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Enzymatic degradation of residual non-cellulosic polysaccharides present on dew-retted flax fibres

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Summary. A mixture of Pectional AC and Ultrazym, and enzyme extracted from cultures of *Ceraceomyces sublaevis* was the most suitable enzyme preparation for depolymerising non-cellulosic materials present on dew-retted fibre at 45° C. All the enzyme treated roves produced high quality yarns compared with the yarns spun from untreated roves. Fluidity of all the yarns spun from enzyme treated roves was low, suggesting that the enzymes have not affected the cellulose fibres. The use of polysaccharide-degrading enzymes for the removal of non-cellulosic material present on flax fibre may be more energy efficient than traditional caustic boil treatment (using NaOH) for removing residual non-cellulosic polysaccharides.

Introduction

Polysaccharide-degrading enzymes produced by saprophytic fungi are involved in dew-retting (Allen 1946a, b; De Franca et al. 1969; Sharma and Robinson 1984) and the retting of desiccated flax stem induced by the application of glyphosate (Brown 1984; Brown and Sharma 1984; Brown et al. 1986; Sharma 1986a). The range of polysaccharide-degrading enzymes, produced by the fungal colonisers on flax tissue, is important with respect to the degree to which stem tissues are degraded. During water retting of flax, fibre separation is brought about by pectinase enzymes produced by bacteria (Avrova 1975; Avrova and Goshko 1973; Chesson 1978; Kapitonova et al. 1972).

Preliminary reports revealed that enzyme mixtures of pectinases and hemicellulases have been shown to be capable of retting flax stems in vitro (Sharma 1987a).

The flax fibre generally has a high content (26%) of non-cellulosic matter (hemicellulose, pectin, lignin, fat and wax) (Turner 1949; Sharma 1986b). Little attention has been given to upgrading coarse flax fibres by enzymatic hydrolysis of the non-cellulosic component of fibre. The aim of the present study was to investigate the enzymatic degradation of the residual hemicelluloses, pectins and other non-cellulosic materials left of coarse dew-retted fibre, and their effect on yarn quality number, strength, extension, caustic weight loss and fluidity.

Materials and methods

Roves of dew-retted fibre were obtained from the Spinning Department of Lambeg Industrial Research Association. A rove is defined as a bobbin wound on with fine flax sliver. The roves were prepared on 9 × 4.5 inch bobbins and each held 200g of flax fibre at a density of 35 g/100 cc.

Enzymes used

The commercial enzymes used in this study were Pectinol AC (Rohm and Haas), and Ultrazym and Novozym 249 (Novo Industries). The polysaccharide degrading enzymes were also produced in the laboratory by growing *Ceraceomyces sublaevis* (Brown and Sharma 1986) and a hybrid strain A of *Pleurotus ostreatus* and *P. florida* (Sharma 1987b) on flax shive. The flax shive (100 g) left after scutching desiccated stand-retted flax stems (Sharma 1986a) was first pre-washed in de-ionised water and later wetted with 200 ml of deionised water. The flasks were sterilized for 1 h at 120° C and the cooled flasks inoculated with four agar plugs (3 mm dia) cut out from the leading

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edge of a 7 day old culture plate (MA Oxoid). After 25 days of incubation at 20° C in near total darkness, the enzymes present in the substrate were extracted by macerating in 0.3 M KCl (1 l) for 4 min. The extracts were further clarified filtered and stored at −4° C.

Enzyme assay

Reducing groups released by the activities of pectinases, cellulases and xylanases present in the extracts and commercial enzymes were measured by colorimetric analysis after reaction with 3,5 dinitrosalicylic acid reagent of the reducing groups (Miller 1959) liberated from their respective substrates sodium polypectate (0.5% w/v), carboxymethyl cellulose (0.1% w/v) and xylan (0.1% w/v). Substrates were mixed 2:1 (v/v) with enzyme samples and incubated at 30° C for 1 h.

Laccase activity was assayed using 2,6 dimethoxyphenol as substrate (Haars and Huttermans 1983). Substrate was mixed 2:1 (v/v) with enzyme samples and incubated at 30° C. The reaction was terminated by the addition of 0.5 ml dimethoxy sulphoxide and read at 468 nm in a spectrophotometer (Pye Unicam Ltd).

Enzyme treatment of rove

The three commercial enzymes were tested individually at a concentration of 3.0 g/l. A mixture of the Pectinol Al and Ultrazym was prepared by mixing equal volumes at a concentration of 3.0 g/l. The enzyme extracts of *C. sublaevis* and the hybrid strain of *P. ostreatus* and *P. florida* were tested individually at a concentration of 100 ml/l and a mixture of the two extracts was prepared by mixing equal volumes of the two extracts at a concentration of 100 ml/l. The rove was washed with water (12 l) in a package bleaching unit (Pegg, Samuel Pegg & Son). The water hardness was neutralised by addition of a sequestering agent (Trilon TB BASF Ltd) at a concentration of 2.6 g/l. This treatment swells the non-cellulosic materials retained on the fibre and was continued for 10 min. After this, the test enzyme preparation was added and the treatments were carried out at a range of pH 5.4—6.0 for 2 h at 45° C. Non-ionic surfactants were found to enhance the activity of the polysaccharide degrading enzymes (Sharma, unpublished data). To increase the activity of the enzymes, the non-ionic surfactant Zylasil NW (0.2 g/l) was added in the final enzyme solution. The control treatment was carried out with water sequestrated with Trilon TB (2.6 ml/l) at pH 5.2 for 2 h at 45° C. Each treatment was replicated three times. After the treatment of the roves for 2 h, the package bleaching unit was drained and the rove given 2 cold rinses (10 min) with water.

NaOH treatment of rove

To compare the effects of enzyme treatment at low temperature (45° C) with traditional NaOH treatment, NaOH (2 g/l) treatment of rove was carried out at 45° and 95° C for 2 h and the methods described above were followed. After the treatment, the rove was rinsed at 60° C for 10 min and later rinsed in cold water three times. This treatment was replicated three times.

Wet spinning

Enzyme-treated, NaOH-treated and control roves were spun to a count range of 52—57 lea in a wet spinning frame (Mackie & Sons Ltd). Lea is defined as an unit for measuring the number of 300 yard long cuts in a pound of yarn and very fine yarn has high yarn count (80 lea or more). One bobbin (300 g) of yarn was spun from each rove and dried at 60° C for 4—5 h.

Yarn testing

Yarn samples were conditioned for at least 4 h at 20° C and 65% RH (Anon. 1974). The tensile strength of the yarn was given as the mean of 100 breaks/sample using an automatic yarn tester (Zellweger Ltd, Uster, Switzerland). The quality number of the test yarn was calculated according to the methods of the British Standard Institute (Anon. 1974). Quality number is derived by multiplying yarn count in a lea by yarn strength in ounces. Yarn count is the number of 300 yard long cuts in a pound of yarn. Yarn strength is defined as the force required to break the yarn (gf). Extension (%) of yarn is expressed as a percentage of the initial length.

Caustic weight loss

Caustic weight loss was determined by treating 3 g of yarn with 100 ml of 2% NaOH solution at 100° C for 4 h. After redrying to constant weight, the loss in weight was calculated from a mean of 3 sample replicates. The weight loss was determined to study the effect of the enzyme treatment on the residual non-cellulosic polysaccharides.

Fluidity

The sample of yarn was divided up into a fiberous state and cut up into 1 cm lengths. The fluidity test was carried out in a controlled environment maintained at 20° C and 65% RH. The details of this method was reported by Butterworth and Elkin (1929). The non-cellulosic components of the fibre samples were extracted by boling in 2% NaOH for 4 h. Later, the fluidity (n) of the solution of yarn sample (2 g) in cuprammonium hydroxide (100 ml) was determined to study the effect of the enzyme treatment on cellulosic component of the test yarn.

Results

Analysis of the enzymes

The commercial enzymes tested in this investigation contained a wide range of polysaccharide-degrading enzymes. Novozym 249 contained polygalacturonase (PG), pectin-lyase (PL), xylanase and traces of cellulase. PG, xylanase and traces of PL were present in Pectinol AC. Ultrazym contained xylanase as the main component of the preparation along with traces of PG. Laccase was not detected in any of the three commercial enzymes (Table 1).

The enzymes extracted from the colonised flax shive contained a wide range of polysaccharide-degrading enzymes. Culture extracts of *C. sublaevis* contained mainly xylanase and laccase along

Table 1. Analysis of the three commercial enzymes and two enzyme extracts from shive colonised by *P. ostreatus* × *florida* (POF) and *C. sublaevis* (CS) for the prescence of polygalacturonase (PG), pectin-lyase (PL), xylanase, cellulase and laccase

	Novozym 249	Pectinol AC	Ultrazym	POF	CS
PG	835	850	275	475	250
PL	100	100	0	175	150
Xylanase	350	205	2100	650	1500
Cellulase	100	0	0	120	120
Laccase	0	0	0	10.2	8.3

PG and PL activities = $\mu g\ ml^{-1}\ h^{-1}$ galacturonic acid equivalent, xylanase and cellulase activities = $\mu g\ ml^{-1}\ h^{-1}$ glucose equivalent and Laccase activities are expressed as 1 unit = amount of enzyme which produced an increase in absorption at 468 nm for 0.01 min^{-1} at 30° C

with traces of PG, PL and cellulase. Xylanase, PG and laccase were detected in high concentrations along with traces of PL and cellulase in extracts of *Pleurotus* (Table 1).

Yarn testing

All the seven enzymatic treatments gave yarns with high quality numbers than that of the control. The co-efficient of variation was also lower for yarns from the enzyme-treated roves compared with the control (Table 2). Among the commercial enzymes, a mixture of Pectinol AC and Ultrazym was the most effective in removing residual pectins and hemicellulases left on the dew-retted fibres.

Of the two culture extracts, enzymes produced by *C. sublaevis* were the most effective in depolymerising the residual non-cellulosic materials left on the fibre (Table 2). The quality number of yarn samples spun from roves treated with an enzyme mixture of Pectinol AC and Ultrazym and NaOH at 95° C, was marginally better than the quality number of the yarn spun from roves treated with extracts of *C. sublaevis* (Table 2).

Caustic weight loss

The reduction in weight loss was significantly ($P < 0.01$) lower for all the yarns spun from NaOH (high temperature) and enzyme treated roves compared to the control yarn. The enzyme extract of *C. sublaevis* was most effective in the removal of the residual hemicelluloses, pectin and lignin as shown by the low % caustic weight loss (Table 3).

Fluidity

The fluidity of the yarns spun from all NaOH and enzyme-treated roves revealed that the NaOH treatment and enzymatic depolymerisation of the polysaccharides did not significantly ($P < 0.01$) reduce the chain lengths of cellulose and as a result fluidities for the test yarns were low (Table 3).

Discussion

Improvements in yarn quality number resulting from enzymatic or NaOH treatments correlated with low caustic weight losses. Fluidity of all the test yarns was also low confirming that the cellulose chains were not damaged by the treatments. Since a dew-retted fibre can lose as much as 25% of its weight on boiling in 2% NaOH solution, it is evident that a high proportion of residual hemi-

Table 2. Extent of enzymatic hydrolysis measured as yarn quality number (YQN), co-efficient of variation (CV) and strength of the yarn spun at a range of 52—57 lea from roves treated with Novozym 249 (N249), Pectinol AC (PAC), Ultrazym (U), mixtures of PAC and U, enzyme extracts from shive colonised by *P. ostreatus* × *florida* (POF), *C. sublaevis* (CS) and mixtures of POF and CS; NaOH treatment of roves at 45° C (LOW) and 95° C (HIGH) and control

	N249	PAC	U	PAC+U	POF	CS	POF+CS	LOW	HIGH	Control
YQN	1553	1334	1501	1659	1335	1620	1400	1400	1665	1298
CV	17.8	19.6	20.6	21.8	20.1	19.8	20.3	17.7	19.6	25.3
Strength	28.3	25.4	26.3	27.8	25.2	28.0	27.0	26.3	28.8	24.0
Lea	55.0	53.0	57.0	57.0	53.0	57.0	52.0	54.0	57.0	54.0
% Ext.	2.8	2.5	2.6	2.8	2.5	2.8	2.7	2.3	3.0	2.4

Uster test length 50 cm
Time to break 20.3 s
100 breaks/sample

Table 3. Effect of the enzymatic hydrolysis of the residual non-cellulosic and cellulosic components present on dew-retted fibre measured as caustic weight loss (CWL) and fluidity (F) respectively of the yarn spun from roves treated with Novozym 249 (N249), Pectinol AC (PAC), Ultrazym (U), mixtures of PAC and U, enzyme extracts from shive colonised by *P. ostreatus* × *florida* (POF), *C. sublaevis* (CS) and mixture of POF and CS; NaOH treatment of the rove at 45° C (LOW) and 95° C (HIGH) and control

	N249	PAC	U	PAC+U	POF	CS	POF+CS	LOW	HIGH	Control	S.E.
CWL (%)	20.0	20.2	21.2	15.8	20.2	18.3	17.1	22.3	19.3	25.3	0.264**
F	1.4	1.0	1.0	1.1	1.3	1.1	1.4	1.2	1.5	1.0	0.064**

** $P=0.01$ levels of significance

celluloses and pectins have to be removed before bleaching the rove. Traditionally this has been done by boiling the dew-retted fibres in either NaOH or NaOH and ash boil followed by a peroxide bleach (Turner 1954). Of the enzymes tested some in this study, can equally hydrolyse the polysaccharides present on fibre and can produce yarns of high quality number with a low coefficient of variation (20% or less). Since a NaOH boil is carried out at range of 95° C for 2—3 h, there may be an economic advantage in using polysaccharide-degrading enzymes for the removal of non-cellulosic materials at low temperature (45° C) range. The greatest savings in energy are most likely to accrue from the economic use of energy in the wet processing of textiles particularly in the heating of process water (Anon. 1979). Enzymatic softening and upgrading of low grade mesta and jute is widely used in Indian jute mills, by depolymerising residual pectins and hemicelluloses the polysaccharide degrading enzymes can enhance the quality of low grade jute fibre and consequently improve the handling and processing of jute fibres (Ghosh and Dutta 1980).

High concentration of hemicellulase along with low levels of pectinase and laccase would easily hydrolyse the hemicellulose (16.5%), pectin (2—3%) and other non-cellulosic materials (Turner 1949; Sharma 1986b) present on flax fibres. The concentrated enzymes like Novozym 249 may not be necessary for hydrolysing residual hemicelluloses and pectins. The enzymes released by *C. sublaevis* may contain the right concentration of hemicellulase, pectinase, cellulase and laccase to remove the residual polysaccharides and produce yarn of high quality number.

Due to the limited number of bobbins of rove used in this experiment for spinning yarns, yarn breakage data were not obtained. The quality number of yarn spun from the control roves was significantly inferior to those of the enzyme treated roves. This confirms the effectivenes of the enzyme treatment. Residual non-cellulosic polysaccharides present on water-retted fibre can also be removed by enzymatic depolymerisation. Similarly, green flax fibre can also be softened by enzymatic depolymerisation leading to improvements in the handling and spinning qualities of the fibre (Sharma unpublished data).

If the polysaccharide degrading enzymes can be produced economically, the overall savings in energy and materials is likely to be more. This aspect of enzymatic treatment requires further investigation.

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