI revolutions of refining, this charge maintained an increase in

tensile strength of approximately 5%.

The enzymatic treatment of the longer, coarser fraction enhanced both fiber collapsibility and flexibility. This effect is evident in the increases in sheet density for the unbeaten fibers. Also in support of this observation are the results obtained from confocal laser scanning microscopy analysis of handsheet cross sections (31).

The increases in density also indicated that the treatments increased the relative bonded area within the handsheets. As a result, the treatments improved handsheet consolidation and the physical parameters of the handsheets. The exception was tear strength, which was adversely

affected by the increase in relative bonded area. In essence, the improvements were obtained at the expense of fiber strength, which also adversely affected the tear index (27–29). However, it has been suggested that the tear index is an industrially irrelevant paper parameter (32).

Handsheet modifications could be achieved at much lower enzyme dosages if the treated fibers were subjected to refining stages. Although increases in fiber collapsibility and flexibility could explain the improved handsheet properties of the unrefined fibers, the reductions in the pulp freeness of the refined fibers undoubtedly contributed to the modifications in some of the handsheet properties.

While the industrial fractionation process substantially enriched the reject stream with long, coarser fibers, it by no means generated a homogenous stream of pulp fibers. Thus, the efficacy of fractionation can substantially influence the extent of the modifications possible with enzyme treatment. It may be necessary to improve the fractionation process before this technology can be implemented successfully.

CONCLUSIONS

Industrial fractionation technology was used in combination with cellulase treatments to specifically target the long, coarse fibers. This combined approach resulted in alterations in handsheet parameters derived from a market kraft pulp high in Douglas-fir. It also reduced both yield losses and the amount of enzyme required. Although there are some shortcomings, such as reduced intrinsic fiber strength and lower tear strength, the approach resulted in increases of as much as 10% in tensile strength over the corresponding control furnishes.

The utilization of this technology may offer several benefits. Sheet smoothness could be improved, which could improve paper printability. The treatment could also reduce the refining energy required to attain target in-plane paper strength, such as tensile strength. Furthermore, the integration of an enzymatic treatment stage with refining could provide a means of incorporating coarse feedstocks such as Douglas-fir into the manufacture of some fine paper products.

METHODS AND MATERIALS

Pulp

A dried, fully bleached kraft pulp was obtained from the Crofton mill (Fletcher Challenge, Crofton BC, Canada). Approximately 85% of this pulp consists of Douglas-fir.

Pilot plant fractionation

Fiber length fractionation was performed with a small industrial pressure screen, a Hopper PSV-2100, in PAPRICAN's pilot plant in Vancouver. The screen rotor was a four-bladed, 1.5 in. foil operated at a constant velocity (1300 rpm), driven by a 30 kW motor. The screen plate was un-contoured (or smooth) with a 15% open area. The apertures were 0.085 in. in diameter and were conically drilled.

The screen was equipped with pressure transducers, magnetic flowmeters, and actuated control valves on the feed, accept, and reject streams. Valve positions and motor speeds were controlled by a process computer that also recorded the pressure and flow information.

The screen was operated at a target accepts flow rate of 2000 L/min. A constant accepts flow rate and a constant rotor speed maintain a constant average fluid velocity through the screen plate apertures and upstream of the screen. The reject rate was fixed by targeting a rate of 500 L/min.

The pulp was pumped through the pressure screen from a 10,000 L feed tank at a feed consistency of about 1.2%. The starting freeness was approximately 670 mL CSF.

After a single pass through the screens, the accepts were enriched in fractions of short fiber length, and rejects were enriched in long fiber fractions. The accepts and rejects streams were directed into two separate holding tanks.

Enzyme characterization

Novozyme SP342 from Novo Nordisk (Bagsvaerd, Denmark), an enzyme preparation derived from *Humicola insolens*, was used for all cellulase treatments. Its activity was determined on carboxymethylcellulose (1% CMC, Sigma, St. Louis), xylan (1% Birchwood xylan, Sigma), filter paper (No. 1 Whatman), and mannan

(1% locust bean gum and 1% guar gum, Sigma) according to methods described previously (33-35).

Proteins in solution were quantified using the bicinchonic acid assay (36). The enzyme preparation contained 46.7 mg protein/mL. It possessed cellulase activities (CMC, 143.5 IU/mL; filter paper, 9.9 IU/mL), as well as relatively high xylanase activity (2869 IU/mL) (where 1 IU = 1 μ mol/min of liberated hydrolysis product of the specified substrate).

Enzymatic treatment of fractionated pulp

The rejects fraction at 3% consistency in a 50 mM phosphate buffer (pH 7.0) was treated with enzyme for 1 h at 50°C under continuous agitation. These treatments were carried out over a range of enzyme dosages, which were based on the addition of enzyme as measured in CMC activity units per gram of oven-dried fiber.

We stopped the reactions by placing the pulp samples in a boiling water bath for 15 min. The pulps were drained. After secondary acid hydrolysis, the filtrates were analyzed for solubilized carbohydrates by high-performance anion-exchange chromatography, using a CarboPac PA-1 column and a Dionex DX-500 system (Dionex, Sunnyvale, CA).

Control pulps were similarly treated with equivalent amounts of enzyme inactivated (15 min. boiling). One control was run for each of the different enzyme dosages. The results for the enzyme dosages were pooled and averaged.

After enzymatic treatment, the rejects fraction was recombined with the untreated accepts fraction. These fractions were recombined at the same ratio as the ratio in which they were derived from the original unfractionated pulp. We used this furnish in making handsheets. In some cases, the furnish was refined in a PFI laboratory refiner (TAPPI T 248 cm-85) before handsheets were made.

Physical pulp testing

The handsheets were prepared and tested according to TAPPI test methods. The white water was recycled to ensure that both the primary and secondary fines were included in the handsheets. Pulp freeness was measured at 20°C according to TAPPI T 227 om-94.

Fiber analysis

Fiber length distributions and fiber coarseness were determined by passing representative samples through a Kajaani FS-200 fiber analyzer. **TJ**

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LITERATURE CITED

1. LeBlanc, P. and Harrison, R., *TAPPI* 58(4): 85(1975).
2. Wakelin, R. F. and Corson, S. R., *TAPPI 1995 International Mechanical Pulping Conference Proceedings*, TAPPI PRESS, Atlanta, p. 257.
3. Scott, G. M. and Abubakr, S., *Prog. Paper Recyc.* 3(6): 50(1994).
4. Guss, D. and Meier, A., *TAPPI 1997 Recycling Symposium*, TAPPI PRESS, Atlanta, p. 447.
5. Moss, C. S., *TAPPI 1996 Recycling Symposium*, TAPPI PRESS, Atlanta, p. 387.
6. Pekkarinen, T., *TAPPI 1985 Pulping Conference*, TAPPI PRESS, Atlanta, p. 37.
7. Ali, S. F., *73rd Annual CPPA Meeting*, CPPA, Montreal, 1987, Preprint No. 73, p. 1.
8. Floccia, L., *TAPPI 1988 International Pulp Bleaching Conference*, TAPPI PRESS, Atlanta, p. 181.
9. Eul, W., Süß, H. U., and Helmling, O., *TAPPI 1988 International Pulp Bleaching Conference*, TAPPI PRESS, Atlanta, p. 169.
10. Floccia, L. and Voillot, C., *Paper Technol.* 37(3): 44(1996).
11. Abubakr, S. M., Scott, G. M., and Klungness, J. H., *Tappi J.* 78(5): 123(1995).
12. Clark, L. E. and Iannazzi, F. D., *TAPPI* 57(11): 59(1974).
13. Bliss, T., *Pulp & Paper* 61: 104(1987).
14. Seifert, P. and Long, K. J., *TAPPI* 57(10): 69(1974).
15. Häggblom-Ahnger, U. and Hautala, J., *Paperi Puu* 78(6-7): 386(1996).
16. Dobbins, R. J., *TAPPI* 61(10): 90(1978).
17. Mansfield, S. D., Wong, K. K.Y., de Jong, E., and Saddler, J. N., *TAPPI J.* 79(8): 125(1996).
18. Mansfield, S. D., Wong, K. K.Y., de Jong, E., and Saddler, J. N., *Appl. Microbiol. Biotechnol.* 46(4): 319(1996).
19. Mansfield, S. D. and Saddler, J. N., *J. Pulp Pap. Sci.* 25(3): 1999.
20. Clark, T. A., Allison, R. W., and Kibblewhite, R. P., *Appita J.* 50(4): 329(1997).
21. Kibblewhite, T. P. and Clark, T. A., *Appita J.* 49(6): 390(1996).
22. Stone, J. E., Scallan, A. M., Donefer, E., and Ahlgren, E., *Advances in Chemistry Series* 95: 219(1969).
23. Fan, L. T., Lee, Y.-H., and Beardmore, D. H., *Biotechnol. Bioeng.* 22: 177(1980).
24. Grethlein, H. E., *Bio/Technology* 3: 155(1985).
25. Nazhad, M. N., Ramos, L. P., Paszner, L., and Saddler, J. N., *Enzyme Microb. Technol.* 17(1): 68(1995).
26. Bendzalova, M., Pekarovicova, A., Kokta, B. V., and Chen, R. B., *Cellulose Chem. Technol.* 30(1-2): 19(1996).
27. Seth, R. S., and Page, D. H., *Tappi J.* 71(2): 103(1988).
28. Seth, R. S., *Materials Research Society Symposium Proceedings* 197: 125(1990).
29. Seth, R. S., *Materials Research Society Symposium Proceedings* 197: 143(1990).
30. Seth, R. S., *Tappi J.* 78(10): 177(1995).
31. Mansfield, S. D., Dickson, A. R., and Saddler, J. N., *Proceedings of the 7th International Conference on Biotechnology in the Pulp and Paper Industry*, Technical Section, CPPA, Vol. 1, 1988, p. 189.
32. Seth, R. S., *Tappi J.* 79(1): 170(1996).
33. Wood, T. M. and Bhat, G. R., *Methods Enzymol.* 160: 87(1988).
34. Bailey, M. J., Biely, P., and Poutanen, K., *J. Biotechnol.* 23: 257(1992).
35. Gübitz, G. M., Hayn, M., Urban, G., and Steiner, W., *J. Biotechnol.* 45(2): 165(1996).
36. Stoscheck, C. M., *Methods Enzymol.* 182: 50(1990).