Modification of a commercial radiata pine kraft pulp using carbohydrate degrading enzymes

R. PAUL KIBBLEWHITE* AND KEN K.Y. WONG†

There is a need to improve the papermaking qualities of radiata pine kraft pulp to retain and/or improve its competitive advantages in the market place; the present work evaluates the potential of using carbohydrate degrading enzymes for this purpose. Six enzymes are included in the study: five endoglucanases and one xylanase. The effects of enzyme treatment on a commercial pulp are assessed by determining carbohydrate solubilization; fibre dimensions of both unrefined and refined fibres for never dried pulp; and handsheet property interrelations.

Xylanase treatment selectively increases handsheet tear index relative to apparent density and tensile index, with the retention of fibre strength. It appears that the selective removal of xylan from fibre surface layers affects fibre bonding. When fibre cross-sections are compared to control fibres after PFI refining, changes are achieved with nearly all enzymes examined. The endoglucanase treatments cause fibre strength degradation and decreases in handsheet mechanical properties, even at low enzyme applications.

Keywords
kraft pulp, radiata pine, enzymes, fibre properties, handsheet properties

Recent research has evaluated the potential of using enzymes to improve the quality of certain fibre sources (1-10). For radiata pine kraft pulps, preliminary research has described the response of laboratory unbleached pulp (high coarseness, slabwood) to treatment with several carbohydrate degrading enzymes (6,8):

- Endoglucanase treatment lowers both fibre and handsheet strengths substantially. The extent of degradation differs for the three endoglucanases studied.
- Xylanase treatment seems to yield stiffer kraft fibres with surfaces of low water affinity and bonding potential. This occurs with the retention of intrinsic fibre strength as indicated by relative viscosity and wet zero span tensile index. Tear index is selectively increased.

The responses of low coarseness, thinnings pulp and bleached pulp were apparently less prominent (6).

The present study is designed to verify these observations using a commercial pulp of medium coarseness and lower doses of enzyme. For endoglucanases, the lower enzyme loadings should reduce the extent of fibre damage. The effects of enzyme treatment are assessed by determining carbohydrate solubilization; fibre dimensions of never dried and dried/rewetted pulps; and handsheet property interrelations. In addition, changes to fibre dimensions after PFI refining are described for the first time for enzyme treated pulp. Six enzymes are examined: five endoglucanases and one xylanase.

EXPIMENTAL
Pulp
Never dried (slush), unbleached (Kappa no. 22.2), radiata pine kraft pulp of medium coarseness (0.226 mg/m) was obtained from Tasman Pulp and Paper Mill (Kawerau, New Zealand) in 1995. The pulp was produced using a conventional continuous kraft cooking process. The pulp was not washed further before enzyme treatment. Pulp composition was determined using sulfuric acid hydrolysates following Tappi T222 om-88 for Kason lignin, Tappi UM250 for acid soluble lignin, and the method of Pettersen and Schwandt (11) for carbohydrates: 3.2% Kason and 0.5% acid soluble lignin; 0.6% arabinosyl, 0.4% galactosyl, 78.6% glucosyl, 6.8% xylosyl and 5.2% mannosyl residues; 0.8% ash.

Enzymes
Four enzymes from the fungus Trichoderma reesii were provided by Genencor International (Palo Alto, USA): endoglucanases EG A, EG B and EG C; xylanase.

Two enzymes from the fungus Humicola insolens were provided by Novo Nordisk (Bagsvaerd, Denmark): endoglucanases SP A and SP B.

The activity of endoglucanase and xylanase (Table 1) was assayed on 1.8% carboxymethyl cellulose and 0.9% birchwood xylan (Sigma, St. Louis, USA), respectively, using methods modified from that of Bailey et al. (12). Further assays with these substrates, as well as 0.45% locust bean gum galactomannan (Sigma), were used to evaluate the level of contaminating activities in each enzyme preparation.

Enzyme treatments
Pulp at 5% pulp concentration was treated at 50°C and pH 5 or 7 for 2 h with enzyme loadings based on their activity. Low loadings of endoglucanases were selected to minimize pulp degradation as indicated by the relative viscosity and wet zero span tensile index of the pulps. The control pulp was treated at pH 6 in

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* Senior Scientist, Member Appita
† Project Leader, Member Appita
PAPRO NZ, Forest Research, Private Bag 3020, Rotorua, New Zealand.

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Fig. 1 Diagram of fibre cross section illustrating fibre dimensions.
the same manner as the enzyme treated pulps, except that no enzyme was added. Carbohydrate solubilization was determined through analysis of treatment filtrates (8) and the relative viscosity of pulp was determined according to TAPPI method T 230.

Pulp and fibre analyses

Handsheets were prepared and pulp physical evaluations made in accordance with Appita standard procedures. Wet zero span tensile strengths of pulps were determined according to AS/NZS 1301.459 rp: 1998. The load applied during pulp refining with the PFI mill was 3.4 N/mm. Pulps were refined at 10% pulp concentration for 500, 1000, 2000, and 4000 rev. Results are reported on the o.d. bases. The tensile – density plots were fitted to an exponential growth model, while the tear – density plots were fitted to a first order exponential decay model.

### Table 2

<table>
<thead>
<tr>
<th>Treatment conditions</th>
<th>Carbohydrate solubilized, *</th>
<th>Enzyme</th>
<th>Viscosity, mPa.s</th>
<th>WZST†</th>
<th>N.m/g</th>
<th>FSP‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
<td>Glu</td>
<td>Xyl</td>
<td>Man</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control pH 6</td>
<td>0.02</td>
<td>0.00</td>
<td>0.02</td>
<td>0.00</td>
<td>34.7</td>
<td>137</td>
</tr>
<tr>
<td>EG A (58 nkat/g, pH 5)</td>
<td>0.05</td>
<td>0.02</td>
<td>0.02</td>
<td>0.00</td>
<td>30.2</td>
<td>132</td>
</tr>
<tr>
<td>EG B (147 nkat/g, pH 5)</td>
<td>0.16</td>
<td>0.03</td>
<td>0.12</td>
<td>0.00</td>
<td>32.0</td>
<td>134</td>
</tr>
<tr>
<td>EG C (25 nkat/g, pH 5)</td>
<td>0.04</td>
<td>0.02</td>
<td>0.02</td>
<td>0.00</td>
<td>29.4</td>
<td>117</td>
</tr>
<tr>
<td>SP A (8 nkat/g, pH 7)</td>
<td>0.07</td>
<td>0.03</td>
<td>0.03</td>
<td>0.00</td>
<td>30.1</td>
<td>127</td>
</tr>
<tr>
<td>SP B (4580 nkat/g, pH 7)</td>
<td>0.65</td>
<td>0.20</td>
<td>0.08</td>
<td>0.29</td>
<td>30.9</td>
<td>137</td>
</tr>
<tr>
<td>Xylanase (1800 nkat/g, pH 6)</td>
<td>0.98</td>
<td>0.00</td>
<td>0.89</td>
<td>0.00</td>
<td>34.9</td>
<td>141</td>
</tr>
</tbody>
</table>

LSD§

* Carbohydrate residues: Glu = glucose; Xyl = xylose; Man = mannose. 
† Total is the sum of glucosyl, xylosyl, mannosyl, arabinosyl and galactosyl residues.
§ Cross-section fibre dimensions of fibre thickness, fibre width, wall area and wall thickness were measured using image processing procedures (Fig. 1) (15). Measurements were made on never dried fibres, and dried and rewetted fibres from handsheets (AS/NZS 1301.209rp-89). The product of fibre width x fibre thickness represents the minimum fibre cross-section rectangle and is an indicator of cross section size. The ratio of width: thickness is an indicator of the collapse potential for the dried and rewetted fibres, and of fibre shape for the never dried fibres. Length weighted fibre length and fibre coarseness were determined with a Kajaani FS 200 instrument using TAPPI T271 pm-91.

### RESULTS AND DISCUSSION

The present report summarizes the responses of a commercial, medium coarseness Kraft pulp derived from radiata pine to treatment with six carbohydrate degrading enzymes. The work was part of a study that also examined a commercial, low coarseness pulp (16). In agreement with previous observations (6), enzyme derived effects were found to be more clearly evident and consistent for the pulp with higher coarseness.

### Effects of enzyme treatment on pulp

The object of treating Kraft pulp with enzymes is to enhance certain properties while retaining others, in particular fibre strength. For this reason the enzyme loadings in this study were selected so that fibre strength losses were minimal (Table 2). Despite such an approach only treatments with endoglucanase SP B and xylanase can be considered to have had minimal effects on fibre strength as measured by wet zero span tensile index. All other enzyme treatments caused the pulp to be degraded to some degree, although pulp viscosity was well maintained.

Pulp carbohydrate was solubilized to different extents by the enzyme treatments, with the xylanase and SP B treatments being most effective (Table 2). Xylan was selectively solubilized by the xylanase treatment, while both glucan and glucomannan were solubilized by the SP B endoglucanase treatment. Substantial amounts of xylan were also solubilized by endoglucanases EG B and SP B. Fibre saturation points consistently increased with all enzyme treatments.
Fibre property response

Fibre wall properties: The properties measured included microfibril angle, presence and condition of the S1 and S3 layers, and incidence of wall delamination. Except for microfibril angle, these properties were different for control and enzyme treated pulps but similar for all enzyme treated pulps. Enzyme treatment reduced the amount of intact S1 and S3 walls and increased wall delamination. No changes to microfibril angle occurred with enzyme treatment. These results are in agreement with trends observed in a previous study (6).

Fibre length: Fibre length was marginally lower after enzyme treatments, but some values were within the 0.05 mm least significant difference limits (Table 3). Since control and enzyme treated pulps were subjected to the same processing, large changes in fibre length were not expected unless there was severe attack by enzymes.

Fibres dried and rewetted from handsheets of unrefined pulp: The degree of fibre collapse, as measured by the fibre width:thickness ratio of fibres dried and rewetted from handsheets, is a good indicator of the quality of radiata pine kraft pulp (17). Fibre collapse was unchanged after enzyme treatments (16). Most of the other fibre dimensions were also similar, at the 95% level of significance, for the enzyme treated and control pulps.

Never dried fibres of unrefined pulp: Fibre width:thickness ratios were unchanged by enzyme treatment (Table 3). Fibre wall areas and wall thicknesses were, however, smaller for many of the enzyme treated pulps than for the control pulp. Thus, never dried fibre walls could be selectively contracted or densified by these treatments. The increase in fibre saturation points (Table 2) and contraction in fibre wall areas (Table 3) of enzyme treated medium coarseness pulps requires comment since opposing trends for wall porosity are indicated. The situation remains unresolved but could be related to the limitations of the measurement procedures for characterizing the dimensions and structural organizations of fibre walls, and the distributions and gradients of pores (18,19).

Response to PFI refining: Changes to fibre dimensions were also used to determine the responses to refining of the never dried pulps (Fig. 2,3,4):

- For the control pulp, fibre cross-section size (fibre width × fibre thickness) decreased abruptly after initial refining of 500 rev, reverted to nearly its original value after 1000 rev, and then decreased with additional PFI refining (Fig. 2), in agreement with previous research (20). A similar pattern could be seen for the pulp treated with EG A and xylanase, although the initial drop in fibre cross-section size was less prominent. For pulp treated with any of the other enzymes, there was no recovery of fibre cross-section size after intermediate levels of pulp refining.

- The response of fibre wall area during the refining of the control pulp (Fig. 3) was similar to that of fibre cross-section size. The contraction of fibre walls with initial refining was followed by wall delamination and expansion.

Fig. 2 Response of medium coarseness kraft pulp to refining after enzyme treatment – fibre cross-section size (width x thickness).

Fig. 3 Response of medium coarseness kraft pulp to refining after enzyme treatment – fibre wall area.
with further refining (20). Pulp treated with EG A or xylanase showed a similar development of fibre wall area, but the wall area was smaller before refining or did not decrease as much during initial refining. An unexplained anomaly was seen in the pulp treated with EG C, where there was an increase rather than a decrease of wall area after initial refining.

- Fibre cross-section shape (width:thickness) indicates the extent of deviation from roundness or squareness as a function of PFI refining (Fig. 4). Fibres in the control pulp collapsed abruptly with initial refining and reverted partly to their original shape with further refining, as noted previously for PFI refining of never dried kraft pulps (20). Trends were similar but less abrupt for pulp treated with xylanase, as well as that treated with endoglucanase SP A and SP B. Pulp treated with the EG endoglucanase showed the abrupt fibre collapse with initial refining, but the extent of collapse was retained with intermediate levels of refining.

**Hands sheet Property Interrelationships**

Apparent density is an indicator of fibre arrangements and packing densities in handsheets, while interrelationships among tensile index, tear index and apparent density are indicators of the influences of different fibre properties (6,17). Thus, a decrease in handsheet tensile index at a given apparent density can indicate a decrease in fibre strength, a decrease in fibre bonding, and/or changes in fibre dimensions that modify packing arrangements and densities within fibre webs. For example a loss in interfibre bonding was seen after treatment with xylanase because there was a decrease in tensile strength at a given apparent density (Fig. 5) but good retention of fibre strength (Table 2). Xylan is concentrated within the outer layers of kraft fibre walls (21) and its selective removal can be expected to lower the intensity of interfibre bonds in handsheets. Such an explanation of the effects of xylanase treatment is probably an over simplification because the smaller fibre cross-section area and wall area, and marginally more collapsed fibres (Table 3), could have resulted in an increase in apparent density of handsheets.

Pulp treatment with EG B, EG C and SP A showed minimal losses in tensile strength for given apparent density values (Fig. 5). The loss in tensile strength after treatment with EG C seemed to correspond to the large loss in fibre strength (Table 2). There was no evidence for an increase in tensile strength, as reported in the long fibre fraction of Douglas-fir kraft pulp after treatment with a crude commercial cellulase (7).

Hands sheet tear index at given tensile index is considered to be an indicator of web toughness or runnability on paper machines and printing presses (17). The xylanase treatment selectively increased tear resistance when compared to apparent density (Fig. 6). This increase in tear index was proportionately greater than the loss in tensile strength, for the medium coarseness pulp used in the present study and a low coarseness pulp (16). An increase in the tearing resistance of kraft pulp after xylanase treatment has also

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**Fig. 4** Response of medium coarseness kraft pulp to refining after enzyme treatment – fibre cross-section shape (width:thickness).

**Fig. 5** Handsheet tensile index versus apparent density relationships for enzyme treated pulp.
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REFERENCES


Continued page 311.