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Biobased Fiber Production: Enzyme Retting for Flax/ Linen Fibers

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Flax (*Linum ustitatissimum* L.) is the source of natural fibers that provides biobased products for a variety of existing markets, but considerable processing and cleaning is required. Flax fibers, and bast fibers generally, are produced in the outer regions of the stem between bark and inner core tissues and require retting, which is the microbial separation of fiber from nonfiber tissues, as the first and most limiting stage of processing. Enzyme retting offers a method to overcome disadvantages of the current method, i.e., dew-retting, for high- and consistent-quality fibers with tailored properties for specific applications. Using chemical analyses, microscopy, and microspectroscopy, sites of carbohydrates, aromatics, and waxes plus cutins were identified in flax stems and their relationship to effective enzyme retting determined. Aromatics occur mostly in the inner, core tissues, with the fibers containing only small amounts located sporadically in cell corners of fiber bundles. Therefore, effective retting using enzymes to separate the aromatic-containing tissues from the fibers, but not to degrade aromatic compounds per se, is required. Waxes and cutin in the epidermal regions are effective barriers to enzyme penetration, and mechanical disruption facilitates enzyme penetration into the stems. Pectinases, with chelators to remove Ca^{++} and destabilize pectin molecules, remove matrix compounds holding fibers within the stem and have been used in effective formulations to ret flax stems.

KEY WORDS: Aromatics; waxes; pectins; retting; fiber.

INTRODUCTION

Flax (*Linum usitatissimum* L.) is the commercial source of both oil seed (i.e., linseed oil and nutritional oils containing 3-omega linolenic acid-rich oil) and of bast fibers for textile linen and, more recently, of biobased fiber composites [6, 7, 8, 18]. Linen has occupied a prominent place in textiles for centuries, and recently linen products have enjoyed a renaissance, especially in blended yarns [6]. Although traditional linen in Europe is constructed with long-line fibers, many industry analysts indicate that the largest use for U.S. textiles will be as short staple fibers blended with cotton or other fibers. Per capita consumption of flax in the United States leads that of other Western countries. Therefore, new methods for processing and characterizing fiber should be considered in light of these factors.

In addition to linen products for textiles, use of biobased fibers in value-added nonwovens and composite products is gaining substantial interest. The potential markets for flax in composites [9] have increased, led by possible value-added products for the automotive industry [18]. Seed flax straw, generally considered coarser than required for textiles, is an option for production of technical-grade fiber for composites [17]. Large amounts of seed flax straw occur as a by-product of the linseed indus-

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Table I. Compositional Analysis of Bast Tissue Manually Separated from Shives

Chemical component (N = 5)	Amount present (mg/g)
Carbohydrate	
Uronic acid	0.19 ± 0.13
Rhamnose	6.98 ± 1.0
Arabinose	11.08 ± 3.4
Xylose	11.96 ± 1.4
Mannose	29.0 ± 7.1
Galactose	24.3 ± 4.2
Glucose	505.1 ± 85.1
Total aromatics	2.4 ± 1.3
Wax plus cutin	14.0 ± 7.9

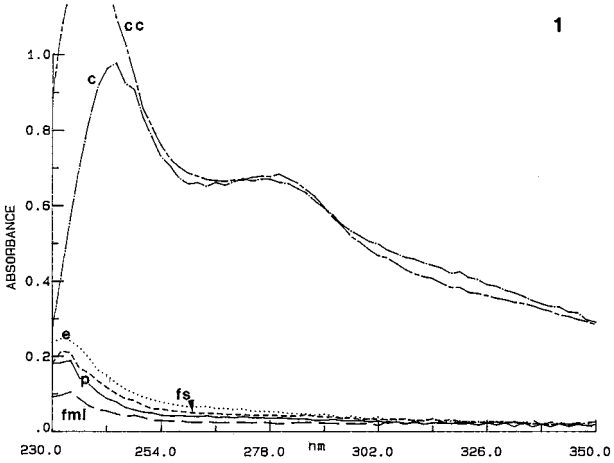

Fig. 1. Ultraviolet absorption microspectrophotometry showing lack of absorbance indicative of aromatic constituents in all tissues except cuticle and some cell corners in the bast fiber bundles. c = cuticle, cc = cell corner, p = parenchyma, e = epidermis, fs = fiber secondary wall, fml = fiber middle lamella.

try, e.g., > 1 million metric tons annually from western Canada [8], and constitute a major environmental problem for disposal, with most of this residue burned. Fiber from seed flax straw has strength and coarseness that make it an ideal choice for biobased fiber composites, and improved processing potentially could improve properties and add value to the product. Therefore, the substantial value from use in composites along with the opportunity to solve an environmental disposal problem has rekindled a reevaluation of the application of seed flax residue for biobased products with a higher value than pulp and paper.

Fibers in the commercially important bast plants, e.g., flax, hemp, kenaf, and ramie, are produced in the outer regions of the stem between the bark and the inner core tissues. The fibers, which exist in groupings of individual fibers as bundles, must be separated from nonfiber tissues for use. This process is called retting and frees the fiber from contaminants and further separates fibers into smaller bundles and single fibers. Retting is the major problem in processing flax [21]. Water retting, which depends on fermentation by anaerobic bacteria and results in good-quality fibers, was formerly used until environmentally unacceptable fermentation waste caused this practice to be discontinued in Western countries several decades ago [23]. Currently, dew retting, which depends on indigenous, aerobic fungi to colonize pulled plants in the fields, is the accepted practice in Western countries and accounts for much of the linen used in textiles, especially high-quality fibers produced in Europe. Dew retting suffers from several disadvantages, including (1) restriction to geographical regions that have the appropriate moisture and temperature ranges for retting, (2) coarser and lower-quality fiber than with water retting, (3) poor consistency in fiber characteristics, and (4) occupation of agricultural fields for several weeks [25]. The heavily contaminated fiber is particularly cited as a problem in U.S. cotton textile mills. Enzymes have been considered for some time as a potential replacement for dew retting flax [25], but costs and other factors have to date prevented commercial development of enzyme retting.

Recently, the Agricultural Research Service of the U.S. Department of Agriculture began a project to explore the reestablishment of a U.S. flax/linen fiber industry for textiles and composites. Toward that goal, we developed

Table II. Histochemistry of Flax Bast Tissue

Histochemical stain	Major component to identify	Rx in tissues			
		Cutin	Epidermis	Parenchyma	Fibers
Ruthenium red	Pectins	+	+	+	+
Acid phloroglucinol	Aromatics	0	0	0	—
Chlorine sulfite	Aromatics	0	0	0	0
Oil red	Wax	+	0	0	0

+, 0, — = positive, negative, in particular cell corners, respectively.

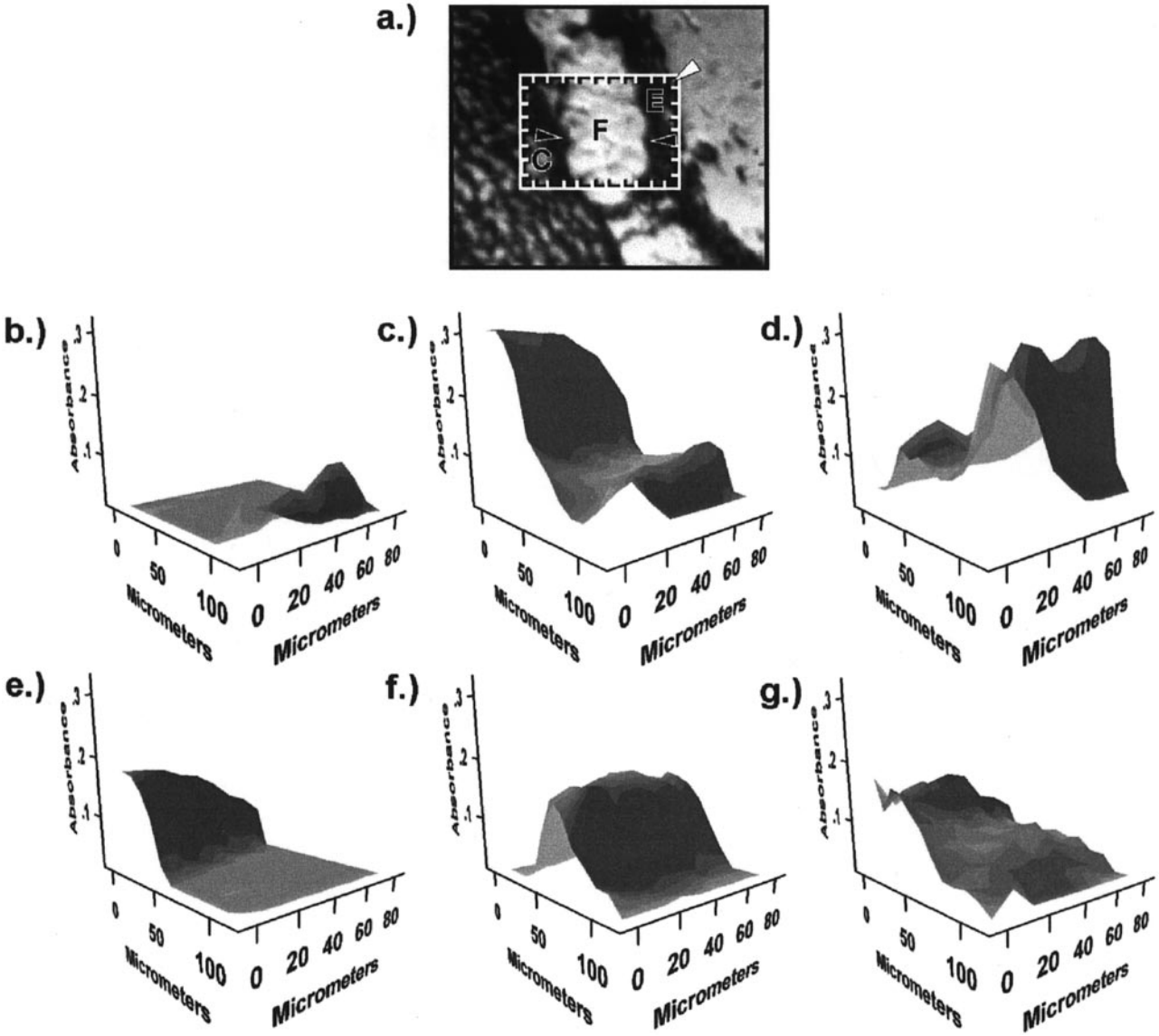

Fig. 2. Mid-infrared maps of flax stem cross section using point-by-point modes for specific wavelengths diagnostic for various chemical constituents. (a) Visible image showing location of the anatomical features for the successive profiles mapped at specific wavelengths. (b) Waxes mapped at 2850 cm^{-1}. (c) Pectic acids/esters mapped at 1740 cm^{-1}. (d) Pectate salts mapped at 1608 cm^{-1}. (e) Aromatics, e.g., lignin, mapped at 1508 cm^{-1}. (f) Cellulose mapped at 1336 cm^{-1}. (g) Acetylated structures, e.g., hemicellulose, mapped at 1250 cm^{-1}.

an enzyme-retting process [4] that has since been tested in small, pilot-plant amounts. Herein, we discuss structural and chemical factors in flax determined by various methods that are pertinent to developing an enzyme-retting method.

CHEMICAL ANALYSIS

Compositional analyses, by gas liquid chromatography as described Morrison *et al.* [20] from a variety of flax sources, indicate types and amounts of components within the bast tissue that had been manually separated from the inner core tissue, i.e., shives (Table I). Glucose amounts were considerably greater than those of carbohydrates representative of pectin and/or hemicellulose. Glucose, as a marker for cellulose, indicates the high level of this structural polysaccharide that predominates in the fiber of this tissue. It is well known that the flax fibers, while high in cellulose, inherently contain sugars other than cellulose [19]. While pectinaceous and hemicellu-

losic sugars are removed during retting, an evaluation of a retted flax sample showed that glucose, mannose, and galactose increased by 50%, 27%, and 8%, respectively, in the residue [2]. Components such as galactan chains and arabinogalactan-proteins occur in secondary walls and have been suggested as important for imparting strength in flax fibers [10]. While carbohydrates predominate in the bast tissue, small levels of aromatics and wax plus cutin also occur [2] (Table I).

HISTOCHEMISTRY

The use of ruthenium red to stain for pectin, acid phloroglucinol and chlorine-sulfite for aromatic constituents, and oil red for waxes in the cuticle provides general information on the location of major components in flax stems (Table II). Ruthenium red, while an indicator—but not specific—for pectins, showed that this constituent was prevalent throughout the bast tissue. Microscopic examination indicated that the pectin occurred primarily in the middle lamella of fibers rather than in the secondary layers. The most intensely stained region using ruthenium red was the cambium layer, which separates bast and core tissues [2]. Lignin, or some form of aromatics identified with acid phloroglucinol, sporadically occurred in cell corners in the fiber bundles. Other work has shown that lignin did not bind fibers together that were subjected to mechanical forces such as those used in cleaning (not shown), therefore suggesting that these aromatics do not play a major role in maintaining fiber bundle integrity. However, heavily localized areas of aromatics could influence fiber properties, as recently suggested [11].

ULTRAVIOLET ABSORPTION MICROSPECTROPHOTOMETRY

Aromatic compounds, such as lignin, can be investigated for their locations within tissues using ultraviolet (UV) absorption microspectrophotometry [1]. UV absorption analysis of thin cross sections of flax indicated that the cuticle and particular cell corners in fiber bundles absorbed UV illumination, with λ_{max} near 280 nm; cell walls of the epidermis and parenchyma and fiber cell walls lacked any evidence of aromatics (Fig. 1). Results showing that aromatics were confined to sporadic sites in cell corners of bast fiber bundles confirmed histochemical observations. Aromatic constituents also were present in the cuticle, as indicated by UV absorption but not indicated by histochemistry in this study, and likely contributed to the total phenolic content reported for bast fiber (Table I).

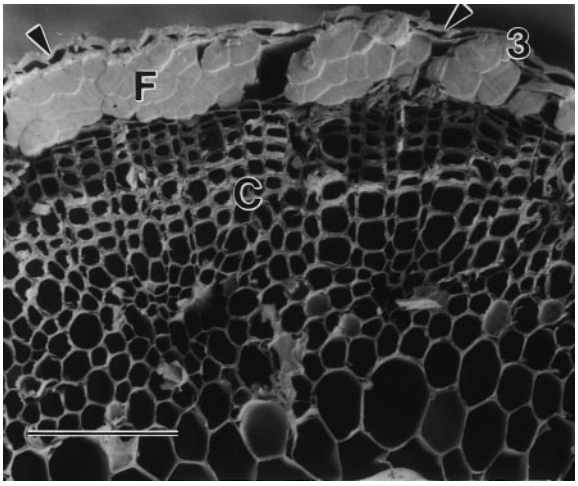

Fig. 3. Scanning electron micrograph of cross section of flax stem showing tissue arrangement with fiber bundles (F) between the cuticle-covered epidermis (arrows) and the inner, lignified core tissues (C). Bar = 100 μm. From Henriksson *et al.* [12].

MICROSPECTROSCOPY

A method in addition to histochemistry and UV aborption microspectrophotometry used to link the chemical and structural features of flax is mid-infrared microspectroscopy. This method has been applied in the point-by-point and mapping modes to flax cross sections. The point mode produces a spectrum at a specific point in the tissue [24]. The mapping mode links individual points, each containing an entire spectrum, as pixels in a matrix. From this matrix, profiles are produced for each component that can be distinguished by a specific functional group [13]. An example of the mapping of a thin cross section of flax is shown in Fig. 2. The visible image (Fig. 2a) reveals the location of the anatomical features, and the successive profiles map the location of waxes (Fig. 2b); pectic acids/esters (Fig. 2c); pectate salts (Fig. 2d); aromatics, e.g., lignin (Fig. 2e); cellulose (Fig. 2f); and the acetylated structures, e.g., hemicellulose (Fig. 2g). Maps of the important constituents in flax bast confirm results from other methods and further provide comparative levels of components within different tissues that could influence retting and fiber properties. This method has been recently used to analyze the influence of these compounds on enzyme-retting of flax [14].

STRUCTURAL AND CHEMICAL INFLUENCES ON ENZYME-RETTING OF FLAX

Flax fibers are located between the epidermis/cuticle barrier and the inner, lignified core (i.e., shive) components of the stem (Fig. 3). Effective retting separates

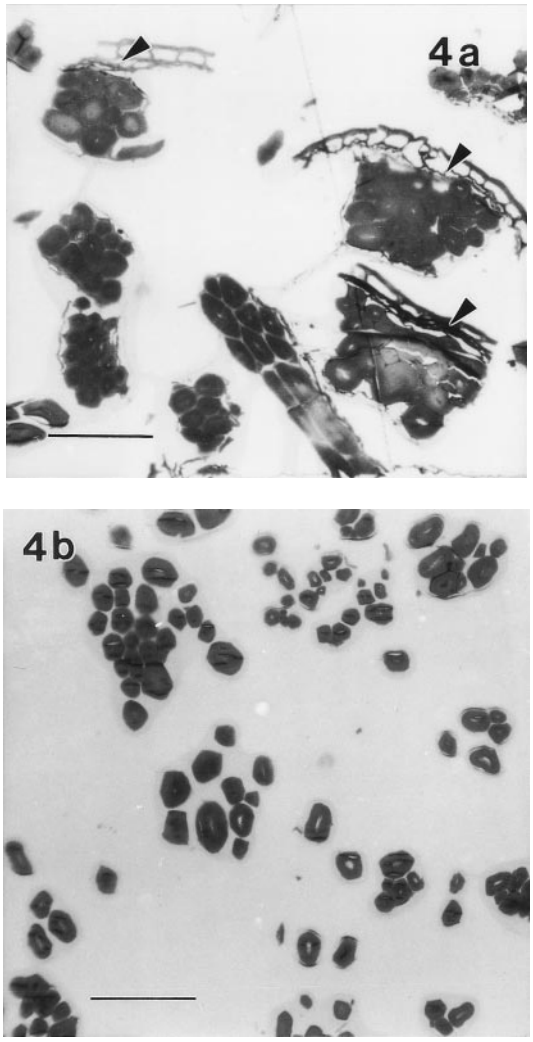

Fig. 4. Light micrographs of flax bast tissue. Bar = 100 μm. (a) Unretted and mechanically treated to disrupt stem integrity, showing fibers mostly in bundles with epidermis/cuticle often attached (arrows). (b) Retted with 0.05% Flaxzyme (commercial product from Novozymes, Franklinton, NC; v/v as supplied) + 50 mM EDTA showing smaller bundles and ultimate fibers. From Akin *et al.* [3].

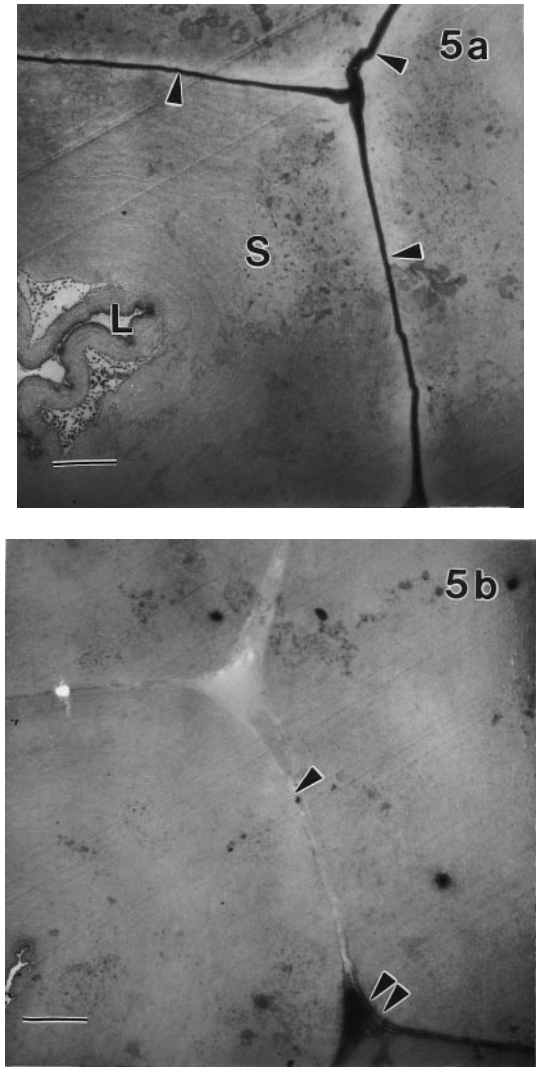

Fig. 5. Transmission electron micrographs of flax stems. Bar = 2 μm. (a) Unretted flax fibers showing cell lumen (L), secondary layer of the cell wall (S), and electron-dense middle lamella (arrows). (b) Retted with 0.05% Flaxzyme + 50 mM EDTA, showing loss of material apparently due to the enzymes in portions of the middle lamella (arrow) with residual material left in the cell corner (double arrows). From Akin *et al.* [3].

fibers from the nonfiber components, resulting in smaller units of bundles and ultimate fibers (Fig. 4a,b). For separation of the fibers, the middle lamella is degraded, freeing ultimate fibers (Fig. 5a,b). An enzyme-retting procedure has been developed [4], and fibers have been produced in pilot-plant amounts and evaluated for quality properties [5]. Pectinase-rich enzyme mixtures effectively retted flax. Lignin did not appear to be a primary limitation in retting, although the presence of aromatics may reduce effectiveness or quality after further processing. The epidermis/cuticle component often appears to be particularly resistant to removal and is contained within the processed fibers. Such a phenomenon occurred in commercially graded high- and low-quality fibers, in which low-quality fibers were observed with high amounts of large fragments of epidermis/cuticle associated with fiber, resulting in a coarser material (Fig. 6a, b); low-quality fibers also had higher levels of waxes, e.g., 1.2 vs 0.7% [20]. In flax hypocotyls, both acidic polygalacturonans and calcium levels are higher in the epidermal than in cortical regions [16, 22]. Mid-infrared microspectroscopic mapping confirmed this result, showing the pectate salts are primarily localized at the parenchyma and epidermal regions (Fig. 2d). The anionic sites of the acidic

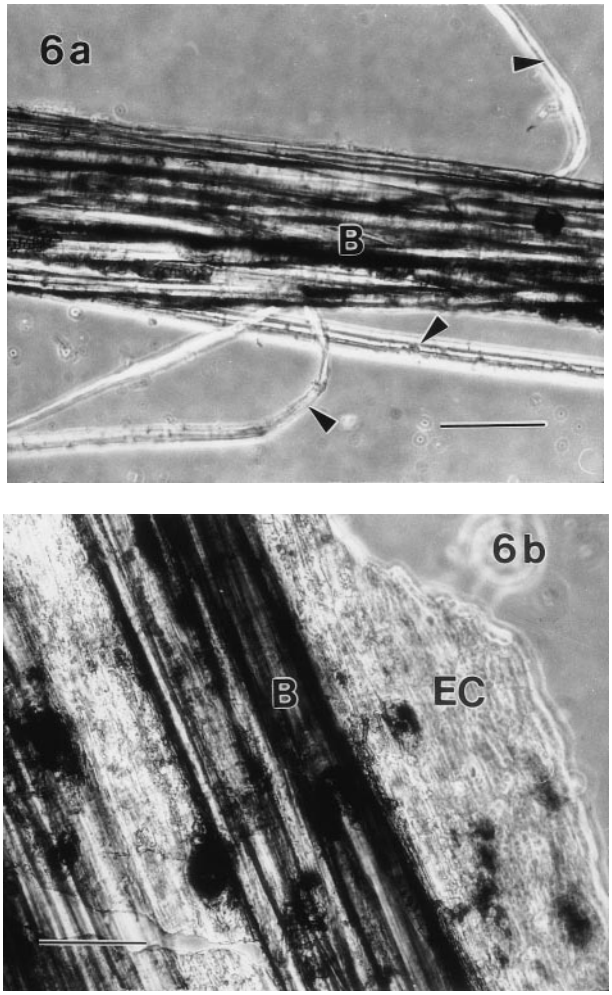

Fig. 6. Light micrographs of commercial samples of flax fiber. Bar = 100 μm. (a) High-quality grade showing bundle (B) with separating fibers (arrows). (b) Low-quality grade showing fiber bundles (B) still attached to epidermis/cuticle (EC), forming a large and poorly retted fragment. From Morrison *et al.* [20].

pectins in flax hypocotyls are largely compensated by Ca^{++}, which stabilizes pectins within epidermal tissues [15]. Further, calcium inhibited tissue disorganization by endopolygalacturonase [15, 25], suggesting that Ca^{++}-linked pectin molecules provide a chemical and physical barrier that limits enzyme-retting with pectinase. Our spray enzyme-retting method [4] attempts to overcome this barrier by physical disruption through crimping the stems and by including calcium chelators (e.g., ethylenediaminetetraacetic acid) that presumably remove Ca^{++}, thereby destabilizing pectin molecules and facilitating enzyme retting. However, it is clear that the cuticle/epidermis barrier is formidable and can produce a major problem in particular samples.

CONCLUSION

Structural and chemical characteristics determine the effectiveness of retting of flax stems. Lignin in the bast tissue does not appear to be a major problem for retting, but aromatics associated with fibers could reduce processing efficiency or reduce fiber quality. The epidermis/cuticle is a formidable barrier to enzyme-retting, preventing the penetration of enzymes into the internal bast tissues. In the new enzyme-retting method, this barrier is mechanically disrupted, and chelators are included with pectinase-rich enzyme mixtures to improve retting. Such an approach is thought to be particularly effective in degrading Ca^{++}-stabilized pectins primarily located in the epidermal regions. Optimization of the retting method should be continued, and further knowledge of the structure and chemistry of the bast tissues will reduce the cost of enzyme formulations and improve specific properties of the fibers.

REFERENCES

1. D. E. Akin and R. D. Hartley (1992) *J. Sci. Food Agric.* **59,** 437–447.
2. D. E. Akin, G. R. Gamble, W. H. Morrison III, L. L. Rigsby, and R. B. Dodd (1996) *J. Sci. Food Agric.* **72,** 155–165.
3. D. E. Akin, L. L. Rigsby, and W. Perkins (1999) *Textile Res. J.* **69,** 747–753.
4. D. E. Akin, R. B. Dodd, W. Perkins, G. Henriksson, and K.-E. L. Eriksson (2000) *Textile Res. J.* **70,** 486–494.
5. D. E. Akin, J. A. Foulk, R. B. Dodd, and D. D. McAlister III (2001) *J. Biotechnol.* **89,** 193–203.
6. V. S. Borland (2000) *America's Textile Industries* **June,** 66–70.
7. J. F. Carter (1993) *Cereal Foods World* **38,** 753–759.
8. K. W. Domier (1997) *Euroflax Newsletter* **2,** 8–10.
9. K. W. Domier (1998) in *Proc. 57th Flax Institute of the United States*, North Dakota State Univ., Fargo, North Dakota, pp. 81–84.
10. R. Girault, F. Bert, C. Rihouey, A. Jauneau, C. Morvan, and M. Jarvis (1997) *Int. J. Biol. Macromolecules* **21,** 179–188.
11. A. Gorshkova, V. V. Salnikov, N. M. Pogodina, S. B. Chemikosova, E. V. Yablokova, A. V. Ulanov, M. V. Ageeva, J. E. G. Van Dam, and V. V. Lozovaya (2000) *Ann. Bot.* **85,** 477–486.
12. G. Henriksson, K.-E. Eriksson, L. Kimmel, and D. E. Akin (1998) *Textile Res. J.* **68,** 942–947.
13. D. S. Himmelsbach, S. Khalili, and D. E. Akin (1998) *Cell. Mol. Biol.* **44,** 99–108.
14. D. S. Himmelsbach, S. Khalili, and D. E. Akin *J. Sci. Food Agric.* (in press).
15. I. His, A. Driouich, and A. Jauneau (1997) *Plant Physiol. Biochem.* **35,** 631–644.
16. A. Jauneau, M. Quentin, and A. Driouich (1997) *Protoplasma* **198,** 9–19.
17. F. Kolla (1997) *Euroflax Newsletter* **2,** 10–11.
18. D. Lepsch and J. W. Horal (1998) in *Proc. 1998 Society for Automotive Engineering International Congress and Exposition*, Society of Automotive Engineers, Iac., Warrendale, Pennsylvania, pp. 87–94.
19. G. J. McDougall (1993) *Carbohyd. Res.* **241,** 227–236.
20. W. H. Morrison III, D. E. Akin, D. S. Himmelsbach, and G. R. Gamble (1999) *J. Sci. Food Agric.* **79,** 3–10.
21. B. E. Pallesen (1996) *Ind. Crops Prod.* **5,** 65–78.

22. C. Rihouey, A. Jauneau, A. Cabin-Flaman, M. Demarty, F. Lefebvre, and C. Morvan (1995) *Plant Physiol. Biochem.* **33,** 497–508.
23. H. S. S. Sharma and C. F. Van Sumere (1992) *Genetic Engin. and Biotechnol.* **12,** 19–23.
24. D. Stewart, G. J. McDougall, A. Baty (1995) *J. Agric. Food Chem.* **43,** 1853–1858.
25. C. F. Van Sumere (1992) in H. S. S. Sharma and C. F. Van Sumere (Eds.), *The Biology and Processing of Flax*, M. Publications, Belfast, Northern Ireland, pp. 157–198.