# Plant Factors Influencing Enzyme Retting of Fiber and Seed Flax

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Retting, which is the microbial activity through which bast fibers are released from nonfiber tissues, is the limiting factor in flax processing. The objective of this work is to identify chemical and structural characteristics in a variety of fiber and seed flax types that influence enzyme retting in a recently developed method. Analyses of flax retted in a series of tests, including two enzyme rettings in some cases, indicated that lignin did not limit the separation of fibers from shive and showed that pectinases in enzyme-retting mixtures could ret fiber and seed flax. However, mature stems, such as that in flax produced for seed, had greater amounts of cutin and wax in the cleaned fiber product, suggesting that the cuticle could be a greater antiquality factor in seed versus fiber flax. With seed flax, the fraction of finer fibers produced during retting was significantly lower than with fiber flax. Results indicated that enzyme retting could be used to obtain flax fibers from seed flax stem residues and add value to this agricultural material.

**Keywords:** *Flax; retting; aromatics; lignin; cuticle; waxes; cutin*

## INTRODUCTION

Flax (*Linum usitatissimum* L.) is likely the oldest textile fiber known, with evidence of production dating back 7000 years or more (*1*, *2*). Except for a small company that began in the 1990s and is no longer in operation, flax fiber has not been produced commercially since the 1950s in the United States, and all linen and flax fiber used in textiles is imported. Flax is grown as an oilseed crop, with Canada as the major supplier, and a small percentage of the straw is used for specialty paper and pulp (*3*). The linseed industry in the United States, although greatly down-sized from previous years, has shown increased production in the past 2−3 years (J. F. Carter, personal communication). Recent interest in possible nutritional benefits, for example, lignans and omega-3 fatty acids (*4*), in flaxseeds bodes well for future increased production of this crop. Considerable interest exists now in the United States and Canada for production of flax fiber for use in textiles and a variety of high-value products (*5*). The potential markets for flax in composites (*6*) have recently increased, led by environmentally friendly and value-added components for the automotive industry (*7*). Vast amounts of seed flax straw occur as a byproduct of the linseed industry, for example, >1 million metric tons annually from western Canada (*3*), and constitute a major environmental problem for disposal. Improved processing potentially could add value to the residue and create new markets. Therefore, the substantial value from use in composites along with the opportunity to solve an environmental disposal problem has rekindled a reevaluation of seed flax residue for applications with a higher value than pulp and paper.

Retting, which is the separation of fibers and fiber bundles from nonfiber tissues in stems of bast plants such as flax, is the major problem in processing flax (*8*). With the cessation of water retting several decades ago due to pollution from fermentation wastes, dew retting is the current commercial method of choice (*9*). However, disadvantages of dew retting are many, including (1) dependence on particular geographical regions that have the appropriate moisture and temperature ranges for retting, (2) coarser and lower quality fiber than with water retting, (3) poor retting consistency, and (4) occupation of agricultural fields for several weeks (*10*). Enzymes have been considered for some time as a potential replacement for dew-retting flax (*10*), but costs and other factors have prevented commercial development of enzyme retting. Recently we developed an enzyme-retting process with reduced enzyme levels compared with those previously used (*11*), but further work is needed to optimize the method and reduce costs. Our objectives in this work are twofold: (1) to identify chemical and structural characteristics in a variety of fiber and seed flax types that limit enzyme retting by our method and (2) to determine the potential for enzyme retting of seed flax straw residue as a potential source of high-quality fiber for industrial use.

## MATERIALS AND METHODS

**Flax Samples.** A diverse set of flax varieties grown in a number of locations for study included fiber and seed varieties: Ariane grown commercially for fiber in The Netherlands (Ariane NL 95); Ariane grown in Maine for commercial fiber (Ariane ME 97); Ariane grown for a pilot test in South Carolina, December 1998 to May 5 or 27, 1999, and harvested early for fiber or late, respectively, as a mature crop for seed and fiber (Ariane SC 99); Ariane grown in 1999 at the Carrington Research and Extension Center, North Dakota State University, Carrington, ND, and harvested for fiber and as a mature crop for fiber and seed (Ariane ND 99); Neche and Omega grown for seed in 1999 at Carrington, ND (Neche ND 99 and Omega ND 99); Natasja grown in South Carolina

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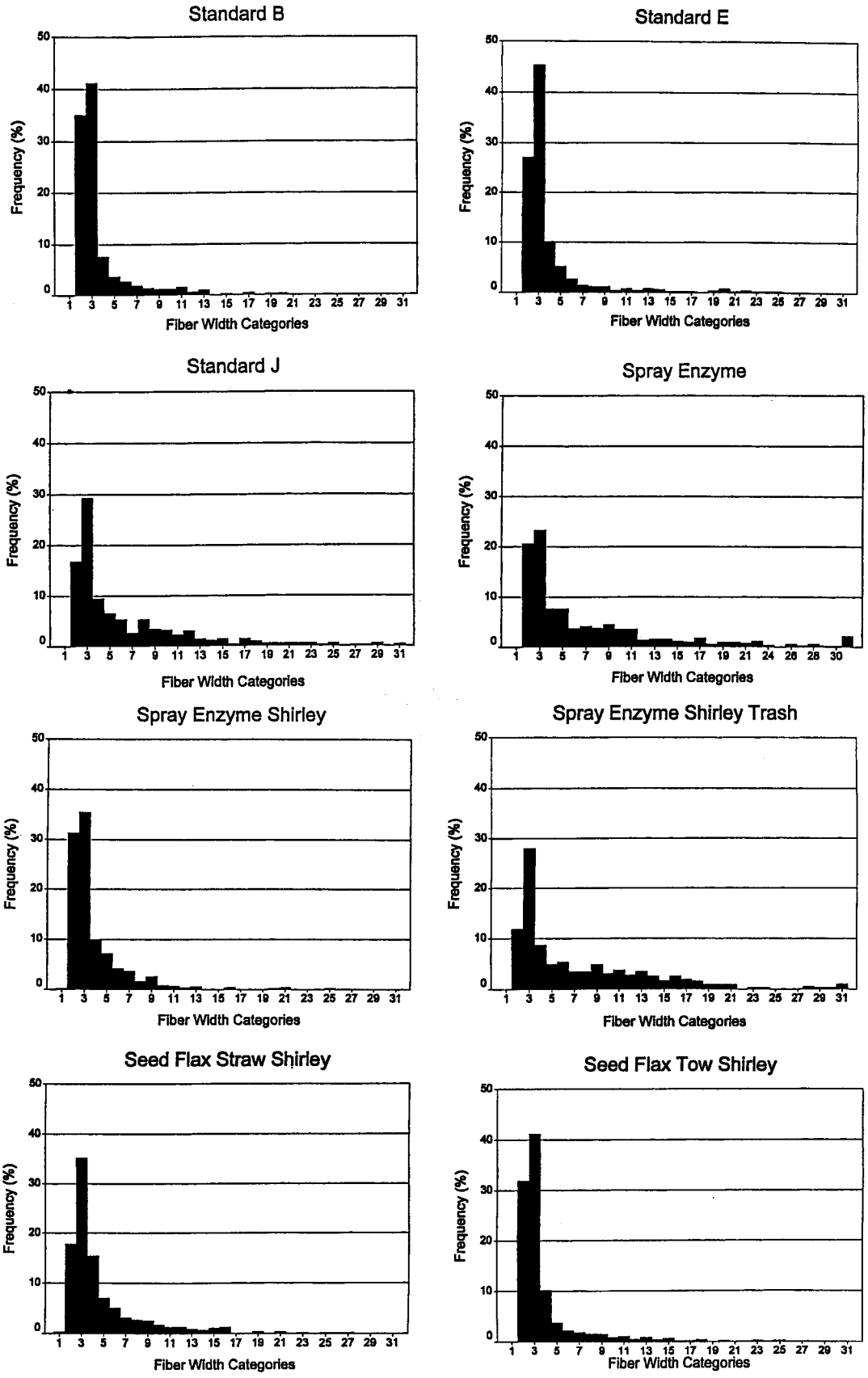


**Figure 1.** Image analysis of flax fiber width distribution using light microscopy ($N$, ~500 fibers or fiber bundles). Frequency percent is the number of fibers or fiber bundles occurring within the width categories, which when multiplied by 10 gives the width in micrometers (i.e., width category of 1 = 10 $\mu$m, etc.). Standards B, E, and J represent ISO fiber standards from the Institut Textile de France, Lille, France, for finest, middle grade, and coarsest fibers, respectively. All other samples are enzyme-retted. "Shirley" indicates the fibers were cleaned through the Shirley Analyzer. "Trash" is the residue after passing through the Shirley Analyzer. Tow is commercially prepared flax for pulping.

**Table 1. Chemical Analyses of Cleaned Flax Fibers[a] Retted by Various Enzyme Formulations**

| sample | enzyme[e] level (%) | EDTA level (mM) | Shirley-cleaned yield (%) | amount of constituents (mg g$^{-1}$) | | |
|---|---|---|---|---|---|---|
| | | | | aromatics | waxes | cutin |
| ND[b] seed flax | 0.05 | 50 | 25.3 ± 1.0ef | 0.6 ± 0.4a | 5.8 ± 1.2a | 6.9 ± 1.6a |
| ND[b] seed flax | 0.05 | 25 | 23.6 ± 1.0f | 0.7 ± 0.1a | 5.4 ± 1.1a | 5.6 ± 0.7a |
| Ariane SC 99 fiber[c] | 0.05 | 50 | 30.7 ± 8.8de | 0.9 ± 0.4a | 3.7 ± 4.2a | 1.6 ± 0.5cd |
| Ariane SC 99 fiber[c] | 0.05 | 25 | 37.9 ± 0.3bc | 0.7 ± 0.1a | 6.8 ± 3.0a | 3.5 ± 1.1b |
| Ariane SC 99 fiber[c] | 0.3 | 50 | 61.4 ± 0.7a | 0.7 ± 0.2a | 5.1 ± 3.3a | 1.6 ± 0.5cd |
| Ariane SC 99 fiber[c] | 0.3 | 25 | 58.7 ± 1.1a | 0.7 ± 0.0a | 5.9 ± 1.1a | 1.2 ± 0.3d |
| Ariane SC 99 fiber[c] | dew-retted | | 43.0 ± 1.1b | 0.8 ± 0.1a | 6.1 ± 0.9a | 2.7 ± 0.3bc |
| Ariane SC 99 fiber and seed[d] | 0.05 | 50 | 32.3 ± 0.3cd | 0.8 ± 0.1a | 11.4 ± 1.5b | 3.4 ± 0.7b |

[a] Enzyme-retted fibers cleaned and cottonized with commercial equipment and cleaned through the Shirley Analyzer one time. Average ± standard deviation of three replicates. Values within columns followed by different letters differ at $P \leq 0.05$. [b] Combined for seed and then harvested. [c] Grown from December to May 5, 1999. [d] Grown from December to May 27, 1999. [e] Viscozyme L (Novozymes, Franklinton, NC) included vol/vol as supplied.

for fiber during the winter and harvested in spring 1995 (Natasja SC 95) and also in 1997 (Natasja SC 97); Flanders seed flax combined for seed removal and not dew-retted (Flanders seed flax); seed flax tow poorly dew-retted and mechanically processed to shorten fibers and reduce shive content to ~50% for pulp and paper (commercial seed flax tow); an unidentified variety of seed flax grown commercially in North Dakota and combined for seed and partially weathered after harvesting (ND seed flax); a mixture of Belinka and Elise at about 50:50 grown for pulp in Spain in 1998 (Belinka/Elise Sp 98); and Jordan, Laura, Neche, and Omega grown in the winter in South Carolina and harvested as a mature seed crop in May 2000 (Jordan SC 00; Laura SC 00; Neche SC 00; Omega SC 00).

**Chemical Analysis.** Ground samples of fiber and shive were treated with 4 M NaOH for 2 h at 170 °C, adjusted to pH 2.5 with 2 N HCL, and extracted with ethyl ether. Sample and base were heated in a screw-capped Teflon vial. The solution was purged with nitrogen, and the vial was capped and placed in a steel reaction vessel containing 7 mL of water. The vessel was sealed and placed in an oven at 170 °C for 2 h. After acidification and extraction with diethyl ether, the ether layer was dried and the residue treated with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA). Analyses of aromatics, fatty acids, dihydroxy fatty acids, and alcohols were carried out as previously described (*26*) on a Finnigan 9001 gas chromatograph using a 0.25 mm i.d. × 30 m DB-5 capillary column (0.25 μm film thickness). Because 8,16-dihydroxyhexadecanoic acid is the predominant hydroxy fatty acid associated with cutin, this acid was used as marker to evaluate the amount of cutin remaining on fiber samples. Cell wall sugars were analyzed as their alditol acetates by gas chromatography according to the method of Hoebler et al. (*13*) with the initial digestion time increased from 30 to 90 min at 40 °C. The column used was a 0.25 mm i.d. × 30 m DB-225 capillary column (0.15 μm film thickness).

**Enzyme Retting.** The spray enzyme retting method (*11*) was used for retting 50 g and larger samples. Briefly, stems were crimped with fluted rollers at ~80 N and sprayed (or in some cases soaked for 2 min) to completely saturate stems with enzyme formulation containing (unless otherwise noted) 0.05% Viscozyme (v/v as supplied by Novozymes, Franklinton, NC) or a similar pectinase-rich enzyme mixture plus 25 or 50 mM EDTA at pH 5.0 for 24 h at 40 °C. The Fried test, an in vitro method for evaluating separation of fiber from shive by enzyme retting, was also employed (*10*, *14*). Briefly, sections usually from the middle of the stem 10 cm in length from 12 plants were placed in tubes with enzyme solution as above for retting on a rotary device. After retting, liquid was decanted, boiling water was added, and the stems were shaken in precise movements. The fibers were scored independently from 0 (no fiber separation from stems) to 3 (fibers separated from stems) using standard images, usually by two scorers.

**Light Microscopy.** An Olympus BH-2 light microscope was used in bright field or phase contrast mode. For histochemistry of lignin, acid phloroglucinol or chlorine sulfite stains (*15*) were used. For analysis of fiber widths by image analysis, fibers were stained for ~30 s with toluidine blue (1:10 aqueous dilution of a 1% solution in 1% aqueous sodium borate) for contrast, and the frequency of fibers in 10 μm width intervals was determined. Fibers were imaged through a 2.5× lens on the light microscope with the Optimas 6.1 imaging system (Media Cybernetics, Silver Spring, MD).

**Cleaning of Fibers.** Enzyme-retted flax stems were cleaned by hand-carding and some samples by the Shirley Analyzer (SDL America, Inc., Charlotte, NC), which uses a rotating drum and air flow to separate fine fibers from coarse fibers and trash. One series of samples was cleaned and then cottonized using commercial cleaning systems (Unified Line and La Roche systems at Ceskomoravsky len, Humpolec, Czech Republic) (*16*).

**Determination of Fiber Properties.** Flax fiber was analyzed for fineness by micronaire (*17*), which was modified to use 5.0 g fiber samples based on calibration with flax fineness standards (Institut Textile de France, Lille, France) as reported (*18*).

**Statistical Analysis.** Samples were analyzed by a one-way analysis of variance with differences at $P \leq 0.05$ determined by least-squares difference.

## RESULTS AND DISCUSSION

Tests were carried out to evaluate the Shirley Analyzer as a method to measure the yield of fine fibers and, therefore, to compare retting efficiencies for various flax types. Image analysis and light microscopy of a series of samples showed that Shirley cleaning changed the distribution of fiber bundle widths, resulting in a higher frequency of fine fibers, especially in the 10−30 μm interval, and a reduction of coarser fibers (Figure 1). The greater amount of fine fibers was always obtained in the first pass through the Shirley Analyzer, with progressively less fine fiber available from the residue in successive passes (not shown). This result suggested that Shirley cleaning was not a major determinant of fiber fineness but primarily separated finer fibers produced by enzyme retting. Variations in fineness of these samples were confirmed using air flow methods (*17*) designed for cotton fibers but with modifications based on tests with flax calibration standards. For example, Shirley cleaning produced fibers with a micronaire reading of ~4−5, whereas the reading for uncleaned or residue fiber from cleaning was 7.5−8 (*18*).

Specific fiber and seed flax samples were enzyme-retted and then Shirley-Analyzed for yield of fine fibers. Commercially processed fibers from a previous study (*16*) showed that the fiber flax yielded more fine fibers than the seed type. Chemical analysis of these fine fibers indicated that levels of aromatic constituents were small and similar in amounts among samples, but cutin amounts were higher in seed flax (Table 1). To further compare fiber and seed flax types based on response to

**Table 2. Yields of Fiber and Seed Flax Grown in Side-by-Side Tests during the Winter in South Carolina under Identical Conditions for Seed Production**

| | yield[a] | | |
|---|---|---|---|
| | | Shirley analysis | |
| sample | hand-carded % | % hand-carded | % straw |
| fiber type | | | |
| Laura SC 00 | 35.5 ± 0.9a | 40.7 ± 0.9a | 14.4 ± 0.7a |
| Jordan SC 00 | 49.4 ± 5.2a | 48.4 ± 3.7b | 23.8 ± 0.7b |
| seed type | | | |
| Neche SC 00 | 35.6 ± 4.9a | 30.5 ± 1.5c | 12.1 ± 0.4c |
| Omega SC 00 | 39.3 ± 2.2a | 31.2 ± 1.5c | 12.2 ± 0.1c |

[a] Average and standard deviation from duplicate 50-g samples of plants, excluding roots and seed bolls, that were enzyme-retted with 0.05% Viscozyme + 25 mM EDTA, pH 5.0, for 24 h at 40° C. Values within columns with different letters differ at $P \leq 0.05$.

**Table 3. Comparison of Fiber and Seed Flax Grown in North Dakota**

| sample | fiber[a] (%) | Fried test[b] |
|---|---|---|
| Ariane ND 99 (optimal fiber) | 27.6 ± 0.9a | 2.8 ± 0a |
| Ariane ND 99 (mature seed) | 32.7 ± 0.5b | 2.0 ± 0a |
| Neche ND 99 (mature seed) | 26.6 ± 1.3a | 1.3 ± 0b |
| Omega ND 99 (mature seed) | 30.3 ± 0.1c | 1.7 ± 0.3a |

[a] Calculated from fibers in 10-cm stem segments midway of the plant. Stems were enzyme-retted using 0.05% Viscozyme + 50 mM EDTA at pH 5.0 for 24 h. All fibers were then manually separated from shive, freeze-dried, and weighed. Calculated as % fiber = [fiber wt/(fiber wt + shive wt)] × 100. Values within columns with different letters differ at $P \leq 0.05$. [b] Average of two scorers for duplicate retting tubes, each with four replicates, and scored as 0 (no retting) to 3 (fibers separated). Values within columns with different letters differ at $P \leq 0.05$.

enzyme retting, fiber and seed types were grown in a side-by-side study as a winter crop in South Carolina with high seeding rates of ~111 kg/ha to produce fine-stemmed plants (~1.5 and 1.3 mm diameter for fiber and seed types, respectively). Even though the plants were all grown to mature seed production, enzyme retting resulted in higher Shirley-cleaned fiber yields from fiber than from seed types (Table 2). A third comparison was made with Ariane ND 99, harvested for optimal fiber and also at a later maturity for seed and fiber, and the commercial seed types Neche ND 99 and Omega ND 99. Fried tests supported other data (Tables 1 and 2) in that enzyme formulations were less efficient in separating fiber from shives in seed versus fiber types (Table 3). The percentage of lignified cells in cross sections of the fiber bundles, determined with acid phloroglucinol, was not related to differences in retting efficiencies of the flax types. Furthermore, lignified residual middle lamellae of some fibers, as shown on fiber surfaces with acid phloroglucinol, indicated that the presence of lignin did not hold fiber bundles intact when subjected to mechanical cleaning forces. Microscopic evaluation indicated that enzyme retting produced fibers and fiber bundles of diverse sizes in all samples, often with associated cuticle fragments, especially for larger bundles (Figure 2). These analyses plus fine fiber yields suggested that cuticle fragments associated with fiber likely are a more significant factor than lignin in limiting quality in seed flax.

Mature Ariane ND 99 and Omega ND 99, both of which had been enzyme-retted, were enzyme-retted a second time and further evaluated for factors limiting enzyme retting. Fibers that were still attached to shive after the first enzyme retting were mostly separated after the second enzyme-retting procedure, even from

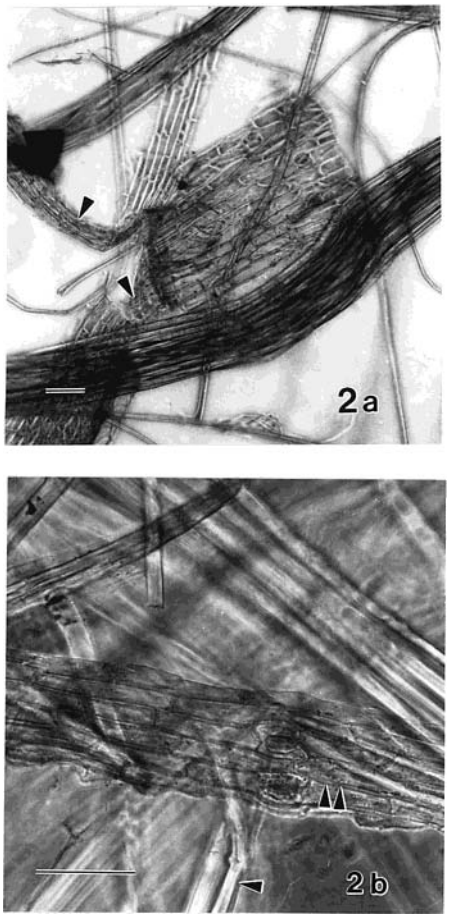


**Figure 2.** Light micrographs of Omega seed flax (SC 00) enzyme-retted and Shirley-cleaned: (a) fiber bundles of diverse sizes and cuticle (arrows) associated with the fiber; (b) enlarged view of fiber mass showing separation of bundles into ultimate fibers in places (arrows) and intimate association of cuticle with fiber bundles in other places (double arrows). Bar = 100 μm.

**Table 4. Fiber−Shive Separation after Two Enzyme Rettings**

| | | fiber separation after second retting | |
|---|---|---|---|
| sample | second retting/ treatment[a] | ranking[b] | % fiber separated[c] |
| mature Ariane ND 99 | enzyme ($N = 30$) | 4.3 ± 0.6 | 87 |
| mature Ariane ND 99 | buffer ($N = 5$) | 4.8 ± 0.5 | 100 |
| Omega ND 99 | | | |
| nonpedicle | enzyme ($N = 20$) | 4.0 ± 0.8 | 68 |
| pedicle | crushed, enzyme | 4.7 ± 0.4 | 96 |
| | not crushed, enzyme | 4.0 ± 2.0 | 67 |
| | crushed, buffer | 4.5 ± 0.9 | 79 |
| | not crushed, buffer | 3.8 ± 1.6 | 58 |

[a] All samples were first enzyme-retted with 0.3% Viscozyme + 25 mM EDTA in water, pH 5.0, at 40 °C for 24 h. The second treatment included enzyme retting of selected shive segments plus attached fiber with 0.3% Viscozyme + 25 mM EDTA in 50 mM sodium acetate buffer, or buffer alone, pH 5.0, at 40 °C for 24 h in test tubes. [b] Shive segment plus attached fibers scored as follows: 5 = fiber completely removed from shive; 4 = fiber slightly clinging to shive; 3 = fiber attached to shive but partially separated; 2 = fiber frayed at ends but mostly attached; 1 = fiber attached. Values are average of two scorers. [c] From scoring as detailed in footnote *b*, (no. of fibers in categories 4 and 5/total no. of fibers) × 100 (average of two scorers).

the more resistant pedicle, which is the small stem holding the seed bolls and often problematic in fiber samples (Table 4). Amounts of wax and cutin, while

**Table 5. Cutin and Wax in Twice Enzyme-Retted Fiber and Seed Flax[a]**

| sample | cutin (mg g$^{-1}$) | wax (mg g$^{-1}$) |
|---|---|---|
| mature Ariane ND 99 attached | 5.9 ± 0.7a | 6.4 ± 0.5a |
| mature Ariane ND 99 unattached | 3.7 ± 0.2a | 3.7 ± 0.2b |
| Omega ND 99 attached | 12.8 ± 1.5b | 10.9 ± 1.6c |
| Omega ND 99 unattached | 8.8 ± 2.0c | 8.1 ± 1.4a |

[a] Average ± standard deviation of three replicates. Values within columns with different letters differ, $P \leq 0.05$.

**Table 6. Fiber−Shive Separation after Two Enzyme Rettings**

| | | fiber separation after second retting | | | |
|---|---|---|---|---|---|
| | | test 1[b] | | test 2[c] | |
| sample | first retting[a] | ranking[d] | % fiber separated[e] | ranking[d] | % fiber separated[e] |
| Ariane ME 97 | SER | 4.9 ± 0.4a | 100 | 4.6 ± 0.5a | 100 |
| Ariane NL 95 | DR | 3.6 ± 1.3b | 57 | 2.8 ± 1.5a | 20 |
| Natasja SC 97 | SER | 4.9 ± 0.4a | 100 | 3.6 ± 1.9a | 60 |
| Flanders seed flax | SER | 3.6 ± 1.0b | 57 | 3.6 ± 1.9a | 60 |
| seed flax ND | SER | ND[f] | | 3.6 ± 1.3a | 40 |
| Belinka/Elise Sp 98 | UR | ND | | 5.0 ± 0a | 100 |

[a] SER, spray enzyme retted; DR, dew-retted by commercial methods; UR, unretted. [b] Seven samples per treatment. [c] Five samples per treatment. [d] Shive segment plus attached fibers enzyme-retted with 0.05% SP 249 + 50 mM EDTA, pH 5.0, at 40 °C for 24 h. Scored as follows: 5 = fiber completely removed from shive; 4 = fiber slightly clinging to shive; 3 = fiber attached to shive but partially separated; 2 = fiber frayed at ends but mostly attached; 1 = fiber attached. [e] From scoring in footnote *d*, (no. of fibers in categories 4 and 5/total no. of fibers) × 100. [f] Not determined.

greater overall in seed flax, were still larger in the fibers that resisted enzyme retting compared to enzyme-freed fibers (Table 5).

Analysis of several other fiber and seed flax types that were twice enzyme-retted showed that most fibers separated from shives (Table 6). Detailed evaluation of Ariane ME 97 and ND seed flax after the second enzyme retting indicated only small amounts, that is, 8.3% of Ariane and 4% of seed flax, still had fibers attached to shive, further showing that most fibers were separated from shive fragments after the second enzyme retting. The few fibers that had been still associated with shive after the second retting, but manually removed before analysis, showed no lignin present by histochemistry with acid phloroglucinol or chlorine sulfite staining. However, cuticle was still present with some fibers. No differences occurred in the amount of aromatic constituents between the fibers separated by enzyme retting and those manually separated from attached shive, but wax and cutin contents were greater in the latter samples (Table 7). Amounts of arabinose, as well as rhamnose in Ariane, were greater in manually separated fibers, but levels of other sugars were similar (Table 7). This difference suggests that that pectin- or hemicellulosic-like compounds still held fibers and nonfiber tissues together in these regions.

Sharma et al. (*19*) reported that lignin, as well as noncellulosic polysaccharides, lipids, and certain minerals, influenced variations in fiber quality. McDougall et al. (*20*) indicated the negative effect of lignin on linen quality. Lignin is well-established as a limiting factor in the biodegradation of plants (*21*, *22*), and concern as to its influence on the enzyme retting of flax, especially in mature samples, is warranted. Aromatics are well-known to occur in the middle lamellae of sporadic sites in fiber flax bundles, but most of these compounds exist in the shives (i.e., inner, woody core cells) (*23*, *24*). Our data indicate that the lignin does not appear to prevent fiber−shive separation during enzyme retting, even for more mature, seed flax stems; a high proportion of this component in a fiber sample would likely indicate residual shive left after poor retting. However, other, extractable aromatic compounds have recently been shown to limit or inhibit enzyme activity (*25*), and this influence requires further research. Furthermore, heavily localized areas of aromatics that remain on retted fiber could influence properties, as recently suggested (*24*), or processing efficiency.

Cutin and wax in the fiber fraction also arise from poor retting and appear to be particularly problematic in seed flax and mature plants, where the cuticle often remains with the fiber. Previous studies (*12*) of different commercial grades showed that poorly dew-retted fiber and poorer quality yarns contained greater amounts of these cuticular components. Recent data from Morrison et al. (*26*) showed that cutin is not inherently a part of the fiber constituents but is associated with the cuticle/epidermal structure. Therefore, associating these components with nonfiber trash and, therefore, poor retting seems to be warranted.

However, the cuticle, containing wax and cutin, once breached by enzymes may not necessarily limit enzyme retting but may act as a marker for how well enzymes have separated fiber from nonfiber components. In flax hypocotyls, both acidic polygalacturonans and calcium levels are higher in the epidermal than in cortical regions (*27*, *28*). The anionic sites of the acidic pectins in flax hypocotyls are largely compensated by $Ca^{2+}$, which stabilizes pectins within epidermal tissues (*29*). Furthermore, calcium inhibited tissue disorganization by endopolygalacturonase (*28*, *29*), suggesting that $Ca^{2+}$-linked pectin molecules provide a chemical and physical barrier to pectinase. Our data showing that pectin-type carbohydrates remained with the fibers most resistant to enzyme retting may be significant with regard to this point. The spray enzyme retting method (*11*) attempts to overcome the cuticle/epidermis barrier by crimping to physically disrupt stems and by including calcium chelators to remove $Ca^{2+}$, thereby destablizing pectin molecules and facilitating enzyme retting. However, it

**Table 7. Chemical Compounds Remaining in Flax Enzyme-Retted Two Times[a]**

| | mg g$^{-1}$ | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | carbohydrates | | | | | |
| sample | total aromatics | cutin | wax | rhamnose | arabinose | xylose | mannose | galactose | glucose |
| Ariane ME 97 attached[b] | 0.9 ± 0.7a | 6.4 ± 0.4a | 5.2 ± 0.1ab | 5.0 ± 0.4a | 4.8 ± 0.3a | 7.7 ± 0.4ab | 30.4 ± 1.9a | 31.9 ± 1.3a | 585 ± 33a |
| unattached | 0.8 ± 0.3a | 3.9 ± 1.1b | 3.0 ± 0.8a | 3.3 ± 0.4b | 4.2 ± 0.3b | 7.1 ± 0.5a | 30.1 ± 3.3a | 31.8 ± 3.7a | 599 ± 62a |
| seed flax ND attached[b] | 1.8 ± 0.3a | 13.3 ± 0.8c | 9.6 ± 0.9c | 3.8 ± 0.2b | 5.5 ± 0.1c | 8.4 ± 0.2bc | 29.2 ± 0.6a | 24.2 ± 0.7b | 525 ± 17a |
| unattached | 1.7 ± 0.6a | 10.9 ± 0.8d | 6.9 ± 1.7bc | 4.0 ± 0.4b | 4.4 ± 0.0ab | 9.2 ± 0.1c | 30.9 ± 1.9a | 24.7 ± 1.1b | 557 ± 25a |

[a] Average ± standard deviation of duplicate samples. Values within columns with different letters differ at $P \leq 0.05$. [b] Fibers had remaining shive manually removed before analysis.

is clear that the barrier is formidable, and data presented herein suggest that fiber/cuticle fragments present a particular problem in mature flax grown to seed production.

## CONCLUSIONS

1. Lignin, although present in large amounts in shive but at significantly lower levels in fibers, does not appear structurally to directly limit enzyme retting per se. However, residual lignin on retted fibers could reduce quality. The role of other, more soluble aromatic compounds, which have recently been shown to limit inhibit enzyme activity, and their levels in fiber and seed flax requires further assessment.

2. Cuticular fragments appear to be associated with poorly retted (coarser) fibers and likely present a particular problem for enzyme retting of mature, that is, seed flax, plants. Different enzyme formulations and/or better methods of mechanical pretreatment may improve retting of these samples.

3. Results indicate that seed flax grown in commercial operations in North America, where climate is not conducive to dew retting, could be enzyme-retted to provide fiber for industrial applications, with yields perhaps less than that for fiber types. Therefore, this material, most of which is presently burned, could potentially be enzyme-retted and cleaned to appropriate standards to provide technical grade fibers for various industrial uses.

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