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Enzymatic Modification of Flaxseed Fibers

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ABSTRACT: Flaxseed (*Linum usitatissimum* L.) fibers were modified by oxidoreductive and cellulolytic enzymes. The lignin amount and intrinsic plant peroxidase activity was evaluated by histochemical and spectrophotometric assays. Peroxidase activity was not found from bast fibers. The flaxseed fibers were further separated and treated with laccase to conjugate the model compounds, that is, the hydrophobic gallate molecules on fiber surfaces. Laccase was able to slowly oxidize fiber-conjugated phenolics, but no fundamental changes in fiber cell surface structure or notable coupling of the applied hydrophobic gallate molecules onto the fibers occurred, as revealed by Fourier transform infrared spectroscopy. The reactivity of the mature fibers was further investigated using cellulolytic enzymes. Cellobiohydrolase (CBH) and endoglucanase (EG)-rich enzyme preparations were applied to reach a hydrolysis degree of 1−6% (of the fiber dry matter) using a standard enzyme dosage. The CBH mixture altered the fiber surface morphology distinctly, and SEM images illustrated fibers in which the cellulose fibrils seemed to be loosened and partially hydrolyzed. In contrast, the effect of the EG-rich preparation without CBH activity was notable on the fiber surface, polishing the surfaces. The cellulolytic treatments were potentially interesting for specific enzymatic modifications of flax fiber surfaces, whereas the approach to use oxidoreductive enzyme treatments on mature linseed fibers offered little potential, obviously due to the low lignin content of the fibers.

KEYWORDS: *flaxseed, fiber, peroxidase, laccase, cellulase, lignin, surface modification*

INTRODUCTION

Flax (*Linum usitatissimum* L.) is a globally important crop, which provides both seeds (linseeds and linseed oil) and fibers (flax fiber) for multiple industrial applications. Flax breeding has resulted in stems with extensively elongated bast fibers, whereas the flaxseed (linseed) cultivars are bred mainly for seed/oil production, with typically rigid stems and shorter bast fibers. Flaxseed could be used as a dual crop, that is, for both seed and technical fiber production, but the attractivity of dual crop fibers for industry has been low due to the rigid characteristics of fibers. The reduced quality of these fibers, especially at the time of harvesting the seeds, has been attributed to the increased deposition of lignin and waxes at seed maturation phase.[1−3] There is a general demand to increase the value of whole plant crops for different sectors, especially those interested in replacing present raw materials with renewables. The utilization potential of the flax bast fibers can be increased with improved processing technologies of the stem fraction.[4] Upgrading of the surface characteristics of flax fiber would create value-added products to be utilized in products requiring strong fibers with moderate length, such as in composites, specialty papers, and nonwoven fabrics.

Various chemical and enzymatic methods to functionalize lignocellulosic fibers or fiber surfaces have been proposed, including targeted functionalization of lignin-containing fibers using oxidative enzymes.[5−8] The chemistry of flax fiber shows unique features with a very high content of crystalline cellulose with tightly bound galactans[9] and a low amount of condensed lignin, the structure of which appears to be highly enriched in guaiacyl units.[3,10] These characteristics make the upgrading of flaxseed stem by enzymatic means challenging. In plant cell walls, lignin is polymerized predominantly by peroxidases.[11] It has been proposed that the low amount of lignin in flax bast fibers is linked with the accumulation of peroxidases into the xylem cell walls by entrapping these enzymes and limiting the lignification in the fiber cell wall.[12,13] Characterization of flax fiber surfaces has revealed that the surface of fibers consists of an abundant coverage of waxes, but no lignin is present.[14,15] However, grafting of phenolic compounds on fiber surfaces of flax by oxidative coupling by enzymes, especially by laccases, has been achieved,[8,16] indicating the presence of reactive phenolic groups on the surface of flax fibers. The principle of the method is based on the formation of radicals on fibers, along with simultaneous radical activation of the compound to be coupled, leading to radical coupling reactions. Thus, the functional compound should, in addition to possessing the desired functional property, also act as a substrate for the oxidative enzyme. The method therefore mimics biosynthetic polymerization of lignin in the plant cell walls.

Traditionally, the fiber fraction of flax stem is separated from the woody core of the stem (shive) and other tissues by the retting process, in which the stems, cut in the field, are partially hydrolyzed, particularly by the enzymatic action of soil microbes.[2,17] Further retting results in even single fibers. The mechanical strength properties of separated fibers would

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inevitably become weaker after a prolonged process, due to prevailing cellulolytic and hemicellulolytic activities.[18,19] However, enzymes acting on the main carbohydrates in fibers can be chosen to obtain targeted modifications in the fibers. Cellulases have been traditionally classified into two major groups: cellobiohydrolases (CBH) and endoglucanases (EG). CBHs act both on crystalline and amorphous areas of cellulose, whereas EGs act on amorphous areas only.[20] Cellobiohydrolases have been observed to increase the outer fibrillation of coarse mechanical fibers.[21] Endoglucanases have been frequently used in the textile and pulp and paper industries to polish fiber surfaces.[22]

In this work, the potential of in planta peroxidases as catalysts to activate and modify the surfaces of mature flaxseed fibers was evaluated. In addition, functionalization of the surfaces of flax fibers by coupling of hydrophobic gallate as model compounds by externally added laccases was further investigated. Modification of fiber surfaces after laccase treatments was analyzed by Fourier transform infrared (FTIR) spectroscopy. Finally, mechanically cleaned bast fibers were treated by various hydrolytic enzymes to record the potential of these biocatalysts for flax fiber modifications.

MATERIALS AND METHODS

Raw Materials. Flaxseed fibers (*L. usitatissimum* L.), cultivar Laser, were obtained from a mature field cultivation in southern Finland in 2009. The raw material was collected, defoliated, and kept at 22 °C until used for histochemical analyses. Mechanically purified fiber material from cultivar Laser collected in the year 2005 was used for chemical analyses, short-term retting, and hydrophobization experiments.

Chemical Composition of the Fibers. The bast fiber material was homogenized with an IKA A10 basic analytical grinder mill (IKA-Werke GmbH & Co. KG, Staufen, Germany) to a maximum particle size of 1 mm. Prior to analysis, the dried and milled samples were extracted with acetone in an automatic Soxtec 2050 extractor (FOSS Analytical, Hilleroed, Denmark) for 0.5 h. Lignin and carbohydrates were analyzed according to National Renewable Energy Laboratory Laboratory Analytical Procedure (NREL LAP) methods (Determination of Structural Carbohydrates and Lignin). The amount of carbohydrates formed after acid hydrolysis was determined as reducing sugars by using the dinitrosalicylic acid method[23] at 540 nm. Reducing sugars were analyzed as three replicates of the samples and standard errors calculated. One of the replicates (the middle one) was chosen for further monosaccharide analyses. The monosaccharides were analyzed by high-performance anion exchange chromatography with pulsed amperometric detection as described.[24]

Enzymes and Enzyme Activity Measurements. Laccase from *Thielavia arenaria* (TaLcc1), heterologously produced in *Trichoderma reesei*, an endoglucanase-rich preparation (Biotouch C800), and a cellobiohydrolase CBH I (CB10096A3)-rich preparation, originating from *Acremonium thermophilum*, were all obtained from Roal Ltd. (Rajamäki, Finland). Pectinase from *Aspergillus aculeatus* was obtained from Sigma-Aldrich (Brøndby, Denmark). Pectinase activity was recorded as described,[25] using 0.4% polygalacturonic acid (Megazyme International, Wicklow, Ireland) as substrate.

To determine the peroxidase activity of the flax fibers, the fibers were rinsed with water, air-dried prior to extraction, and powdered by an IKA A 11 Basic mill (Kinematica, Staufen, Germany). The powdered fibers were supplemented with 30% (w/w) polyclar AT (Serva, Heidelberg, Germany), in ice-cold 50 mM sodium acetate buffer, pH 5.0, containing 1 M NaCl, one complete protease inhibitor tablet per 100 mL (Roche Diagnostics), and 10 mM sodium borate.[26] Samples were extracted for 45 min on ice, after which they were centrifuged. The supernatant was used for the activity measurements. Peroxidase activity from flax fibers was determined at pH 5.4 using guaiacol as substrate.[27] Laccase activity in the samples was analyzed by measuring the change in absorbance prior to hydrogen peroxide addition. The presence of peroxidase activity was also detected from thin tissue sections.

The laccase activity was determined by 2,6-dimethoxyphenol as described.[28] Oxidation of fibers by laccase was followed by oxygen consumption measurements, performed in duplicate. In the method, the reaction chamber was filled with 50 mg of finely cut bast fibers in 1.5 mL of sodium citrate buffer, pH 5.0. The chamber was closed with a tight-fitting plunger, and laccase (10 nkat/mg fiber) was added via a small capillary hole in the plunger with a Hamilton syringe. The consumption of oxygen (nmol/L) was followed during an incubation period of 6 h at 23 °C. The rate of O_2 consumption was taken from the linear portions of Oxygraph recordings and was calculated using Oxygraph software (Hansatech Instruments Oxygraph, King's Lynn, UK).

Enzymatic Treatments. The pectinase treatment was applied to plastic vessels containing 0.1% (w/v) NaCl and 400 mg of bast fibers at a consistency of 0.3%. Fibers were incubated in water bath at 50 °C for 2 h. After the treatment, the solid fibers were thoroughly washed with water and air-dried.

The cellulolytic treatments were carried out in 2 L flasks (working volume of 50 mL) in 50 mM sodium citrate buffer, pH 5.0, with a fiber consistency of 4%. The treatment time was 2 h and temperature, 45 °C. Enzyme loadings were based on total protein content, measured according to the Lowry method.[29] Preliminary determination of the degree of hydrolysis of flax fibers was based on the amount of sugars released in the treatment liquid, analyzed as reducing compounds with the dinitrosalicylic acid reagent, using glucose as standard.[23] The degree of hydrolysis obtained per dry matter of flax fiber was used to calculate the loading of enzymes to obtain a particular degree of hydrolysis. After hydrolysis, the substrates were boiled for 2 min to inactivate the enzymes. Finally, the fibers were filtered and washed with distilled water.

Hydrophobization of Fibers. Gallic acid dodecyl alcohol ester (dodecyl gallate) or gallic acid octyl alcohol ester (octyl gallate) was used as functional compound. The hydrophobic compounds were used in dispersion or solubilized in 40% ethanol. Dispersions with gallates were produced at 5% consistency.[30] Bast fibers were oxidized by TaLcc1 laccase in 0.5% consistency in 0.1 M citrate−0.2 M phosphate buffer at pH 4.5 for 30 min at room temperature using a laccase dosage of 100, 1000, or 10000 nkat/g dry fiber. The reference treatments were carried out under similar conditions but without the addition of laccase. After activation of the fibers, gallate was added to the reaction, either as dispersion or as solvent-solubilized form, and the reaction was continued for 60 min. After the reaction, the mixture was washed with acetone (2 × 10 min).

Light Microscopy. Stems were frozen at −20 °C before sectioning with a Leica CM 3050 S cryo-microtome (Leica Microsystems GmbH, Wetzlar, Germany). Fresh 20 μm thick slices were stained according to standard procedures.[31] Alcian blue and safranin were used for detection of cellulose and lignified cells, respectively. The presence of peroxidase activity was detected from the thin tissue sections by 0.03% 3,3′-diaminobenzidine (Sigma-Aldrich) and 10 mM H_2O_2 in 50 mM sodium acetate buffer, pH 5.0, containing 4 mM $CaCl_2$ and 10 mM $Na_2B_4O_7$. A Leica DMLB 2500 stereomicroscope (Leica Microsystems GmbH) was used for measurement of fiber length and thickness.

FTIR Spectroscopy. FTIR spectra of fibers were obtained with a Bruker Optics Tensor 27 spectrometer (Ettlingen, Germany) equipped with an attenuated total reflectance diamond crystal. Fiber material was placed directly onto the diamond crystal, and infrared spectra were recorded between 400 and 4000 cm^{-1} at a resolution of 4 cm^{-1}. Sixty-four interferograms were added to obtain a high signal-to-noise ratio spectrum. The spectra were baseline corrected for further analysis.

Other Analyses. The color of the bast fibers was determined as CIELAB lightness values (L^*) using a Chromameter CR-210 equipped with a CIE illuminant C (Minolta, Japan) with the light source adjustment D_{65}. The standard viewing observer angle 10° was used during the measurement. The enzyme-treated fiber surfaces were

analyzed by scanning electron microscope JSM-840A (JEOL Ltd., Tokyo, Japan). Before the analysis, the samples were coated with a thin gold layer using a sputter to avoid sample charging under the electron beam.

RESULTS AND DISCUSSION

Histochemical Staining of Lignin and Peroxidases. Staining of flax stem cuttings by safranin (Figure 1A) indicated

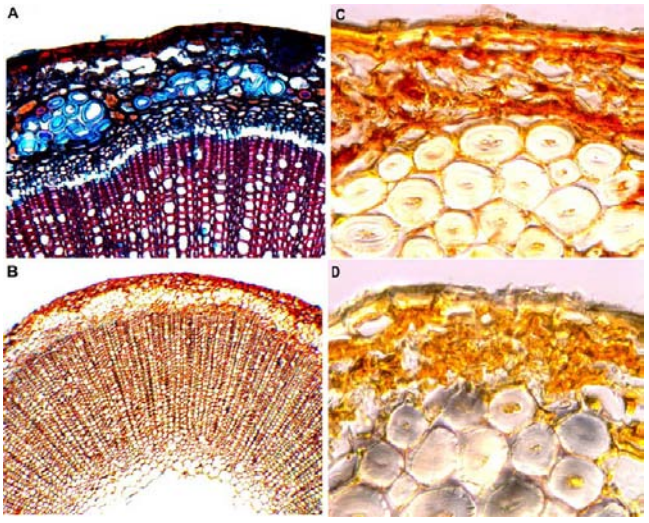

Figure 1. (A) Flax stem visualized by light microscopy after safranin–alcian blue staining. Nonlignified cellulosic fiber bundles in the phloem are clearly visible as blue, and lignified cortex and inner stem parts with lignified tracheids are stained red with safranin. (B) Stem cutting stained with diaminobenzidine and hydrogen peroxide to reveal peroxidase activity as brown precipitate. (C) Same as (B) with high magnification. Fiber bundles are not stained. Note pale yellow background in the control stem without DAB addition (D).

localization of stem lignin on the cortex of the stem, in agreement with chemical analysis of flax shives, which contain a significant amount of lignin, about 26% of the dry matter.[32] This cortex part was intensely labeled with oxidation products of 3,3′-diaminobenzidine (Figure 1B,C), indicating strong peroxidase activity and potential occurrence of lignin due to peroxidase activity in the tissue . In contrast, phloem fibers were completely devoid of 3,3′-diaminobenzidine staining (Figure 1C,D) and, thus, no peroxidase activity could be observed. Peroxidase activity from buffer-extracted fibers was also measured, but no activity could be detected (data not shown). This result is in accordance with the histochemical staining for lignin and also with the very low content or absence of lignin in bast fiber tissues. The negative 3,3′-diaminobenzidine-staining in flax bast fibers was clearly different from results obtained with the xylem tracheids of spruce (*Picea abies* L.), for example, where strong 3,3′-diaminobenzidine-staining in tissue sections and peroxidase activity in tissue extracts were observed even after long-term storage of the wood material.[31] Lignin is initially formed in the middle lamellae and primary cell walls, including xylem vessel elements and phloem fibers, as has been clearly visualized in many plant systems, for example, in spruce.[33] We could, however, not reveal lignin or polymerizing peroxidases in flax fibers using a similar methodology as used for spruce (Figure 1). The result is corroborated by earlier findings of the low lignin content in bast fibers.[3,34] In the xylem, lignification appears to proceed via enzymes present in the cell wall,[1,35] and peroxidases may even be cross-linked to the cell wall through tyrosine–lignin bonds.[36] It can be assumed that the in-flax-fiber peroxidases were not present or were inactive in the mature fibers, in contrast to spruce tracheal elements. Our data from the analysis of the acid-insoluble portion of the biomass indicated the presence of lignin also within the bast fibers of flax (Table 1). The clearly detectable amount of lignin

Table 1. Physical and Chemical Parameters of Flax Fibers

parameter	reference value[48]	Laser 2005 (this study)
length (mm)	4–77	30–40
diameter (μm)	5–76	15 ± 4
cellulose (%)	64	57
hemicellulose (%)	15	16
pectin (%)	2	2
lignin (%)	2	7
extractives (%)	not reported	1.1
microfibrillar angle (deg)	5	nd
degree of crystalinity (%)	91	nd
tensile strength (MPa)	345–1100	nd
S/G ratio	0.3–0.5	nd

in the fiber fraction (7%) probably overestimates the lignin content in these fibers. It cannot be excluded that a small portion of lignin-rich shives may have been present as contaminants in the analyzed fiber fraction, explaining the results.[34] The gravimetric method for determining acid-insoluble lignin residue is not a specific method for lignin, as the residue may contain proteins or other acid-insoluble cell wall materials.[17,37] Specific analytical methods for investigations of fiber surfaces, such as X-ray photoelectron spectroscopy and time-of-flight secondary ion mass spectrometry, have revealed neither lignin nor other aromatics on the surface of flax fibers.[14,15]

Laccase-Aided Functionalization of Fibers by Gallates. Laccase, applied as a low dose (10 nkat/mg fiber), clearly oxidized the acetone-washed bast fibers, as revealed by the consumption of oxygen in the bast fiber suspension (Figure 2). The increased consumption of oxygen in the presence of laccase indicated that flax fiber material contains aromatic hydroxyls, possibly present in lignin or in other aromatic phenolic compounds, such as ferulic and sinapic acids,[37] that can be oxidized by laccases. Slow consumption of oxygen was also detected even without the addition of laccase. The

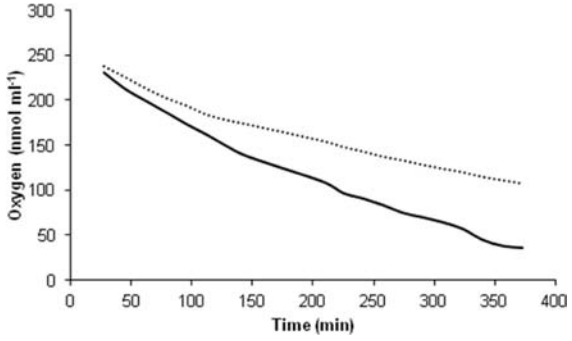

Figure 2. Oxidation of flaxseed fibers by TaLcc1 laccase (solid line) by measuring the consumption of dissolved oxygen. The concentration of fibers was 3%, and the amount of laccase used was 10 nkat/mg fiber. The reference treatment of flaxseed fibers without laccase is shown (dashed line).

functionalization experiments of flax fibers were conducted using the hydrophobic gallate compounds, dodecyl gallate and octyl gallate, as model molecules, and by using laccase, using activities of either 100 nkat (low dosage), 1000 nkat, or 10000 nkat (high dosage) per milligram of fiber in 50% ethanol. Only minor modifications in flax fibers were observed, as depicted by the FTIR spectra (Figure 3).

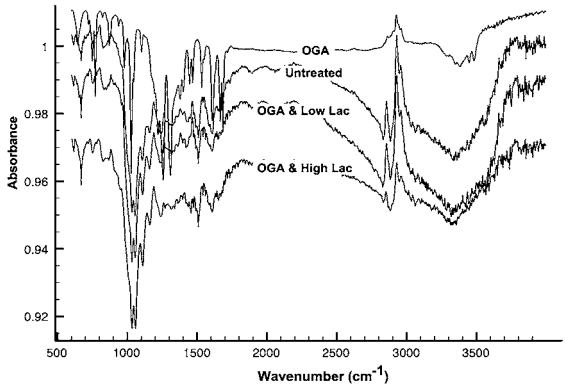

Figure 3. FTIR spectra of octyl gallate (denoted OGA) and flax fibers treated with octyl gallate and with two dosages of laccase (low, 100 nkat/g fiber; high, 10000 nkat/g fiber).

FTIR spectroscopy is a powerful technique to reveal changes in hydroxyl, carbonyl, and ether vibrations in polymers. Minor alterations, such as those in the region 1420−1500 cm^{-1} that showed lower vibration in treated fibers with the high laccase dose, had however occurred (Figure 3). This region was unaltered in the treatment with a low amount of laccase. The absorbance bands at 1464 and 1376 cm^{-1} are assigned to $-CH_3$ asymmetric and C−H symmetric deformations of lignin, respectively.[38] These bands were weakened in fiber treatments with a high laccase dose, indicating that laccase, when used at high dosages, may have removed methyl groups from aromatic phenolics during radicalization reactions,[39,40] however, without expected coupling reactions with the supplied gallate molecules. The assignment of the spectrum of the untreated fiber revealed a typical banding pattern, with strong bands in the region 2800−2920 cm^{-1}, arising from CH stretching in aliphatic methyl and methylene groups.[41] The same region is seen also in pure octyl gallate with a long aliphatic fatty acid chain, and most probably this banding in fibers is a result of fatty acids remaining in fibers even after acetone wash. The spectroscopic region below 1700 cm^{-1} forms a complex banding pattern and is contributed by various vibration modes, both from aromatic skeleton vibrations and also from carbohydrate originating vibrations at 1000−1300 cm^{-1}. The band at 1510 cm^{-1} has been assigned to condensed aromatics,[42] which absorbs with equal intensity in all flax fibers, regardless of the treatment conditions (Figure 3). In summary, the FTIR spectra of fibers, treated with laccase alone or supplemented with octyl or dodecyl gallate (spectra of octyl gallate treatments as a representative gallate shown in Figure 3) were very similar, suggesting that no notable coupling of octyl or dodecyl gallate to the fibers had occurred, leading to fundamental changes in fiber cell surface structure. This is expected due to the low amount and/or poor accessibility of aromatic compounds on the fiber surfaces. It remains to be shown if flax fibers may be modified by compounds with stable, covalent linkages, to increase the hydrophobicity and thus their compatibility with, for example, composite materials. Moreover, condensed structures might have hindered the action of the low redox type TaLcc1 laccase to fully convert lignin moieties onto radicals to react with the gallate. Lignin in flax bast fibers is predominantly polymerized as highly condensed structures, for which a low syringyl/guaiacyl lignin ratio and a relatively high H unit content are typical.[3]

Previously, it has been shown that laccases retain fairly well their activity in ethanol solutions.[43] Solvent or dispersion systems are needed to improve the enzymatic oxidation of poorly soluble substrates. For comparison, coupling studies by the dispersion method were also used. No apparent coupling of gallate compounds and even less modification of laccase-activated fibers with gallates by FTIR were, however, observed with the dispersion method (data not shown). The laccase-catalyzed method of functionalizing lignocellulosic fibers has been successfully used in the hydrophobization of thermo-mechanical pulp fibers[44] and eucalyptus pulp fibers for sizing applications with a reducing of the water absorption rate of the pulp,[45] as well as in the treatment of flax alkali pulps, when gallate was used as a mediator compound for laccase to reduce the hexenuronic acid content of the pulp.[46] Interestingly, the alkali-modified lignin moiety present in flax soda-anthraquinone pulp was not reactive with the laccase of *Trametes villosa*, whereas hexenuronic acid was.[46] In this study, the laccase TaLcc1 efficiently oxidized different gallate compounds, resulting in oxidized brownish products, which were further able to polymerize and were precipitated in the solution, thus further limiting the reactivity of gallate with the radicalized fiber (data not shown). Grafting of phenolic compounds to flax by laccase has been obtained by very low laccase dosage during a long reaction time.[16] As laccases tolerate well high organic solvent concentrations,[43] the use of organic solvents with low toxicity serves as an attractive way to improve the technique for efficient functionalization of bast fibers when using water-insoluble molecules, such as gallates.

Treatments of Fibers by Hydrolytic Enzymes. The flaxseed fibers were further treated by various hydrolytic enzymes to obtain potentially interesting, tailored modifications on the fiber surfaces. The treatment of fibers with a cellobiohydrolase-rich preparation (CBH) at a moderate dosage of 4 mg protein/g fiber substrate resulted in efficient release of sugars, whereas the endoglucanase-rich (EG) enzyme mixture was only poorly able to liberate sugars from the flax fibers (Table 2). This result was in accordance with expectations.[47] The CBH synergistically with some background endoglucanases activities is able to hydrolyze not only the amorphous regions but also crystalline parts of cellulose (Table 1), that is, both abundant forms of cellulose in the bast fibers of

Table 2. Effects of Cellobiohydrolase, Endoglucanase, and Pectinase Enzyme Preparations on the Degree of Hydrolysis of Flax Fibers, Measured as the Amount of Released Reducing Sugars after 2 h at 50 °C

	protein	degree of hydrolysis (% of dm)	CIELAB L^* value[a]
CBH	4	5.7	65 ± 2
EG	4	0.8	66 ± 2
pectinase	4	1.8	66 ± 1
reference		0	55 ± 2

[a]The color of the fibers was measured by Cielab color parameters. The L^* value for white is 100.

flax.[48] On the other hand, the action of EG at the same protein dosage was, without synergistic contribution of significant CBH activities, restricted to the amorphous parts of cellulose chains. The EG treatment, however, seemed to result in an obvious cleaning effect of the fiber surfaces (Figure 4B), and no visual damage, such as breaking of fibers, could be observed at the degree of hydrolysis of 3%.

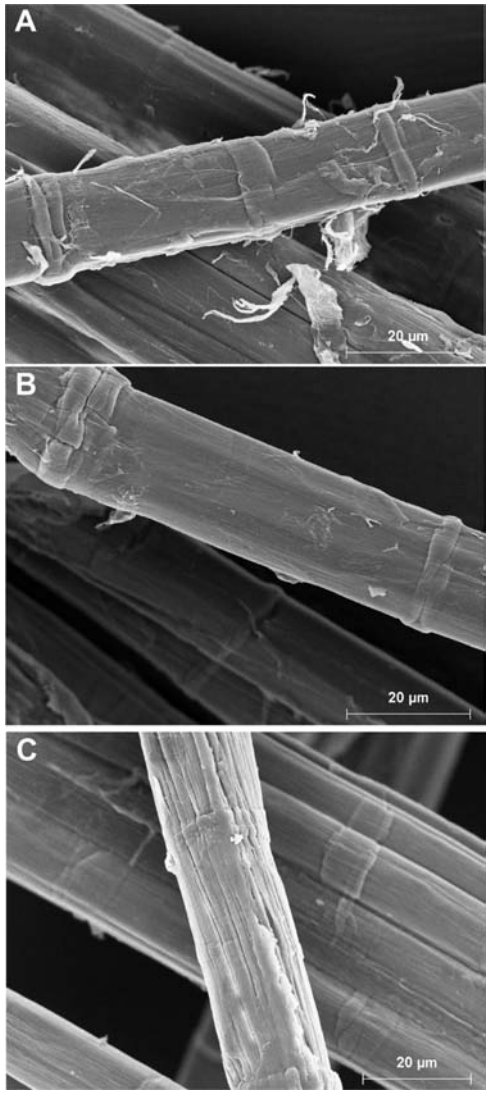

Figure 4. Scanning electron micrographs of bast fibers after treatment with cellulolytic enzymes: (A) reference fiber without hydrolytic treatment; (B) treatment with EG, degree of hydrolysis 3.7% of fiber DM; (C) treatment with CBH, degree of hydrolysis 4% of fiber DM.

Various hydrolases have been used to decorticate the stem, separate the fibers from the surrounding tissue of the stem, and defibrillate the fiber bundles.[2,18,19] The compositional study of flax fibers indicated the recalcitrant structure of flax fibers, even for chemical treatments,[47] possibly influenced by the steric hindrance or covalent linkages between pectin and cellulose.[42] The action of the CBH-rich enzyme preparation hydrolyzed efficiently the surface of fibers (Figure 4C). Cellulose fibers running along the longitudinal axis, reflecting the low microfibrillar angle typical for bast fibers (Table 1), became clearly visible. The highest degree of hydrolysis obtained was 5.7%, shown in Figure 4C, and the treatment could further be optimized for the treatment parameters. Thus, the CBH treatment for fiber applications retaining pectic material in fibers warrants further studies.

Pectins in flax fibers may contribute to some of the physical and structural properties important for their industrial use and even affect the strength properties of fibers.[49] The pectinase preparation used consisted mainly of polygalacturonase, but contained minor amounts of side activities, such as xylanase and endoglucanase.[24] Pectinase was able to release moderate amounts of sugars but, contrary to our expectations and the reported effect of pectinase activity on flax,[2,50] no marked changes in fiber bundle arrangement, such as defibrillation, could be recorded at a degree of hydrolysis of 1.7%, and the fibers remained rather coarse, stiff, and harsh (data not shown). The measured CIELAB L^* values were slightly improved by all enzyme treatments, indicating removal of colored components on the fiber surface (Figure 1). We assume that, although pectinolytic activity may contribute to dew-retting on the field,[50] other additional factors, such as the presence of suitable Ca^{2+} ion chelating compounds, may significantly contribute to retting. Especially, modification of fiber surfaces can be accomplished in a short-term process with other reaction mechanisms, such as specific hydrolytic and oxidoreductive reactions.

In this investigation, the enzymatic modification of flax bast fibers was studied by various means with the aim to exploit in planta peroxidases or added laccases for functionalization of fiber surfaces or to use hydrolytic enzymes for specific modification of fibers. Although peroxidase activity most probably is involved in the developing stems of flax, mature fibers did not possess peroxidase activity. Thus, the proposition for the utilization of in planta peroxidases in functionalization of the mature fiber material appeared to be unsuccessful. Composition of flax fiber cell walls is distinct from many other lignocellulosic materials, and typical techniques to efficiently functionalize fiber surfaces may offer only limited possibilities on bast fibers, due to the low reactivity of fibers for laccase-mediated oxidation. CBH-rich preparations showed promising options for fiber surface modification. The EG treatment seemed to result in an obvious cleaning effect of the fiber surfaces, and no visual damage, such as breaking of fibers, could be observed when the degree of hydrolysis was kept low. Both EG- and CBH-type cellulolytic enzymes may be potentially interesting enzymes for modifications of flax fiber surfaces in applications, such as in composites, where their positive effect on composite performance may be facilitated.

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS USED

FTIR, Fourier transform infrared; CBH, cellobiohydrolase; EG, endoglucanase; TaLCC1, *Thielavia arenaria* laccase 1.

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