INFLUENCE OF PECTINOLYTIC ENZYMES ON RETTING EFFECTIVENESS AND RESULTANT FIBER PROPERTIES

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Enzymes have the potential to provide an improved method to ret flax for textile fibers. Retting is the separation or loosening of fiber bundles from the cuticularized epidermis and the woody core cells. New commercial pectinase products were evaluated both with and without ethylene-diaminetetraacetic acid (EDTA) for retting efficiency. The Fried Test identified the most efficient enzymes and best retting conditions. All enzymes retted flax stems better in the presence of 18 mM EDTA. Pectinases that also contained cellulases reduced fiber strength, whereas those without cellulases effectively retted flax without substantial strength loss. Viscozyme, which has been used extensively in our enzyme-retting research, and several pectinolytic enzymes were compared in pilot plant scale tests. Texazym BFE and Bioprep 3000 L retted flax as well as Viscozyme in this system, and the fibers had higher tenacity. The monocomponent nature, commercial availability and price, and ability to ret flax in combination with EDTA at high pH indicated a potential advantage for Bioprep 3000 L in these tests. Retting with different enzymes and formulations resulted in fibers with different properties, thereby leading to protocols for tailored fiber characteristics.

Keywords: Flax; Fiber; Enzyme; Retting; Fiber quality

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INTRODUCTION

Bast fibers, which form in the cortical regions of certain plants like flax, require retting in order to obtain commercial fibers for textile and other applications. Retting is the separation or loosening of fiber bundles from the cuticularized epidermis and the woody core cells and subdivision to smaller bundles and ultimate fibers. Microbial activity during retting causes a partial degradation of the components that bind tissues together, thereby separating the cellulosic fibers from non-fiber tissues. Earlier work has clearly indicated the requirement of pectinases in flax retting (Sharma 1987a; Van Sumere 1992). Two methods employed for retting flax at commercial levels using pectinolytic microorganisms are water- and dew-retting (Sharma and Van Sumere 1992). Water-retting traditionally depended upon anaerobic bacteria, such as *Clostridium spp*., that live in lakes, rivers, ponds, and vats to produce pectinases and other enzymes to ret flax. The stench from anaerobic fermentation of the plants, extensive pollution of waterways, high drying costs, and putrid odor of resulting fibers resulted in a move away from anaerobic water-retting in the mid 20th century to dew-retting. Dew-retting is the

result of colonization and partial plant degradation by plant-degrading, aerobic fungi of flax stems, which are harvested and laid out in swaths in fields. The highest quality linen fibers are produced in Western Europe, which now uses dew-retting, but concern exists within this industry about low and inconsistent quality. Mainland China is currently expanding into flax production. Daenekindt (2004) reported that 80% of the fiber production in China is by warm water-retting and 20% is from "postponed" dew-retting; fiber yield is reported to be low and quality is moderate.

In the 1980's, substantial research was undertaken to find an enzymatic replacement for dew-retting of flax for linen in Europe (Sharma and Van Sumere 1992). A wide range of commercially available enzyme mixtures containing polygalacturonases, pectin lyase, hemicellulases, and cellulases were screened for retting. Novozym 249 was found to be the most suitable for retting, with conditions of 55°C for 20 h and a straw:liquid ratio of 1:11. Flaxzyme, a product from Novo Nordisk, was developed from this work and was reported to produce fibers with good yield and quality (Van Sumere and Sharma 1991). The yield and hand of enzyme-retted fibers was as good or even better than those from high-quality water-retting. A disadvantage, however, was potential lower fiber strength due to the continued activity of the cellulases in the mixtures. Treatment with an oxidizing agent, such as sodium hypochlorite, or reagents giving a high pH that denatured the enzymes prevented the continuing cellulolytic activity. Research on enzyme-retting led to a series of patents and to a semi-industrial scale trial (Van Sumere 1992), but no commercial system was developed.

Interest has continued on use of enzymes for retting and bast fibers, including flax, with considerable interest at major international conferences (Gűbitz and Cavaco-Paulo 2001; Hardin et al. 2002; Kozlowski et al. 2005). The Agricultural Research Service of US Department of Agriculture began a project on enzyme retting in the mid 1990's towards improving flax fibers that could be used in short staple spinning systems for the US textile industry. The objective of this work was, therefore, not long line fiber for traditional linen, but instead short staple fibers for blending with cotton and other fibers. The requirements to maintain long fiber length and other restrictions necessary for traditional linen could be avoided, and new methods could be explored to produce a total fiber product from diverse sources of flax. Results from several studies have been reviewed (Akin et al. 2004). From available enzyme sources evaluated at the time, Viscozyme L appeared to be the most useful. This enzyme in combination with ethylenediaminetetraacetic acid (EDTA) was extensively evaluated, and a spray enzyme retting method was developed (Akin et al. 2000).

Fibers produced from various formulations and flax sources were evaluated and ranked in test yarns (Akin et al. 2001). Use of Viscozyme and EDTA became the basis on which other products and protocols were compared within our laboratory. Earlier work indicated that 0.05% to 0.3% of the commercial product Viscozyme L with about 18-25 mM EDTA, pH 5.0, 40°C for 24 h resulted in an appropriate retting formulation and method (Akin et al. 2002; Akin et al. 2001). Progressively higher levels or longer incubation times with Viscozyme weakened the fibers. While washing and drying by the methods used prevented continued action of cellulases (Akin et al. 2004), their presence initially in the mixture weakened fibers. Eliminating cellulases and strength loss from commercial retting enzymes became a goal.

Recent advances in enzyme technology have resulted in new products and processes for textiles (Akin and Hardin 2003), including enzymes with potential to improve retting of flax (Antonov et al. 2005; Kozlowski et al. 2005). Further work involving these and other pectinolytic enzyme products (Brühlmann et al. 2000) could improve current retting methods. The objective of research reported herein was to screen new pectinolytic enzymes for retting efficiency, using a series of tests. The more likely candidates were further evaluated by retting in larger amounts and processing in the USDA Flax Fiber pilot plant, using commercial type cleaning equipment. Fibers were assessed for fiber yield, fine fiber yield, and properties important for textile applications. Finally, chemical costs were compared based on available information.

MATERIALS AND METHODS

Enzymes tested for retting are listed in Table 1. The conditions used were those recommended by the suppliers for optimal pH and temperature, and in most cases the recommended levels were used. Cellulase (Sigma Cat. #C8546) at 20 U/ml, pH 5.0 and 37° C was used as a positive control in weakening fibers in the study for microscopic analysis of fiber structure. While information from the sources often include amount of enzyme per weight of substrate, our percentages are v/v proportions for the solutions used in incubations of the flax substrate. Specific conditions are identified in experiments where pH, temperature, or enzyme formulations were varied. Other chemicals used in formulations included chelators and surfactants.

Table 1. Enzymes for Retting of Flax.

Product name	Source	Purpose / activity	Conditions		
			Required amounts	pH range	Temp. range
Texazym BFE	Inotex Ltd., Dvůr Královi, CZ	Elementarization of bast fibers. Degrades pectin layers binding elementary fibers.	2-10% owf	5-9	50-60°C
Texazym DLG	Inotex Ltd., Dvůr Královi, CZ	No impact on cellulase. Decomposition of hemicellulases & lignin partially in bast fiber. Elementarize bast fibers with BFE.	1-5% owf	4.5-5.5	60°C
Multifect Pectinase FE	Genencor Int., Rochester, NY	Concentrated pectinase complex containing pectinase, cellulase, hemicellulase, & arabinase activities.	0.01-0.1%	4.2-4.7	30-60°C
Multifect Xylanase	Genencor Int., Rochester, NY	Xylanase, typically used in animal feed. Low cellulase side activity.	25-75ppm	3.5-6.5	50-60°C
Viscozyme L	Novozymes North America, Franklinton, NC	Multienzyme complex for breakdown of cell walls in cereal/vegetable materials & the brewing industry. Contains cellulase, hemicellulase, arabinase & pectinase.	0.05-0.1%	3.3-5.5	25-55°C
Cellulase	Sigma-Aldrich, St. Louis, MO	From *Trichoderma reesei* for breakdown of cellulose	20U/ml	5.0	37°C
Bioprep	Novozymes North America, Inc.	Removes impurities & hydrophobic material from surface of cotton fibers. Alkaline pectate lyase.	0.1%	7-9	50-60°C

Information pertaining to these enzymes was obtained from product sheets of the sources.

EDTA (Fisher Scientific Co., Fair Lawn, NJ) was used at 18 mM concentration. Mayoquest 200, a commercial product with about 38% EDTA (Lynx Chemical Group, L.L.C., Dalton, Georgia) was used with various enzymes as a chelator. Barapon C-108, an amino polycarboxylic acid salt mixture, and Clavodene CIU, a mixture of surfactants (Dexter Chemical L.L.C., Bronx, NY), were used in formulations with Bioprep in some studies.

For Fried Tests, a sample of 'Jordan' flax was used in order to rank various enzyme formulations for the degree of fiber separation, and therefore retting efficiency (Henriksson et al. 1997). Jordan was grown as a winter flax crop near Plains, Georgia, during 2000-2001 and harvested for optimal fiber on April 25, 2001. Very thick or thin stems were removed to have a more uniform material. Twenty centimeter segments were excised from the mid section of over 100 stems, and each twenty centimeter segment was cut in half; the resulting 10 centimeter segments were mixed to provide a uniform substrate in all Fried Tests. Twelve stem segments were placed into each tube for enzyme-retting. Enzyme solutions were added to fill the tubes, which were placed in an incubator on a rotating wheel to continuously mix enzyme and substrate. Samples were prepared for the Fried Test as indicated (Henriksson et al. 1997). The liquid was decanted, the twelve stems were divided into 4 replicates of 3 stems per tube, 8 ml of boiling water was added to the tubes, tubes were aggressively mixed in a vortex mixer (Scientific Instruments Inc., model G-560) for 10 sec., and the tubes were manually shaken (4 up and down strokes). The degree of fiber separation from the stem core was judged independently by two laboratory workers experienced with this method. Fiber separation was scored from 0 (no separation) to 3 (extensive separation) using standard images.

To determine the effect of enzymes on fiber structure and potential degradation using microscopy, Jordan bast tissue from the stem centers was manually separated from core tissues and cut into 1 cm lengths. These bast sections (about 6 mg per tube) were incubated with enzyme formulations. Carded flax fiber tow (about 6 mg per tube of Grade 23, Danforth International Trade Associates, Point Pleasant, NJ) was also incubated in similar enzyme solutions. A portion of the flax tow was subjected to vortex mixing to add physical stress and facilitate observation of any fiber disruption due to enzymes. Enzyme levels for these tests were elevated over normal recommendations and used without chelators to assess any potential fiber breakdown due to enzyme. For microscopic analysis, enzyme-treated fibers were washed and mounted under glycerol on slides and examined under polarized illumination with a 2.5 X objective lens. Enzyme-treated fibers were compared with those incubated in buffer alone to identify criteria that could be used to judge enzymatic breakdown of fibers. Criteria used were: relative amounts of bends and breaks in fibers especially at fibernodes, visual assessment and location of short fibers broken at fibernodes, and presence of long fibers.

The cultivar 'Ariane', which is known for good fiber yield, was used in pilot plant scale enzyme-retting of fibers, which were subsequently processed and characterized for yield and properties. Ariane was grown to full seed maturity in the coastal plain of South Carolina and harvested in May, 1999. This material had been stored inside to prevent any weathering and has been a mainstay for our studies on enzyme retting (Akin et al. 2004). Seeds were removed and stems were crimped through fluted rollers to disrupt the

stem integrity and facilitate interaction of enzymes with tissues. For retting, 150 g samples of crimped straw were briefly soaked (2 min.) in enzyme solutions, drained for 30 sec., and incubated in conditions optimal for enzyme activity. After retting for a selected period of time (usually 24 h), flax straw was washed for 2 min. in water, and air dried. All treatments were carried out in triplicate.

Enzyme-retted, washed, and dried Ariane was processed through the USDA Flax Fiber Pilot Plant (Flax PP) (Akin et al. 2005) in the following order: 9-roller calender 1 X, top shaker 1 X, scutching wheel 1X, 5-roller calender 1 X, top shaker 2 X. After processing through the Flax PP, fiber samples were evaluated for shive content (ASTM D7076-05 2005), based on an NIR model (Sohn et al. 2004), and then conditioned (21°C, 65% relative humidity) before passing through the Shirley Analyzer (SDL America, Charlottesville, NC). Shirley-cleaned fiber, i.e., fine fiber yield, was the basis for determining retting efficiency, as the finer fibers freed from shive and coarse bundles were collected. Shirley-cleaned fibers were subjected to a series of tests to judge quality. Shirley-cleaned fibers were assessed for shive content by the NIR method (ASTM D7076-05 2005). Strength in g/tex and elongation were determined for six trials each replicate using the Stelometer as in the cotton system (ASTM D1445-95 1999). Fineness was determined using airflow, based on a modified cotton micronaire method (Akin et al. 1999) using a standard method (ASTM D1448-97 1999), to give specific surface index (ASTM D7025-04a 2005) for three 5-g samples each treatment.

RESULTS COMBINED WITH DISCUSSION

The retting effectiveness by selected enzymes was initially assessed by the Fried Test for intact stem portions of flax. Texazym BFE alone effectively retted stems of Jordan flax, at 2, 5, and 10 % levels after 24 h; only 10% BFE retted flax at 7 h (Table 2). The addition of EDTA improved retting, showing effective fiber separation at 7 h for 5 % BFE. DLG was ineffective in fiber separation, even with EDTA, by this method.

The effect of retting was further evaluated using BFE, DLG, and Multifect Pectinase FE in several modifications of formulas and retting conditions (Table 3). Longer periods of time improved BFE retting effectiveness. While 1% BFE was effective at 24 h, the addition of EDTA facilitated enzyme retting with all levels of this enzyme. The 2% level appeared to be effective enough to warrant further study, and temperatures in the 50 to 60°C range were more effective than lower temperatures. Incubation of stems with DLG at 5%, even with EDTA, did not result in fiber separation (Table 3). Multifect Pectinex FE was effective at 0.2% with EDTA, but not without the chelator (Table 3); lower levels were less effective even with EDTA.

Based on suppliers' recommendations, combinations of enzymes were tested and included BFE plus DLG and Multifect FE plus xylanase (Table 4). Addition of DLG as high as 0.5% did not improve retting efficiency of 1% BFE plus EDTA by the Fried Test. Similarly, addition of xylanase up to 0.15% to Multifect FE plus EDTA did not improve fiber separation.

Table 2. Enzyme-Retting of Flax by Texazym BFE and DLG using the Fried Test[a].

Enzyme	Amount	Presence of EDTA	Incubation time (h)	Fried Test Score[c] #1	#2
BFE	2 %	-	7	2	1.5
	2 %	-	24	3	3
	5 %	-	7	2.6	1.8
	5 %	-	24	3	3
	10 %	-	7	3	2
	10 %	-	24	3	3
	5 %	+	7	3	3
	5 %	+	24	3	3
DLG	1 %	-	7	0	0
	1 %	-	24	0	0
	2 %	-	7	0	0
	2 %	-	24	0	0
	5 %	-	7	0	0
	5 %	-	24	0	0
	2 %	+	7	0.6	0.8
	2 %	+	24	1.4	0.8

[a] Texazym BFE and DLG from INOTEX Ltd, Dvůr Královi, Czech Republic incubated at 60 °C with midsection stem lengths of Jordan flax at pH 7.0 and 5.0, respectively. BFE 2-10%, pH 7.0, 60 ^{0}C; DLG 1-5 %, pH 5.0, 60 ^{0}C.
[b] Ethylenediaminetetraacetic acid at 18 mM concentration.
[c]Two scorers independently ranked retted stems by standard images. Value is the average of 4 replicates having 3 stems each taken from a single incubation tube.

Table 3. Enzyme-Retting of Flax by Texazym DLG, Texazym BFE, and Pectinase FE using the Fried Test[a].

Enzyme amounts[b]	Incubation conditions	Fried Test Score[c] #1	#2
DLG 3%	pH 5.0, 60 ^{0}C	1	1
DLG 5 %	pH 5.0, 60 ^{0}C	0.3	0.8
DLG 5 % (no EDTA)	pH 5.0, 60 ^{0}C	0	0
BFE 2 %	pH 7.5, 60 ^{0}C	2.5	3.0
BFE 2 %	pH 7.5, 50 ^{0}C	2.0	2.8
BFE 2 %	pH 7.5, 40 ^{0}C	1.5	2.0
BFE 2 %	pH 7.5, 23 ^{0}C	0.3	1.3
Pectinase FE 0.05 %	pH 3.9, 45 ^{0}C	1.0	2.0
Pectinase FE 0.1%	pH 3.9, 45 ^{0}C	1.5	2.4
Pectinase FE 0.2 %	pH 3.9, 45 ^{0}C	2.8	3.0
Pectinase FE 0.2 % (no EDTA)	pH 3.9, 45 ^{0}C	0.5	1.0

[a]DLG and BFE are from INOTEX, Czech Republic. Multifect Pectinase FE is from Genencor International, Inc.
[b]Unless indicated, EDTA was used with enzymes at 18mM.
[c]Two scorers independently ranked retted stems by standard images. Value is average of 4 replicates having 3 stems each taken from a single incubation tube.

Table 4. Enzyme-Retting by Pectinases Plus other Complementary Enzymes using the Fried Test.

Enzyme treatments[a]	Incubation time (h)	Fried Test score[b] #1	#2
1 % BFE - no DLG	4	2	1.8
	8	3	2.8
1 % BFE + 0.5 % DLG	4	2.3	2.0
	8	3.0	3.0
1% BFE + 1.0 % DLG	4	2.3	2.0
	8	3.0	3.0
1 % BFE + 1.5% DLG	4	2.5	1.8
	8	2.8	2.3
0.5 % FE - no Xylanase	4	1.0	1.0
	8	2.0	1.5
0.5 % FE + 0.05 % Xylanase	4	1.0	0.3
	8	2.0	1.8
0.5 % FE + 0.1 % Xylanase	4	1.0	0.3
	8	1.5	1.3
0.5 % FE + 0.15 % Xylanase	4	1.3	1.0
	8	1.3	0.3

[a]Texazym BFE and BFE + DLG incubated at pH 7.5, 60^0C + 18mM EDTA.
Multifect Pectinase FE and FE + Xylanase incubated at pH 3.9, 45^0C + 18mM EDTA.
[b]Two scorers independently ranked retted stems by standard images. Value is average of 4 replicates having 3 stems each taken from a single incubation tube.

The use of Bioprep 3000 L to ret flax was evaluated at various pH and with various additives (Table 5). Bioprep at 0.05% at pH 8 and 9 and with chelators effectively retted flax. Retting was slightly less effective at pH 7.

Table 5. Enzyme-Retting with Bioprep and Various Additives.

Enzyme treatments [a]		Fried Test score[b] #1	#2
Bioprep 0.05%, pH 7.0	No additives	0.8	0.8
	+ 1.83% Mayoquest 200	2.8	1.8
	+ 1.83% Barapon	3.0	2.8
	+ 1.83% Barapon + 0.15% Clavodene	2.9	2.8
Bioprep 0.05%, pH 8.0	No additives	1.0	1.0
	+ 1.83% Mayoquest 200	3.0	2.8
	+ 1.83% Barapon	3.0	2.0
	+ 1.83% Barapon + 0.15% Clavodene	3.0	2.8
Bioprep 0.05%, pH 9.0	No additives	0.8	0.3
	+ 1.83% Mayoquest 200	3.0	2.8
	+ 1.83% Barapon	3.0	3.0
	+ 1.83% Barapon + 0.15% Clavodene	3.0	2.8

[a] Incubated at 55° C
[b]Two scorers independently ranked retted stems by standard images. Value is average of 4 replicates having 3 stems each taken from a single incubation tube.

Many of the enzyme mixtures tested contain multiple types of enzymes active against plant cell walls, including cellulases. Microscopic analysis of fibers after selected days of incubation shows clear signs of variation due to structural changes and breakdown of fibers (Table 6). Figure 1 shows examples from several treatments of fibers used to determine the degree of destruction. The pectinases Texazym BFE and Bioprep resulted in slight to no fiber destruction as indicated by microscopic assessment. In contrast, Texazym DLG and Sigma cellulase were very destructive to flax fibers (Table 6).

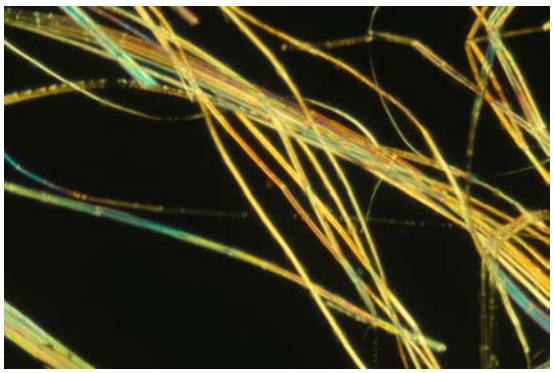

Figure 1 a

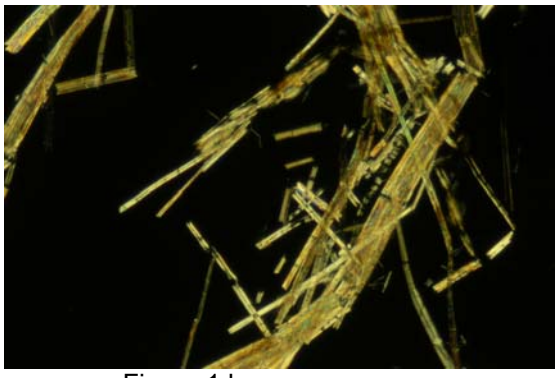

Figure 1 b

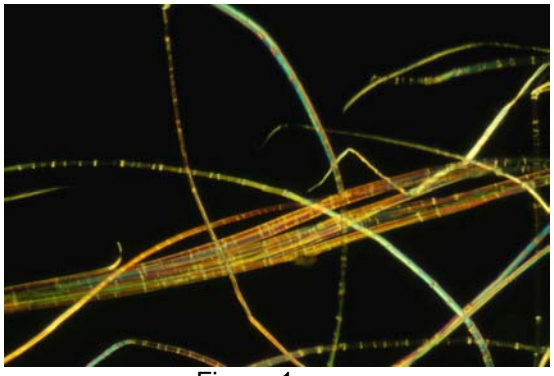

Figure 1 c

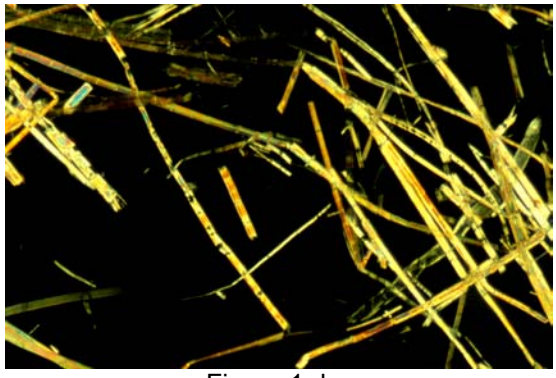

Figure 1 d

Figure 1. Polarized microscopic images showing structural modifications of Grade 23 tow flax fiber incubated with enzymes (2.5 lens for all images). a. Buffer pH 5.0 after 7 days resulting in intact long fibers and fiber bundles. b. Cellulase for 48 h with extensive short fragments due to breakdown at fibernodes. c. BFE 10% after 7 days showing intact long fibers and fiber bundles. d. Viscozyme 0.3% for 7 days showing numerous small fragments due to breakdown at fibernodes.

Further evaluation of the use of these enzymes for retting included the following: the amount of formulation uptake during brief (i.e., 2 min.) soaking, fine fiber yield, cleanliness, and relative cost based on enzyme uptake, fine fiber yield, and available cost information (Table 7). Uptake of the amount of formulation was similar among enzymes and was about 300 ml (ranging from 272 to 408 ml) for 150 g initial fiber weight, giving a liquid to fiber ratio of 2 - 2.7 to 1. Fine fiber yield, i.e., fiber cleaned by 1 pass through the Shirley Analyzer, was highest for Texazym BFE and Bioprep formulations but not significantly different from Viscozyme plus Mayoquest.

Table 6. Strength Loss of Flax Fiber Incubated with Various Enzymes.

	Fiber breakdown at day:[a]						
	------1-------		------2-------		----------7----------		
Enzyme treatment	Jordan	tow	Jordan	tow	Jordan	tow	tow (v)
10 % BFE - pH 7.5, 58 ^{0}C	0	0	1	1	0	0	0
5 % DLG - pH 7.5, 58 ^{0}C	1	1	2	2	2	2	2
5% DLG - pH 7.5, 58 ^{0}C	2	2	2	3	3	2	3
0.3 %Multifect pectinase - pH 3.9, 45 ^{0}C	0	0	1	1	2	2	2
0.3% Multifect Xylanase - pH 3.9, 45 ^{0}C	0	1	0	1	0	1	1
0.3% Multifect Xylanase - pH 5.0, 45 ^{0}C	0	1	1	1	1	1	1
0.3% Viscozyme - pH 5.0, 40 ^{0}C	1	2	1	1	2	3	3
20 U/ml cellulase - pH 5.0, 40 ^{0}C	2	2	2	2	3	3	3
2 % Bioprep - pH 9.0, 55 ^{0}C	ND [b]	ND	ND	ND	0	0	1
Buffer - pH 3.9, 45 ^{0}C	0	1	0	0	0	0	0
Buffer - pH 5.0, 45 ^{0}C	0	0	0	0	0	0	-

[a]Fiber breakdown was determined by microscopic observation of samples. Jordan bast, with shive manually removed, was excised from the center of the stem and cut into 1 cm pieces. Tow was a commercial tow fiber from Europe (Grade 23 supplied by Frank Riccio, Danforth International Trade Associates). A separate sample of tow was vortexed (Fisher Scientific, cat#12-812) for 30 sec at highest speed to add physical stress to fibers. A summary conclusion on breakdown was made from the following criteria: breaks and bends of fibers, amount and location of short fibers broken at nodes, presence of long fibers. Scores areas follows: 0, no breakdown; 1 slight evidence (not conclusive); 2, definite breakdown; 3 extensive breakdown.
[b]Not determined

Shive content in fiber, as determined by the NIR model, showed that fiber that was subsequently Shirley-cleaned contained considerably less shive than that cleaned only by the Flax PP for all formulations. All enzyme-retted and Shirley-cleaned samples were cleaner than unretted fiber, and differences were not large among the enzyme treatments. Based on available information from suppliers, Bioprep and Bioprep plus Mayoquest gave the lowest cost per kg fiber. Since, one pass through the Shirley Analyzer fiber does not represent all the cottonized fiber that would be obtained, a relative cost rather than an absolute value was used to compare treatments. Bioprep only was the lowest cost, and a value of 1.0 was assigned and all other formulations were calculated as an increase over Bioprep (Table 7). Wide variations occurred for the enzyme formulations.

Properties important for application in textile and other industries are shown for fibers derived from the various enzyme-retting methods and cleaned by one pass through the Shirley Analyzer (Table 8). Retting with Texazym BFE and Bioprep, which

reportedly contain only pectinases, resulted in fibers with significantly higher strength than cellulase-containing mixtures, i.e., Multifect Pectinex FE and Viscozyme. Bioprep in all formulations resulted in higher elongation, although values were still small at 2.0 to 2.5%. Retting with Bioprep and Viscozyme produced fibers that tended to be finer than those from other treatments.

Table 7. Yield and Relative Cost for Flax Retted with Different Enzymes*.

	Fiber Yield (% straw)		% Shive (NIR method)		
Enzyme formulation[a]	Pilot Plant	Shirley-cleaned (1 Pass)	Pilot Plant-cleaned fiber	Shirley-cleaned fiber	Relative costs[b] Shirley-cleaned
2 % Texazym BFE + EDTA	36.2 ± 2.4abc	7.0 ± 1.2a	13.8 ± 2.0abc	2.7 ± 0.8bc	5.7
5 % Texazym BFE only	35.3 ± 2.5abc	7.2 ± 0.6a	10.5 ± 0.7c	3.3 ± 0.3b	10.2
0.1 % Pectinase + EDTA	32.9 ± 1.1bc	2.1 ± 0.5c	15.8 ± 1.0abc	3.2 ± 0.3bc	ND
0.2 % Pectinase + EDTA	32.2 ± 1.2c	2.2 ± 0.2c	13.5 ± 3.3bc	1.2 ± 0.7c	ND
0.1% Bioprep only	38.9 ± 0.9a	6.0 ± 1.1ab	19.8 ± 1.7a	4.1 ± 0.2b	1.0
0.1 % Bioprep + EDTA	36.7 ± 3.5ab	7.7 ± 1.4a	18.0 ± 3.5ab	3.7 ± 1.0b	1.4
0.1% Bioprep + B + C	34.3 ± 1.8bc	5.7 ± 1.3ab	14.5 ± 5.7abc	3.2 ± 1.1bc	3.5
0.05 % Viscozyme + EDTA	32.3 ± 2.7c	5.2 ± 2.3ab	14.3 ± 4.5abc	2.9 ± 2.1bc	3.4
Untreated	36.1 ± 4.5abc	3.2 ± 1.5bc	19.6 ± 3.8a	6.2 ± 1.0a	ND

* Values followed by different letters within columns are significantly different at P≤0.05.
[a] Enzymes are listed in Table 1. Mayoquest 200 was added to provide 1.83 % EDTA. Incubations were at optimal conditions for specific enzyme.
[b] Costs are provided to provide relative comparisons with Bioprep the lowest cost enzymatic application.

Table 8. Properties of fibers from flax [a] retted with different enzymes*.

Enzyme formulation [b]	Strength [c] (g/tex)	Elongation [c] (%)	Fineness [d] (SSI)
2.0% Texazyme BFE + Mayoquest 200	36.7 ± 1.5 ab	1.8 ± 0.1 bc	4.69 ± 0.10 ab
5.0% Texazyme BFE only	34.6 ± 2.0 b	1.6 ± 0.1 cd	4.27 ± 0.31 bcd
0.1% Multifect FE + Mayoquest 200	21.6 ± 3.7 d	1.3 ± 0 d	4.33 ± 0.1 bc
0.2% Multifect FE + Mayoquest 200	17.8 ± 2.2 d	0.5 ± 0.3 e	4.12 ± 0.05 bcde
0.1% Bioprep only	33.2 ± 2.4 bc	2.0 ± 0.3 abc	3.79 ± 0.05 cde
0.1% Bioprep + Mayoquest 200	34.9 ± 2.0 b	2.3 ± 0.3 ab	2.95 ± 0.63 f
0.1% Bioprep + Barapon + Clavodene	34.8 ± 4.8 b	2.5 ± 0.1a	3.55 ± 0.48 ef
0/05% Viscozyme + Mayoquest 200	27.6 ± 3.8 c	1.4 ± 0.5 cd	3.60 ± 0.69 def
Unretted	42.0 ± 5.5 a	1.9 ± 0.4 abc	5.20 ± 0 a

* Values followed by different letters within columns are significantly different at P≤0.05.
[a] Ariane flax was grown to seed maturity in South Carolina as winter crop in 1998-1999.
[b] Enzymes and chemicals are used as provided by suppliers under optimal conditions for activity. Mayoquest 200 was used at 18 mM EDTA based on a 38% EDTA content.
[c] Fiber properties determined using a modified test method (ASTM D1445-95 1999a).
[d] Airflow fineness determined using test method (ASTM D7025-04a 2005).

A chronological listing of research for enzyme-retting of flax beginning in 1932 has been reported by Van Sumere (1992). Enzymes screened for retting flax have typically been in mixtures from plant-degrading microorganisms (Sharma 1987a).

Because plant cell walls are complex structures of several components, consideration was given for a combination of polysaccharidases to release clean fibers (Sharma 1992). Residual cellulase activity, however, became problematic, and measures had to be taken to inactivate the enzymes after retting. Recently, we reported on the evaluation of several enzymes for fiber yield and quality, using small pilot plant samples (Akin et al. 2004). Products containing cellulases reduced fiber strength, with greater losses in strength with higher enzyme levels. Microscopic studies have shown that cellulases, and cellulase-containing enzyme mixtures preferentially attack the fibernodes or kink bands in fibers and fiber bundles (Khalili et al. 2002). Flax fibers are weakened by these preferential attacks at these sites, and tenacity is extensively reduced (Akin et al. 2001; Akin et al. 2004). Results also indicated polygalacturonase alone could effectively separate fibers, and the presence of other plant cell wall polysaccharidases may not be required (Akin et al. 2002; Akin et al. 2004; Evans et al. 2002). One objective of our work, therefore, was to find retting enzyme mixtures without cellulases. Until recently, these mixtures were not commercially available at reasonable costs.

The commercial enzymes used in the present study represented a mixture of polysaccharidases, e.g., cellulases and hemicellulases in some, as well as different types of pectinases. Polygalacturonase (PG) and pectate lyase (PL) are both depolymerizing enzymes for pectin; PG catalyzes random hydrolysis of α -1,4 polygalacturonic acid and PL carries out a nonhydrolytic breakdown of pectates and pectinates by a trans-elimination split of the pectic polymer (Sakai et al. 1993). PL is activated by Ca++ and usually is active at higher pHs (e.g., 8-10) than PG. Recent research has shown that alkaline pectinases such as pectate lyase are potentially important for retting bast plants (Antonov et al. 2005; Brühlmann et al. 2000).

The role of Ca++ chelators, such as EDTA, for improved retting is well known (Sharma 1988; Van Sumere 1992). Henriksson et al. (1997) showed that the addition of oxalic acid and EDTA facilitated the action of commercial enzyme mixtures, e.g., Flaxzyme. While the binding capacity for Ca++ of EDTA is greater at alkaline pH, Adamsen et al. (2002) showed that EDTA has substantial Ca++ binding activity even at pH 5, which explained the positive value of EDTA at lower pHs optimal for some enzymes.

The role for Ca++ chelators in flax retting likely arises from destabilizing bridges between Ca++ and polygalacturonic acid, thus leading to disruption of tissues. Flax reportedly absorbs considerable amount of calcium from the soil during growth (Sultana 1992), and analysis of Ariane flax using the inductive coupling plasma (ICP) method showed particularly high levels of calcium in the epidermal/cuticle regions compared to the fiber (300 versus 16 mmol/kg) (Akin et al. 2004). Other work (Jauneau et al. 1997; Rihouey et al. 1995) indicated higher levels of both non-methoxylated pectin and calcium levels in epidermal regions of flax compared to the fibers. This fact suggests that the cuticle/epidermal region of the stem is stabilized with the calcium bridges in addition to the waxy cuticle barrier. The application of EDTA with enzymes has been shown to improve retting, particularly in removing the epidermal/cuticle material from the fibers and fiber bundles (Akin et al. 2004; Akin et al. 1999). The use of EDTA alone has not given particularly good results. The inclusion of EDTA with enzymes, however, has

provided the best retting efficiencies in our protocol (Akin et al. 2004). Results in the present study confirmed this response with the new enzymes screened.

Several past studies in our laboratory have evaluated the effect of Viscozyme L plus EDTA for retting efficiency and for properties of flax fibers (Akin et al. 2000; Akin et al. 2004). Viscozyme L is not marketed for flax retting but is close in nature to Flaxzyme and worked well in our screening. A formulation was developed with 0.05 to 0.3% Viscozyme L plus about 18 -25 mM EDTA from Mayoquest 200. This product was our enzyme of choice for retting, but a characteristic of retting with Viscozyme is the progressive loss of fiber strength with increasing enzyme levels or incubation times. Multifect Pectinase FE, which is not listed for flax retting but for food and feed applications (product information from Genencor International, Rochester, NY), has substantial and progressive cellulolytic activity against flax fibers in our tests. Texazym BFE is reportedly a multi-component product without cellulase activity. Flax retting is one of the stated applications, and industry recommendations are to use it with DLG. Our data confirm the high retting efficiency of BFE with EDTA; the presence of DLG did not improve retting in our tests and alone reduced fiber strength substantially. Use of BFE at pH 7.5 might indicate significant activity by the PL, based on general knowledge of PG and PL enzymes (Sakai et al. 1993). The use of alkaline pectate lyases has been used for retting ramie (Brühlmann et al. 2000). Bioprep, a commercial pectate lyase, provides a potentially important enzyme for retting of flax due to its monocomponent nature, its commercial availability and price, and its ability to ret flax in combination with EDTA at high pH. Bioprep was developed as an environmentally friendly method to scour cotton, thereby replacing the heavy use of alkali. Its usefulness in scouring cotton has been proved (Durden et al. 2001; Etters et al. 2001). The mode of action is the detachment of the cotton fiber cuticle by degradation of the underlying pectin layer. Previous work with Bioprep (Akin et al. 2004) did not show successful retting, but enzyme levels were likely too low. The protocol used in the present study followed closely the experimental recommendation for cotton scouring (personal communication, S. Salmon, Novozymes North America Inc., Franklin, NC). An exception we used was the inclusion of EDTA with the enzyme in a retting formulation.

Of the enzymes tested in the present study, Bioprep plus EDTA provided good retting efficiency, high yields, strong fiber, and the best cost ratios with current economic information. Other conditions must be evaluated for optimization of the protocol. While the retted fiber is stronger with Bioprep than with Viscozyme, both enzymes appear to work well with EDTA in our pilot plant system, and fibers can be tailored with these enzymes for different properties. Sharma (1987b; 1987c) reported that enzymes other than those for retting may be important to remove components on dew-retted flax fibers and add value to low quality yarns. Similarly, fibers retted with Bioprep or Viscozyme might benefit from additional enzyme that remove non-cellulosic residues and improve utilization. Such work with increased enzyme levels remains to be done.

ACKNOWLEDGMENTS

We thank the following companies for providing products used in evaluations: Novozymes North America, Inc., Franklinton, NC; Inotex Ltd., Dvůr Královi, Czech Republic; Genencor International, Inc., Rochester, NY; Dexter LLC, Bronx, NY; and Lynx Chemical Group, LLC, Dalton, GA. We thank the following for excellent technical assistance: Nkaku Kisaalita and L.L. Rigsby, Russell Research Center, ARS-USDA, Athens, GA, and Brad Reed, Cotton Quality Research Station., ARS-USDA, Clemson, SC. Mention of trade names does not constitute an endorsement of one commercial product over another but is used only for identification purposes.

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Article submitted: Nov. 25, 2007; Peer-review process completed: Dec. 31, 2007;
Revised article accepted: Jan. 3, 2008; Published: Jan. 5, 2008.