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Structure and Chemical Composition of Bast Fibers Isolated from Developing Hemp Stem

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Microscopic and chemical changes of hemp bast fibers were studied during the maturation from vegetative to grain maturity stages at both apical and basal regions of the stems. The content of protein was the main factor related to fiber maturation, whereas increased proportions of mannose and glucose and decreasing levels of galactose were also highly significant. Enhanced glucose deposition in apical fibers could be related to the gradual thickening of the fibers, whereas in basal regions the thickness of the fibers nearly reached the maximum at vegetative stages. In contrast, the extent of lignification remained close to 3−4% during plant growth. Hemp fiber lignins were rich in guaiacyl units and would be rather condensed in nature. In addition, the proportion of *p*-hydroxyphenyl units displayed a constant decline during maturation. A progressive chemical fractionation of hemp fibers provided further insights to the occurrence and nature of noncellulosic polysaccharides. Notably, these data pointed out that maturation is accompanied by a significant increase in water- and alkali-soluble components containing glucose- and mannose-related polymers and a decrease in arabinose and galactose components disrupted by diluted hydrochloric acid. Taken together, chemical features of the noncellulosic components suggest that the architecture of hemp fibers differs slightly from that of the more widely studied flax fibers.

KEYWORDS: Hemp; *Cannabis sativa* L.; maturation; cell wall; lignin; glucomannans

INTRODUCTION

Bast fibers are cellulose-rich cells that display high mechanical properties. Apart from their main use in textiles (flax) and paper (hemp), these fibers could offer relevant properties in the scope of new composite materials (*1*−*3*). Bast fibers encircle the core xylem and are so-called extraxylary fibers. Generally speaking, the fibers used for industrial purposes originate from procambium and correspond to sclerenchyma primary cells. In addition, secondary fibers, although in very low proportion, are also generated from cambium in hemp as in other fiber-rich plants. In the plant stem, individual fibers (single fibers) are intimately associated through their middle lamella to form fiber bundles situated parallel to the longitudinal axis of the stem and provided with mechanical support to the plant (*4*). Their morphological features highly contrast with those of xylem fiber (*5*). At maturity, primary fibers reach many millimeters in length (up to 100 mm) and 15−25 μm in diameter. The secondary fibers are generally shorter (∼2 mm long) and thinner than primary fibers (*6*). As in flax, hemp fibers are characterized by thick secondary cell walls that almost fill the lumen at maturity.

In bast fiber plants such as hemp and flax, the chemical structure and spatial organization of the cell wall polymers are quite distinctive from those encountered in xylem fibers. Notably, they contain a considerable amount of cellulose and few noncellulosic polysaccharides, proteins, and lignins (*6*−*9*). Their thick secondary walls are characterized by highly crystalline cellulose, the microfibrils of which deposit almost parallel to the main cell axis (*10*−*12*). Flax fibers contain 5−15% noncellulosic polysaccharide, with galactose being the predominant sugar (*8*). The main encrusting components of the cell walls are described as β-1-4-galactan together with rhamnogalactans. Galacto-glucomannans, xyloglucans, and xylans have also been identified as hemicelluloses of the fiber walls in addition to proteins (*13*−*15*). In contrast to flax fibers, little information is available on the chemical composition of hemp bast fibers. At grain maturity stage, the hemp bark was shown to contain 4% pectins, 4% hemicellulose, 2% lignin, and 75% cellulose. The cellulose content was shown to increase continuously with the age of the plant, whereas, with the onset of flowering, the proportion of hemicellulose starts to decrease in contrast to that of lignin (*16*, *17*). The structure of hemp cellulose was recently reported as intermediate between the highly crystalline cellulose of flax and the semicrystalline one in kenaf (*18*). Regarding pectins, high molecular weight rhamnogalacturonans of type I have been identified, but lignin structure and distribution, as well as the effect of maturation upon the cell wall network, still need further investigation (*19*).

The present investigation aimed at providing some insights to the cell walls of extraxylary fiber during the maturation of

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hemp. To reach this goal, the bast fibers of developing hemp were obtained from apical and basal regions of the stem at defined maturity stages of the plant from the vegetative stage to maturity of the grain. Light microscopy was used to estimate the thickening of the cell wall and to visualize the extent of lignin deposition. In addition to the overall composition of the hemp fiber as polysaccharides, lignin, and proteins, possible changes of the wall network during the maturation were assessed by means of a chemical fractionation of the cell walls.

MATERIALS AND METHODS

Plant Material. The monoecious hemp (*Cannabis sativa*) variety (Fedora 17) was sown on April 19, 2002, at 50 kg/ha and grown under field conditions at Bar sur Aube (France) by professional hemp producers (Institut Technique du Chanvre, Bar sur Aube). Hemp, which consists mostly in female plants, was harvested at four developmental stages: (1) vegetative; (2) flower formation; (3) "full" flowering, when 50% of the plants had open flowers; (4) grain maturity. On the basis of the definition of the growth stages of hemp given by Medivilla et al. (*20*), these stages corresponded to 1012, 2300, 2302, and 3001 decimal codes, respectively. At vegetative stage (stage 1), the whole plants used for all of the harvests were labeled in two field areas (2 × 120 plants). Temperature was measured every hour from sowing to the complete maturity of the grain. Daily mean values were expressed as degrees Celsius per 24 h (°C/day).

At each of the four selected development stages, the height of the stems within the two field areas was measured (30 samples for each area). Basal (10 cm length) and apical (15 cm length) regions were collected from the stem at 5 cm above the ground level and at 15 cm from the apex, respectively. Two sample groups were obtained in relation to the field areas. The bast fiber-rich strips were peeled from the fresh stem, and then the epidermis was scrapped away using a razor blade and checked microscopically. Bundles obtained from the two field areas were further divided in three groups as replicates for chemical analysis.

Microscopic Observations and Cytochemistry. Fresh transverse sections were cut from the bottom extremity of apical and basal regions obtained from three hemp stems using a cryomicrotome. Cytochemical staining of lignin was achieved on 30 and 60 μm sections, respectively, using the Wiesner reagent (phloroglucinol−HCl) (*21*) that stains red the cinnamaldehyde moieties or the Mäule reagent (*22*) that gives a purple-red coloration with syringyl lignin type. Autofluorescence of cell walls under UV exposure was also examined using a 50 W halogen lamp and a filter combination (340 nm excitation wavelength and 430 nm emission light).

Fresh transverse sections were also cut from the bast fiber bundles that were isolated for chemical analysis (three bundles sampled from three stems) to estimate the proportion of secondary fibers based on surface determination in transverse sections. The wall proportion in primary fibers was obtained by measuring the total and lumen surfaces of single cell (15 measurements using 5 cellules within 3 bundles). The wall thickness of the primary fibers was obtained as the average of 3 measurements on 5 cells within 3 fiber bundles of each sample of bast fibers, which in total meant 45 measurements.

All observations and measurements were performed using an Axioskop microscope (Zeiss) plus aAxioCam MRc digital camera and corresponding image analysis software.

Cell Wall Isolation. Fiber-rich material was freeze-dried and ground with a ball crusher (Retsch, MM 2000). The dried material was extracted with 80% ethanol (4 × 5 h, 100 mL/g) at room temperature and then with water at 30 °C (4 × 20 min, 100 mL/g). The final residues were collected by filtration and designated cell wall residues (CWRs).

Carbohydrate Analysis. Acid hydrolysis of fiber CWR was performed on 5 mg samples using 12 M H_2SO_4 (125 μL, 2 h at room temperature) and then 1 M H_2SO_4 (1.625 mL) for 1 h at 120 °C (*23*). These conditions allowed for a more complete hydrolysis of the fiber polysaccharides than methods using sulfuric acid at 100 °C for 2 h (data not shown). Soluble fractions (1 mL) recovered from chemical fractionation were hydrolyzed for 2 h at 100 °C after the addition of 2 M H_2SO_4 (1 mL). Analysis of solid samples and soluble fractions was performed in triplicate and duplicate, respectively; the variation in the analytical measurement was 5%.

Neutral monosaccharides were determined as their alditol acetate derivatives by capillary gas chromatography (Hewlett-Packard) using the method of Englyst (*24*) and inositol as an internal standard. Alditol acetates were separated on an SP2380 column (Supelco; 30 m × 0.25 mm i.d.; 0.20 μm film thickness) at a temperature gradient of 230−250 °C at 2 °C/min (*25*). The carrier gas was helium (1 bar), and detection was carried out by flame ionization. For uronic acid determination, acid-soluble samples were filtered and injected into a CarboPac PA1 anion exchange column (4 × 250 mm, Dionex). Detection was performed by pulsed amperometry (PAD 2, Dionex). A postcolumn addition of 300 mM NaOH was used. Galacturonic and glucuronic acids were quantified using 2-deoxy-D-ribose as the internal standard.

Hemicellulose-related glucose was released by 2 M trifluoroacetic acid (TFA; 1.5 mL) hydrolysis of the fiber cell walls (5 mg) for 1 h at 120 °C (*26*), which does not allow disruption of crystalline cellulose. Glucose was then quantified as its alditol acetate derivative as previously described. The determination of monosaccharides other than glucose did not show significant differences from the Seaman hydrolysis (data not shown).

Protein Contents of Fiber Cell Wall. Protein contents were determined in triplicate by the total N contents (N × 6.25) of 3 mg of ball-milled samples using an elemental analyzer (NA 1500, Carlo Erba) coupled with a mass spectrometer (Fisons Isochrom). The experimental error was <5%.

Phenol Composition of Fiber Cell Wall. CWR lignin content was estimated by a spectrophotometric procedure using acetyl bromide (*27*) and 20 mg of sample.

Lignin characterization was achieved using thioacidolysis and alkaline nitrobenzene oxidation that disrupt the noncondensed intermonomer linkages (alkyl−aryl−ether). Thioacidolysis allows the preservation of the phenylpropanoid skeleton, whereas the carbon side chain is disrupted by nitrobenzene oxidation. Thioacidolysis was performed in triplicate on 10 mg of CWR at 100 °C using ethanethiol/BF_3 etherate/dioxane reagent and tetracosane as an internal standard as previously detailed (*28*). After 4 h of reaction, the mixture was adjusted to pH 3−4 and extracted with dichloromethane. Guaiacyl (G) and syringyl (S) thioethylated monomers were analyzed as their trimethylsilyl derivatives using a gas chromatograph (Hewlett-Packard) equipped with a fused silica capillary DB1 column (30 m × 0.25 mm) and a flame ionization detector. The temperature gradient was 160−280 °C at 2 °C/min (*28*).

Nitrobenzene oxidation was performed as given by Billa et al. (*29*). Sodium hydroxide (5 mL, 2 M) and nitrobenzene (0.5 mL) were added to CWR (20−30 mg) in a Teflon vial enclosed within a stainless steel autoclave, and samples were heated in an oil bath (3 h, 160 °C). After the addition of the internal standard (3-ethoxy-4-hydroxybenzaldehyde), the cooled reaction mixture was diluted with 20 mL of H_2O and extracted with 3 × 25 mL of CH_2Cl_2, to eliminate the nitrobenzene reagent. The aqueous residue was then adjusted to pH 1−2 with 6 M HCl and extracted with 3 × 25 mL of CH_2Cl_2 and finally dried over anhydrous Na_2SO_4. After removal of the solvent, the lignin oxidation products were analyzed by HPLC (Waters) on a Spherisorb (S5 ODS2, C-18, 4.6 × 250 mm, 5 μm, Waters) column. The elution gradient proceeded at 30 °C as previously described (*30*) using a combination of acetonitrile, methanol, and 1‰ orthophosphoric acid in ultrapure water. The lignin oxidation products were quantified at 280 nm using commercial standards. The measurements were performed in triplicate.

Ester- and ether-linked hydroxycinnamates were analyzed after NaOH treatment (10 mL, 4 M) of 40 mg of CWR, for 2 h at 170 °C (*31*). The alkali filtrates were acidified to pH 1, mixed with 3,4,5-trimethoxy-*trans*-cinnamic acid as the internal standard, and extracted three times with 30 mL of ether. The organic phase was dried under reduced pressure and analyzed by HPLC as described above. Wall-bound phenolic acids were quantified at 302 nm using commercial standards. The determination of phenolic compounds was performed in triplicate; the variation in the analytical measurement reached 5%.

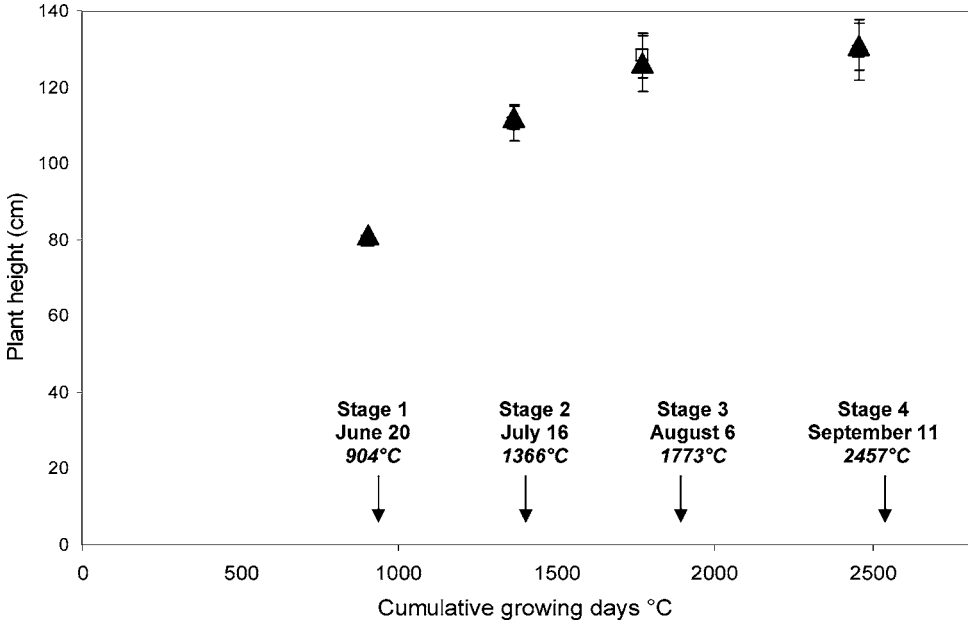

Figure 1. Change in the height of hemp harvested at four developmental stages from vegetative to grain maturity stage. Data are mean values of 30 measurements for the two field areas (area 1, ▲; area 2, □).

Chemical Fractionation of Fiber Cell Wall. Fiber CWRs obtained at different development stages were submitted to a sequential extraction to remove pectin and noncellulose polysaccharides (*25*). CWRs (600 mg) were first extracted with 100 mL of distilled water and continuously stirred in a water bath at 80 °C for 1 h. The water-insoluble residue was retrieved by filtration on a Büchner funnel equipped with a Whatman no. 40 filter and heated with 0.5% ammonium oxalate (100 mL) with continuous stirring in a water bath at 80 °C for 1 h. The resulting residue was recovered as above. The filtrate was dialyzed (cutoff size MW = 3500) against running water for 18 h and then against deionized water for 2 h (× 4). Two extractions with ammonium oxalate were performed to remove pectin polysaccharides. The oxalate-insoluble material was further treated with 0.05 M hydrochloric acid (50 mL) in a water bath at 85 °C for 30 min. The residue was recovered as previously performed, and the extraction was repeated. The pectin-free residues were treated with increasing concentrations of sodium hydroxide to extract the hemicelluloses. The residues from HCl extraction were suspended in 100 mL of 1 M NaOH containing 3 mg/mL $NaBH_4$ for 3 h at 20 °C and continuously stirred, filtered on a Büchner funnel, and washed in water. They were then extracted with 4 M NaOH as previously done. The final residue was neutralized using water and freeze-dried. The alkali extracts and the washes were combined and neutralized with 6 M hydrochloric acid and dialyzed as mentioned above. The filtrate volumes were reduced to 10 mL in a rotary evaporator and stored at −20 °C. These soluble extracts were analyzed for neutral sugars and uronic acids.

Statistical Analysis. Analysis of variance (ANOVA) for the four growing stages was performed independently on apical and basal measurements (SPSS, Chicago, IL). For each measured characteristic, the maturation effect was tested (*F* value), and differences between the growing stage were evaluated by using the Student−Newman−Keuls multiple-range test with a level of significance of 5%. In addition, the lignin characteristics were compared between apical and basal portions using a similar one-way ANOVA test on the average of development stages.

RESULTS

Microscopic View of Hemp Bast Fiber Development. Hemp development can be illustrated by the evolution of the height of the stem on a growing-temperature sum basis (**Figure 1**). The plants reached on average 80 cm at the first harvested stage (vegetative stage). This value markedly increased between stages 1 and 3, from which no drastic change was observed. At grain maturity, hemp stems were nearly 130 cm in height. Microscopic examination of the regions collected at the four developmental stages shows that the extraxylary secondary fibers did not differentiate in the apical region, whereas stem basal regions included secondary fibers even at stage 1. Except for the vegetative stage (stage 1), basal regions had primary fibers almost filled by the thick secondary walls. In apical regions the cell wall surface of a single fiber (transverse section) ranged from 55 to 99% over the hemp development (**Table 1**). In the latter regions, the maturation had the strongest effect on the thickness of the cell wall. Surface and thickness got the highest values in the basal regions irrespective of the growth stage.

Table 1. Histological Features of the Transverse Section of Hemp Primary Fibers[a]

	stage 1	stage 2	stage 3	stage 4	*F* [b]
apical					
cell wall surface[c]	55.1a	59.4a	65.4a	99.3b	40***
cell wall thickness (μm)	2.3a	4.0b	4.6c	8.1d	409***
basal					
cell wall surface[c]	95.5a	99.7b	99.7b	99.9b	33***
cell wall thickness (μm)	10.7a	13.9b	12.6b	14.1b	15***

[a] Values with different letters within a row are significantly different ($P \leq 0.05$). [b] *F* value: maturation effect at $P \leq 0.05$ (*), $P \leq 0.01$ (**), and $P \leq 0.001$ (***). [c] Expressed as a percentage of the surface of a single fiber cross section.

When stained with phloroglucinol, the whole xylem cell walls displayed an intense red color irrespective of the growing stage (**Figure 2a**). In contrast, bast fibers behave distinctively according to the plant stage and the regions considered. A positive reaction was always detected in both primary and secondary fibers from the basal regions as illustrated in **Figure 2** for the vegetative and flowering stages (panels **c** and **d**, respectively). Phloroglucinol did not stain fibers from apical regions at the vegetative stage (**Figure 2a**); a discrete red staining was observed at the flowering (**Figure 2b**) and grain maturity stages (stage 3 and 4). In addition, the area stained by phloroglucinol appeared to be restricted to the middle lamella component and possibly the outermost layers of the secondary walls, which corresponds to the area that gives autofluorescence when illuminated with UV light (**Figure 2e**). In the basal

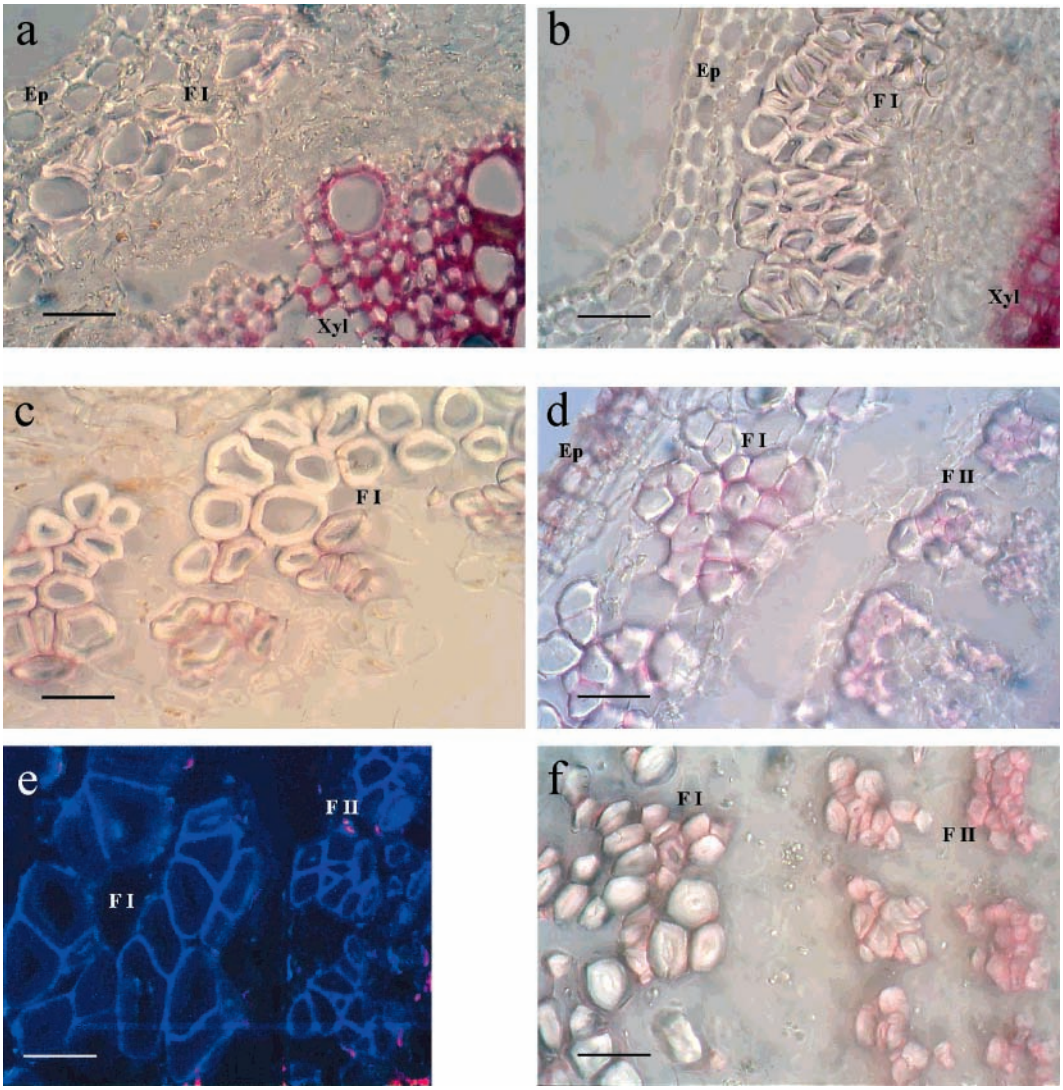

Figure 2. Micrographs of transverse sections of hemp stems isolated at different development stages and stained for lignin using Wiesner (**a**–**d**) and Mäule reagents (**f**) or exposed under UV light (**e**). Xylem cell walls stained red with phloroglucinol irrespective of the stage and the stem regions, as illustrated apical in sections (**a**, **b**). Apical regions were always devoid of secondary fibers, and phloroglucinol did not stain primary fibers in this region at stage 1 (**a**); a very weak staining appeared from stage 3 and was restricted to the primary wall/middle lamella component (**b**). In basal regions, some primary fibers stained red with phloroglucinol at stage 1 (**c**); this staining concerned the whole bundle at stage 3 (**d**) at the cell cementing junctions. A similar pattern was observed with the bundle of secondary fibers that are clearly distinguished at stage 3 (**d**). Lignin deposition was also noted in the basal stem sections following UV light exposure; the blue autofluorescence was mainly concentrated in the cementing component (**e**). Using the Mäule reagent that stains lignin syringyl moieties, the middle lamella component of the primary fibers from basal regions stained positively with the Mäule reagent at the flowering (**f**) and grain maturity stages. Ep, epidermis; Xyl, xylem; FI, primary fibers; FII, secondary fibers. Scale bar = 50 μm.

regions, phloroglucinol staining followed a similar pattern, but some primary fibers stained red by stage 1 (**Figure 2c**); this staining spread to the whole bundle at stage 3 (**Figure 2d**). The Mäule reaction always gave a negative staining with the apical fibers (data not shown). Using this reagent, the secondary fibers of basal regions had a light purple-red coloration at stage 1 (data not shown), whereas the middle lamella component of the primary fibers stained positively with the Mäule reagent at the flowering (**Figure 2f**) and grain maturity stages. Cytochemical staining of lignin thus suggested that extraxylary fibers from apical stem regions would contain a very low amount of lignin; meanwhile, in basal regions, syringyl deposition would be delayed in the primary fibers as compared to the secondary fibers. However, the thick secondary walls appeared to be mostly free of lignin.

Cell Wall Composition. The chemical composition of the hemp fibers was assessed over the growing period regarding their main structural components, that is, carbohydrates, but also phenolics and proteins. A microscopic view confirmed that the hemp fibers (obtained by peeling) were not contaminated with xylem. In addition, fiber-rich material collected from apical regions of hemp stem contained solely primary fibers, whereas samples obtained from basal regions contained 23, 25, 26, and 29% secondary fibers at stages 1, 2, 3, and 4, respectively, based on surface analysis of the transverse sections of bundles collected for chemical studies. Apical fibers were characterized by a significant increase in the CWR proportion during the development, whereas basal fibers displayed weaker variations. The cell walls of the hemp fibers were mainly structural polysaccharides, the level of which ranged from 65 to 84% and from 82 to 85% of the fiber CWR in apical and basal regions, respectively (**Table 2**). The sharp and steady-step increase in polysaccharides of the apical fibers was significantly related to the deposition of glucose, the relative proportion of which was

Table 2. Cell Wall Content and Composition of Outer Fiber-rich Tissues Isolated from Apical and Basal Fragments of Maturing Hemp Stem[a]

	stage 1	stage 2	stage 3	stage 4	F[b]
apical					
CWR (% DM)	75.32a	79.84ab	84.95b	96.01c	66***
total sugar (% CWR)	64.81a	70.27b	75.97c	84.39d	106***
rhamnose	2.17d	1.94c	1.49b	1.13a	90***
fucose	0.49d	0.33c	0.24b	0.13a	52***
arabinose	5.62d	4.64c	3.23b	1.95a	123***
xylose	4.77d	3.77c	2.65b	1.71a	212***
mannose	3.39b	3.01a	3.88c	5.13d	314***
galactose	9.10d	7.26c	5.31b	3.78a	594***
glucose	65.60a	73.00b	78.59c	83.43d	67***
galacturonic acid	7.61d	5.61c	4.29b	2.41a	182***
glucuronic acid	0.50	0.45	0.33	0.25	ns
TFA-glucose (% CWR)	4.67a	8.17b	13.24c	7.68b	173***
protein (% CWR)	7.88c	7.73c	4.82b	1.95a	829***
ferulic acid (μmol/g CWR)	0.24a	0.31a	0.30a	0.59b	50***
lignin (% CWR)	2.91a	3.12a	2.70a	3.58a	2*
basal					
CWR (% DM)	87.35a	92.91b	94.16b	96.51b	17**
total sugar (% CWR)	81.81	82.59	82.88	85.14	ns
rhamnose	1.45b	1.31a	1.27a	1.28a	5**
fucose	0.19b	0.13a	0.15a	0.12a	7**
arabinose	2.79b	2.23a	2.35a	2.06a	5**
xylose	2.80c	2.90ab	3.06b	2.71a	28***
mannose	3.24a	3.88b	4.25c	4.35c	29***
galactose	4.29d	3.63c	3.06b	2.66a	202***
glucose	81.68	83.84	83.65	84.90	ns
galacturonic acid	2.44	1.84	2.00	1.75	ns
glucuronic acid	0.35b	0.21a	0.23a	0.19a	18**
TFA-glucose (% CWR)	6.13a	11.13c	12.50d	9.38b	89.4***
protein (% CWR)	4.81d	3.38c	2.71b	1.60a	231***
ferulic acid (μmol/g CWR)	0.35b	0. 28a	0.42c	0.42c	23**
lignin (% CWR)	4.38	3.92	4.36	4.48	ns

[a] Values with different letters within a row are significantly different ($P \leq 0.05$). [b] *F* value: maturation effect at $P \leq 0.05$ (*), $P \leq 0.01$ (**), and $P \leq 0.001$ (***); ns, not significant.

nearly 1.3-fold higher in the stage 4 samples compared to the stage 1 samples. On the contrary, in basal fibers the levels of total sugars did not vary during the growing period; coincidently, no change was noticeable in the relative proportion of glucose released by sulfuric acid. Following TFA hydrolysis, some glucose was released in noticeable amounts, which reached the highest CWR proportion at stage 3 in both apical and basal regions. Although in much lower content, other minor monosaccharides were quantified. Notably, in both the apical and basal stems the decreasing proportion of arabinose and galactose during the development showed opposite trends to the levels of mannose. In addition, polysaccharides of the apical fibers contained significantly lower proportions of rhamnose, fucose, xylose, and uronic acids at the grain maturity stage versus the vegetative stage. Overall, the maturation effect on polysaccharide composition was strongest for apical regions; however, in all stem regions the most significant variations were displayed for the relative proportions of galactose, TFA-released glucose, mannose, and xylose.

The cell wall protein content showed declining values over the hemp development, especially in the apical regions, where a sharp decrease occurred at the flowering stage (stage 3). Cell wall bound phenolic acids were retrieved almost exclusively as ferulic acid (FA). Although in very low amounts, increased proportions were quantified at the older stages; hence, these variations were significantly related to plant maturation. In contrast, lignin levels, which ranged from 2.7 to 4.4% of the CWR, showed no (basal fibers) or only weak (apical fibers) correlation with maturation. However, the acetyl bromide determinations of bast fibers were significantly distinct between stem regions ($F = 56$ at $P < 0.001$).

Table 3. Monomer Composition of Lignin Determined by Alkaline Nitrobenzene Oxidation and Thioacidolysis of Outer Fiber-Rich Tissues Isolated from Apical and Basal Stem Regions of Maturing Hemp[a]

		stage 1	stage 2	stage 3	stage 4	F[b]
apical						
thioacidolysis	S+G[c]	52.29a	58.51a	177.06c	98.35b	65***
	S/G	0.82	0.87	0.98	0.80	ns
nitrobenzene oxidation	H+V+S[d]	314a	329a	622b	665b	18***
	S/V	0.78b	1.05c	0.68a	0.62a	42***
	H/H+V+S	24.1d	20.9c	13.1b	9.30a	44***
basal						
thioacidolysis	S+G	226.4	178.9	270.5	237.2	ns
	S/G	1.21	1.00	1.15	0.97	ns
nitrobenzene oxidation	H+V+S	456a	568b	528b	683c	34***
	S/V	0.92c	0.85b	0.84b	0.77a	10***
	H/H+V+S	9.4c	7.1b	5.1a	5.1a	22***

[a] Values with different letters within a row are significantly different ($P \leq 0.05$). [b] *F* value: maturation effect at $P \leq 0.05$ (*), $P \leq 0.01$ (**), and $P \leq 0.001$ (***); ns, not significant. [c] S+G: total yields of β-*O*-4-linked syringyl (S) and guaiacyl (G) expressed as micromoles per gram of lignin. [d] H+V+S: total yields of *p*-hydroxybenzaldehyde (H), vanillin (V), and syringaldehyde (S) expressed as micromoles per gram of lignin.

Lignin composition was assessed by means of two chemical reactions (**Table 3**). Thioacidolysis of the basal fiber CWR resulted in the highest yields of ether-linked monomers ($F = 42$ at $P < 0.001$). Total yields (S+G) thus ranged from 53 to 177 μmol/g of lignin and from 179 to 270 μmol/g of lignin in apical and basal fibers, respectively, reflecting a higher proportion of labile-ether-linked lignin structures in basal samples. Moreover, the S/G molar ratio gave the highest values in the basal samples ($F = 19$ at $P < 0.001$), although no significant change could be related to the maturity stage in each of the stem portion. After nitrobenzene oxidation (**Table 3**), three lignin degradation products, 4-hydroxybenzaldehyde (H), vanillin (V), and syringaldehyde (S), corresponding to *p*-hydroxyphenyl, guaiacyl, and syringyl lignin subunits, respectively could be detected in all samples. Traces of phenolic acids were also detected (data not shown). In basal fibers the total (H+V+S) values were higher than in apical samples ($F = 8$ at $P < 0.010$), but this difference is much lower than that observed with the thioacidolysis results. Nitrobenzene oxidation gave rise to S/V ratios that varied from 0.62−1.0 (apical) to 0.77−0.92 (basal) values, which are close to the S/G value obtained with thioacidolysis. Although the cell wall content of H did not vary significantly during hemp maturation, H units represented a decreasing proportion among the lignin degradation products from 24 to 9% (apical) and from 9 to 5% (basal) of the total degradation products. Assuming that (1) acetyl bromide lignin gives a good estimation of the lignin content, (2) the degradation yield of target structures is 100%, and (3) the average molar mass of monolignol is 200, one can estimate that thioacidolysis has permitted characterization of no more than 5% of the polymer. Using nitrobenzene oxidation, nearly 14% of the lignin was analyzed at the most mature stages.

Chemical Fractionation of Hemp Fiber CWR. Chemical fractionation of the fiber CWR was used to estimate the possible change in the extractability of the noncellulosic polysaccharides over hemp maturation. The sequential chemical treatment used aimed at extracting pectin polysaccharides (hot water, ammonium oxalate, and dilute hydrochloric acid) and then hemicelluloses (alkali treatments). Heterogeneous polysaccharide fractions were thus released from hemp fibers. Consis-

Table 4. Total Sugar Content of Chemically Extracted Fractions from Hemp Fibers (Expressed as a Percent of the Initial Sugar Content in CWR)[a]

	apical				basal			
	stage 1	stage 2	stage 3	stage 4	stage 1	stage 2	stage 3	stage 4
H_2O	4.88b	4.28b	4.70b	3.65a	1.69a	4.76b	6.30c	4.50b
oxalate	1.39c	1.07c	0.51a	0.78a,b	0.38a	0.87b	0.99b	0.87b
HCl	7.88c	7.54c	5.26b	1.79a	2.51b	2.63b	2.89c	1.45a
1 M NaOH	6.47b	3.96a	3.46a	3.75a	2.43a	3.02b	3.76c	3.77c
4 M NaOH	10.89b	6.33a	6.10a	5.19a	4.18a	3.98a	3.70a	4.07a
total extracted	31.52d	23.18b	20.03b	15.16a	11.19a	15.26b	17.64c	14.66b

[a] Values with different letters within a row related to apical or basal regions are significantly different ($P \leq 0.05$).

tent with the large proportion of cellulose in basal fibers, lower proportions of sugars were extracted from these samples as compared to apical ones. In addition, fewer amounts of polysaccharides were solubilized during maturation, which was consistent with the declining levels of noncellulosic components from the vegetative to the maturity stage (**Table 4**).

Water-soluble fractions represented increasing proportions during maturation, ranging from 16 to 24% and from 15 to 30% of the total extracted polysaccharides from apical and basal fibers, respectively. The total amount of water-released sugars represented from 1.7% (stage 1, basal region) to 6.3% (stage 3, basal region) of the initial CWR (**Table 4**); stronger variations were observed in basal ($F = 12$ at $P \leq 0.001$) versus apical fibers ($F = 7$ at $P \leq 0.01$). On the basis of their relative monosaccharide composition, water-soluble components became rich in glucose as the fibers became more mature, irrespective of the stem regions (**Tables 5** and **6**). Similarly, the contribution of mannose got larger, especially at stage 4. In contrast, arabinose- and galactose-rich polysaccharides that represented the main water-soluble fractions from apical regions at stage 1 decreased. Such components also declined in the case of the fibers from basal stem regions.

Ammonium oxalate, which is reported to remove Ca^{2+}-associated pectins, was the less effective reagent in removing noncellulosic polysaccharides (**Table 4**). As compared to other treatments, oxalate was the richest fraction in the monosaccharides as rhamnose, galacturonic acid, and galactose, which are indicative of rhamnogalacturonans. Oxalate-released components represented 0.4−1.4% of the initial CWR polysaccharides; this fraction thus accounted for 6% maximum of the total extracted polysaccharides. Consistent with the decreasing quantity of galacturonic acids in the fibers of apical regions, the oxalate was less efficient in removing materials from the cell walls.

Overall, the proportion of HCl-extracted polysaccharides was the lowest in the fiber CWR from basal regions and showed decreasing values from stage 2 (apical regions) or stage 3 (basal regions) (**Table 4**). Variance analysis showed a similar effect of maturation on apical ($F = 111$ at $P \leq 0.001$) and basal regions ($F = 124$ at $P \leq 0.001$). The HCl-soluble polysaccharides decreased approximately from 25 to 12% and from 22 to 10% of the total extracted components from apical and basal regions, respectively. Arabinose and galactose were the main monosaccharides recovered from acid hydrolysis of HCl extracts irrespective of the maturation stage and the stem regions, suggesting the occurrence of arabinogalactans. However a sharp rise in glucose-rich polysaccharides occurred after HCl treatment of the fiber CWR at stage 2 (**Tables 5** and **6**).

With regard to alkali treatments, on average 4 M NaOH disrupted 30% of the total extracted polysaccharides from the fibers isolated from the apical regions (**Table 4**) and was more effective than 1 M NaOH (17−25%). The greatest yields of cell wall alkali-released hemicelluloses were retrieved at stage 1 and then declined; maturation impact was almost similar regarding the amount extracted by the two alkalis ($F = 18$ and 29 for 1 and 4 M NaOH, respectively, at $P \leq 0.001$). In contrast, the amounts of components solubilized from basal fibers displayed only significant variations related to maturation with

Table 5. Monosaccharide Composition of the Chemically Extracted Fractions from Hemp Fibers of the Apical Region (as a Percentage of the Total Amount Extracted by Each Reagent)[a]

		stage 1	stage 2	stage 3	stage 4	F[b]
water	Rha	3.4ab	4.2b	2.4a	4.5b	5*
	Fuc	1.1	1.5	0.2a	0.6a	ns
	Ara	21.1d	12.4c	9.6b	6.8a	248***
	Xyl	9.8c	5.6b	2.9a	2.7a	80***
	Man	10.0b	8.7a	9.7b	27.3c	1023***
	Gal	38.1c	21.8b	16.9a	17.3a	374***
	Glc	14.3a	43.2c	56.9d	38.9b	218***
	GalA	2.3	2.5	1.4	1.8a	ns
	GlcA	nq	nq	nq	nq	
oxalate	Rha	10.6	9.9	11.5	8.9	ns
	Fuc	nq	nq	nq	nq	
	Ara	25.7c	21.4c	14.2b	8.5a	49***
	Xyl	6.3d	3.7c	1.5a	2.1b	21***
	Man	9.7a	8.2a	11.8b	25.8c	269***
	Gal	30.2c	23.2b	17.9a	16.1a	36***
	Glc	9.9a	19.9b	37.5d	32.4c	242***
	GalA	8.0a	13.6c	5.4b	6.3b	5*
	GlcA	nq	nq	nq	nq	
HCl	Rha	4.4a	4.3a	3.4a	9.1b	72***
	Fuc	2.6c	1.2b	1.1a	1.9b	303***
	Ara	52.1c	29.8b	24.3a	31.1b	631***
	Xyl	1.6b	1.3ab	1.0a	1.7c	112***
	Man	0.8a	0.3a,b	1.0b	5.2c	276***
	Gal	32.2c	23.6b	18.9a	36.0a	103***
	Glc	4.2a	34.8c	47.9d	10.9b	1370***
	GalA	2.1	4.6	2.3	4.1	ns
	GlcA	nq	nq	nq	nq	
1 M NaOH	Rha	2.7a	6.0b	4.6b	5.7b	13***
	Fuc	1.7	1.5	1.4	1.4	ns
	Ara	4.3ab	3.8a	4.5b	3.6a	5*
	Xyl	44.1c	40.2b	40.8b	25.8a	136***
	Man	5.4a	4.4a	8.2b	18.8c	339***
	Gal	18.6c	13.8b	11.9ab	10.8a	48***
	Glc	17.3a	22.3b	23.4b	29.6c	53***
	GalA	3.7a	5.7b	3.3a	3.3a	17***
	GlcA	2.2b	2.3b	2.0b	1.0a	10***
4 M NaOH	Rha	1.1a	3.1bc	2.7b	2.6a	6*
	Fuc	2.3c	1.8b	1.5b	0.9a	52***
	Ara	1.5a	1.9ab	2.0bc	2.1c	5*
	Xyl	27.7c	26.2c	19.2b	10.8a	99***
	Man	18.5a	19.2ab	21.2b	24.3bc	7**
	Gal	15.4c	11.2b	10.2b	7.9a	56***
	Glc	32.5c	35.2c	42.0b	50.9a	51***
	GalA	1.0bc	1.4c	1.3c	0.6a	8**
	GlcA	nq	nq	nq	nq	

[a] Values with different letters within a row for each chemical reagent are significantly different ($P \leq 0.05$). [b] F value: maturation effect at $P \leq 0.05$ (*), $P \leq 0.01$ (**), and $P \leq 0.001$ (***); ns, not significant; nq, not quantifiable.

Table 6. Monosaccharide Composition of the Chemically Extracted Fractions from Hemp Fibers of the Basal Region (as a Percentage of the Total Amount Extracted by Each Reagent)[a]

		stage 1	stage 2	stage 3	stage 4	F [b]
water	Rha	3.4c	1.5a	1.3a	1.9b	101***
	Fuc	2.2b	0.8a	0.6a	0.7a	75***
	Ara	13.1c	6.9b	5.1a	4.8a	274***
	Xyl	7.1b	2.8a	2.3a	3.0a	145***
	Man	15.5b	11.5a	12.3a	22.5c	316***
	Gal	32.7d	13.7c	9.3a	12.0b	1568***
	Glc	23.3a	61.8c	68.4d	53.8b	2792***
	GalA	2.7c	1.0b	0.7a	1.2bc	107***
	GlcA	nq	nq	nq	nq	
oxalate	Rha	nq	5.0ab	4.4a	5.7b	222***
	Fuc	8.2b	4.2a	3.2a	3.6a	56***
	Ara	15.3	15.5	16.0	14.4	ns
	Xyl	nq	3.9a	3.4a	3.6a	89***
	Man	20.4c	13.2a	13.1a	18.5b	35***
	Gal	24.7ac	18.9b	16.8a	20.4c	26***
	Glc	23.0a	33.1b	37.3b	26.0a	20***
	GalA	8.7c	6.1ab	5.8a	7.9bc	5*
	GlcA	nq	nq	nq	nq	
HCl	Rha	5.9b	3.7a	3.3a	5.6b	25***
	Fuc	3.2ab	2.6a	2.1a	3.4b	8**
	Ara	34.4b	26.6ab	23.1a	25.7a	47***
	Xyl	3.3b	2.3a	1.9a	3.1b	13***
	Man	2.2a	2.7a	2.2a	4.9b	34***
	Gal	39.1c	25.2b	18.0a	26.1b	468***
	Glc	7.1a	34.9c	47.5d	27.8b	259***
	GalA	4.8c	2,0a	2.0a	3.3b	57***
	GlcA	nq	nq	nq	nq	
1 M NaOH	Rha	nq	2.6b	1.3a	1.8ab	25***
	Fuc	3.6c	3.1b,c	2.4a,b	2.1a	8**
	Ara	3.7b	3.2b	1.8a	2.2a	18***
	Xyl	53.2d	44.4c	40.0b	35.1a	87***
	Man	5.7a	9.8b	16.8c	19.3d	189***
	Gal	12.9d	11.1c	5.6a	7.6b	70***
	Glc	13.5a	20.4b	28.0c	27.1c	475***
	GalA	4.6b	3.8a	2.7a	3.4a	17***
	GlcA	2.6b	1.7a	1.4a	1.4a	6*
4 M NaOH	Rha	0.7	nq	nq	nq	3*
	Fuc	2.8	2.7	2.7	2.1	ns
	Ara	0.6a	nq	nq	2.2b	32***
	Xyl	21.7c	17.6b	16.5b	12.6a	88***
	Man	21.5a	27.2c	22.1a	23.9b	41***
	Gal	9.7b	7.7a	7.6a	7.2a	17***
	Glc	40.9a	43.9b	49.9c	51.1d	71***
	GalA	2.0b	1.0a	1.2a	1.0a	46***
	GlcA	nq	nq	nq	nq	

[a] Values with different letters within a row for apical and basal regions, respectively, are significantly different ($P \leq 0.05$). [b] F value: maturation effect at $P \leq 0.05$ (*), $P \leq 0.01$ (**), $P \leq 0.001$ (***); ns, not significant; nq, not quantifiable.

1 M NaOH ($F = 70$ at $P \leq 0.001$). Alkali fractions were rich in xylans based on the high portion of xylose (**Tables 5** and **6**). Notably, 1 M NaOH was the most efficient in extracting glucuronic acid-rich components such as glucuroxylans, which became less important in fibers during maturation. In contrast, both glucose and mannose monomer received larger proportions in fractions extracted from older samples. The change in the composition of 4 M NaOH soluble fractions displayed a similar trend in the case of fibers from apical regions, whereas for basal fibers the mannose proportion had weaker variations than glucose. On the basis of the high proportion of mannose and glucose, 4 M NaOH would mostly extract glucomannans in addition to xylans. This alkali-soluble fraction became richer in glucomannans as the plant developed. This trend was particularly noted for the fiber CWR obtained from basal regions at the oldest stages as compared to apical ones.

Table 7. Polysaccharide and Lignin Composition of Residues Recovered from Chemical Fractionation of Hemp Fibers from Apical and Basal Fragments of Maturing Hemp Stem[a]

	stage 1	stage 2	stage 3	stage 4
apical				
final residue (% initial CWR)	46.3	46.3	53.4	66.7
polysaccharide composition (% total monosaccharides)				
rhamnose	0.9d	0.6b	0.8c	0.5a
fucose	0.1b	nq	0.01a	nq
arabinose	1.2a	1.1a	1.0a	1.1a
xylose	0.6c	0.5b	0.5b	0.4a
mannose	0.2a	0.3a	0.5b	0.3a
galactose	3.1c	2.3b	2.2b	1.3a
glucose	92.2a	93.9b	94.3c	95.9d
galacturonic acid	1.7d	1.3c	0.7b	0.5a
glucuronic acid	nq	nq	nq	nq
lignin (μmol/CWR)[b]				
H+V+S	5.5a	5.9a	8.9b	13.2c
H/H+V+S	10.0b	10.0b	7.1ab	6.2a
S/V	0.7c	0.7c	0.6b	0.5a
basal				
final residue (% initial CWR)	58.3	59.3	55.7	61.0
polysaccharide (% total monosaccharides)				
rhamnose	0.4a	0.4a	0.5b	0.4a
fucose	0.1a	0.1a	0.1a	0.1a
arabinose	0.9a	0.9a	1.1b	1.1b
xylose	0.4a	0.4a	0.5b	0.4a
mannose	0.4a	0.5a	0.5a	0.5a
galactose	1.3b	1.4c	1.4c	1.2a
glucose	95.7a	95.5a	95.2a	97.7ba
galacturonic acid	0.7b	0.7b	0.7b	0.5a
glucuronic acid	nq	nq	nq	nq
lignin (μmol/CWR)[b]				
H+V+S	11.9a	16.1b	15.7b	17.9b
H/H+V+S	3.2a	3.2a	3.2a	4.4b
S/V	0.7a	0.7a	0.8a	0.7a

[a] Values with different letters within a row for apical and basal regions, respectively, are significantly different ($P \leq 0.05$). nq, not quantifiable. [b] H, *p*-hydroxybenzaldehyde; V, vanillin; S, syringaldehyde, were released by alkaline nitrobenzene oxidation.

Chemically Treated Fibers. The final residues were essentially composed of cellulose as reflected by the high proportion of glucose (**Table 7**). Among minor sugars, galactose and arabinose reached approximately 1−1.5% of the polysaccharides. Given the fact that the final residue is made up of nearly 95% glucose as mostly cellulose, the amount of hemp cellulose will roughly correspond to the yield of final residue recovered after the chemical fractionation. Thereby, apical fibers would contain 46−67% cellulose from stage 1 to stage 4; meanwhile, cellulose levels range from 58 to 61% in samples from basal stem regions. Altogether, soluble and residual polysaccharides ranged from 80 to 90% of the initial CWR polysaccharides. Following nitrobenzene oxidation the proportion of monolignol-derived aldehydes represented nearly 55−65% of the original amounts determined in initial fiber CWR; residual lignin had a lower S/V molar ratio.

Although the sequential fractionation was mostly applied to ground CWR for chemical determination, some fiber bundles (basal stem regions, stages 1 and 4) were similarly treated for microscopic examination. Water, ammonium oxalate, and hydrochloric acid did not appear to efficiently remove the middle lamella, which still gave a positive reaction with phloroglucinol (**Figure 3a**,**b**,**e**,**f**). However, some dislocation of the fiber bundles started to occur following the water treatment; this trend was particularly noted in the case of fibers collected at stage 1 (arrow in **Figure 3a**). After 0.05 M HCl extraction, secondary walls often appeared detached from the middle lamella component in transverse sections of residual fibers at stage 1 (arrow in **Figure 3b**). At stage 4, this observation was more seldom

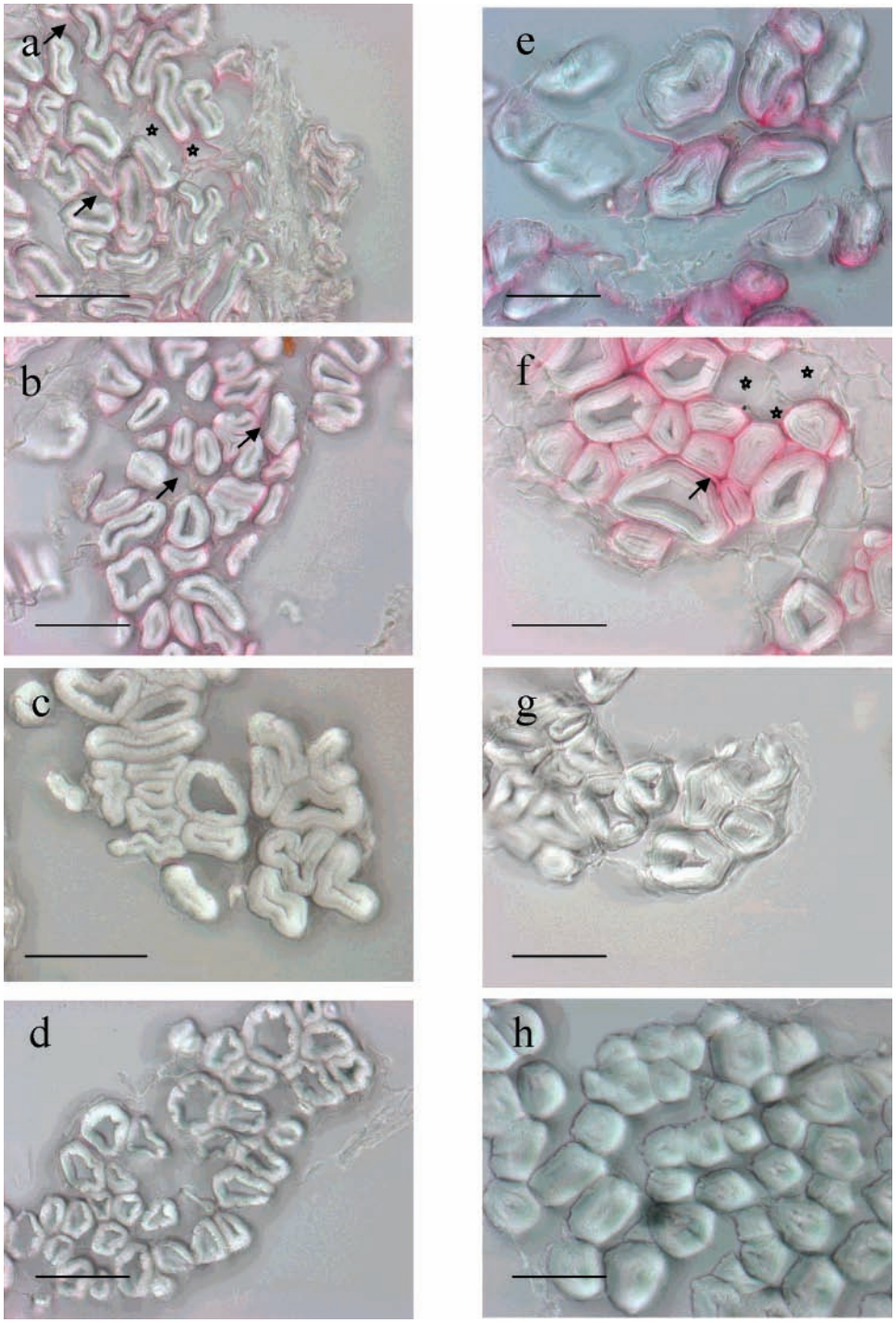

Figure 3. Micrographs of transverse cross sections of chemically extracted fiber bundles from basal regions of hemp stem and stained for lignin using Wiesner. Following the water (**a**, **e**) and the HCl (**b**, **f**) treatments, lignin staining was still observed irrespective of the development stage: stage 1 (**a**, **b**), stage 3 (**e**, **f**). At vegetative stage, extraction of water-soluble components induced partial detachment of the secondary walls from cementing component (black arrow, **a**). Complete detachment was also enhanced by cross sectioning (☆). After the treatment with 0.05 M HCl, the first layer of the thick secondary walls was less cohesive in cells corresponding to stage 3 (black arrow, **f**). Alkali-treated fibers did not stain phloroglucinol irrespective of the development stage: stage 1 (**c**, **d**) stage 3 (**g**, **h**); the middle lamella component almost disappeared. The swollen aspect of the secondary walls after 4 M NaOH treatment (**d**, **h**) indicated that cellulose structure is modified, whereas fewer modifications would occur following treatment using less concentrated alkali (**c**, **g**). Ep, epidermis; Xyl, xylem; FI, primary fibers; FII, secondary fibers. Scale bar = 50 μm.

(arrow in **Figure 3f**), but cells free of secondary walls were observed in the bundle section (open star in **Figure 3f**). After alkali treatment, the fibers did not stain red with phloroglucinol (**Figure 3c**,**d**,**g**,**h**), and the primary wall−middle lamella component had almost disappeared, especially after the 4 M NaOH treatment, which led to the swelling of the secondary walls.

DISCUSSION

Extraxylary fibers displayed changes in both chemical composition and thickening of the walls during hemp maturation from the vegetative to the grain maturity stage. These changes were clearly noted in the fibers collected in apical stem regions,

which showed significant thickening of the secondary wall. Basal fibers displayed limited variations compared to apical fiber during the four stages analyzed mainly for the fact that the basal portion of the stem is in a more advanced stage of development, as reflected by the presence of secondary fibers even at vegetative stage. This finding is somehow distinct from the study of Mediavilla et al. (*32*) that did not observe secondary fibers at vegetative stage. However, this discrepancy may rely not only on different environmental conditions but also on genetic variability. Indeed, the proportion of secondary fibers in the total bast fibers was found to be slightly higher in a monoecious compared to a dioecious cultivar irrespective of the stem portion (*6*). In our study, manual isolation of the bast fibers from the fresh stem led to primary fibers only from apical regions, whereas in basal regions, secondary fibers also accounted for a small fraction (~25%) of the bast fibers in basal regions. Even though chemical characteristics of basal fiber bundles would arise from a mixture of primary and secondary fibers, the proportion of the last ones did not show strong variation along development. In addition, the chemical-free procedure for fiber isolation we used was preferred to maceration involving alkali (*6*) as this procedure would likely induce the release some of the noncellulosic components from primary fibers.

Basically, the thick bast fibers of hemp are mostly composed of polysaccharides, which proportions increased from the vegetative to the grain maturity stages in apical regions. Filling of apical fibers was strongly noted by the enrichment of glucose, the variations of which significantly lay in the evolution of the wall thickness and wall surface proportion. However, the main variation related to maturation concerns the protein content of fiber cell walls whatever the stem region, indicating the possible role of structural protein in the wall network of hemp fibers.

In contrast, the lignin content that did not exceed 4.5% did not show a strong impact on fiber maturation in agreement with a previous study on developing hemp (*6*, *16*). Overall, fibers were more lignified in basal stem regions than in apical stem regions. In the latter ones, acetyl bromide determination allowed the quantification of lignin even at vegetative stages for which phloroglucinol reagent failed to show some lignin deposition. This discrepancy might be linked to a possible weak sensitivity of phloroglucinol in comparison to more specific tools for electron microscopy as shown in flax fibers (*7*, *9*). Additionally, although the acetyl bromide method is an appropriate and sensitive technique for lignin determination (*33*), one must point out that lignin determination in weak lignified plant samples may suffer from interferences with other components such as proteins or hydroxycinnamic acids. Although the low levels of ferulic acid would not significantly contribute to the overall acetyl bromide estimation of lignin, one cannot totally exclude that proteins, which account for 8% of the cell wall in the apical regions, may lead to an overestimation of lignin in these samples. However, UV absorption of aromatic amino acids is strongly reduced after acetylation (*27*), thereby limiting their potential contribution to the acetyl bromide determination.

Both thioacidolysis and nitrobenzene oxidation indicated the presence of a mixed G−S lignin type (S/G and S/V ratios ranging from 0.6 to 1.2) in apical and basal regions. Altogether, lignin characterization highlighted that hemp fibers would mostly contain condensed lignin structures that are not (thioacidolysis) or to a very low extent (nitrobenzene oxidation) accessible to chemical degradation. Indeed, on the basis of the amounts of lignin monomers released by thioacidolysis, only 1−5% of fiber lignin would be noncondensed. In addition, nitrobenzene oxidation revealed the occurrence of significant proportions of H units in hemp fiber lignins. Traces of H units are usually found in the lignin of the dicotyledon annual plants; a high proportion of H monomers was recently reported in lignin of flax fibers, suggesting some similarities between these bast fibers (*9*). Given the high level of protein in apical regions, one could not totally exclude that aromatic amino acid may account for H units; however, such an interference would be of low relevance in the case of basal fibers that contain low amounts of proteins. The occurrence of H unit further argued for a condensed polymer in hemp as the nonmethoxylated monolignol is often related to the deposition of highly condensed lignin (*34*). Accordingly, one may thus hypothesize that these H units are preferentially involved in condensed structures such as diarylpropane and/or resinol bonds that are sensitive to nitrobenzene oxidation (*35*). Even though the relative proportions of H subunits were much higher in apical fibers as compared to basal ones, in both regions the declining relative proportion of H units during maturation was accompanied by a higher range of noncondensed lignin. This trend would indicate that the later stages of lignification correspond to the deposition of a less condensed polymer as reported in maturing dicotyledon wood cell walls (*34*). However, in the case of hemp, such a trend did not lead to an intense syringyl deposition because the ratio syringyl/guaiacyl remained constant (thioacidolysis) or even decreased (nitrobenzene oxidation) during maturation. On the basis of the preferential localization of lignin in the primary wall−middle lamella component of hemp fibers, one could suggest that the presence of high amounts of crystalline cellulose may impair the polymerization process. Indeed, some wall model studies have highlighted that the pre-existing polysaccharides of the wall can affect the pattern of lignin polymerization (*36*, *37*).

As compared to flax, hemp fibers thus appeared to be quite similar with respect to the apparent restricted deposition of lignin in the middle lamella component, but histochemical observations highlighted that the Mäule reagent gave positive staining in contrast to flax (unpublished results). Even though the inner layers of the secondary walls do not react with lignin staining, further studies would be required to address the question of lignin distribution in the secondary walls using more specific and sensitive tools that have allowed detection of a low amount of lignin deposits in the inner layers of highly cellulosic cell walls as flax fibers (*7*, *9*). Hemp fibers are slightly more lignified than flax (*9*, *18*) and display variations regarding lignin structure. First, although found in low amounts at young stages as for flax fibers, the proportion of noncondensed lignin structure tends to increase at the mature stages, which was not observed in the case of flax (*9*). Second, the proportion of H units is lower. Finally, the S/G molar ratio obtained by thioacidolysis (0.8−1.2) is nearly twice the value obtained for flax (0.2−0.4) (*9*). These discrepancies related to lignin may hold a distinct polysaccharide matrix in flax and hemp extraxylary fibers.

Glucose predominates in the cell wall in agreement with the high cellulose content of hemp fibers, which is a common characteristic of flax fibers (*5*, *18*). However, on the basis of the proportion of galacturonic acids, acidic pectins would be less important in hemp as compared to flax. Hence, the calcium chelator reagent was weakly efficient in removing polysaccharides from hemp fibers, whereas pectins and arabinogalactans were the major components released from flax fibers using this reagent. Nevertheless, in both stem regions galactose was the main noncellulosic monomer at vegetative stages. Furthermore, variance analysis clearly showed that the decrease in galactose

was one of the strongest factors related to maturation, suggesting that galactan-containing polymer would be of great relevance in the hemp fiber architecture as previously shown for flax (*38*). Consistently, the declining proportions of water-soluble galactans in mature cells might correspond to β-(1-4)-galactans that occur at the early stages of cell wall thickening as recently suggested (*39*). In addition, the composition of 0.05 M HCl fractions that disrupted the largest part of galactose- and arabinose-rich polysaccharides from hemp fibers may indicate the occurrence of arabinogalactan that is possibly connected to protein (*40*). Finally, the significant contribution of mannose in all fractions can be tentatively assigned to components related to glucomannans, which might be of great importance in the wall network of hemp fibers. Indeed, ANOVA analysis indicated that the increase in the relative proportion of mannose and the concomitant decrease in galactose and in xylose were the main factors explaining fiber maturation in both apical and basal stem regions. In addition, using a mild acid hydrolysis allows us to highlight that glucose would not be solely related to cellulose but also to noncrystalline glucans. Noteworthy was the high proportion of the TFA-released glucose that represented nearly the same total amount of the other monosaccharide in all samples. Our results related to chemical fractionation of the cell walls were in strong agreement with this trend and further suggest that TFA-released glucose would essentially arise from water- and alkali-soluble polysaccharides such as glucomannans and (galacto)glucomannans. Change in the composition of the soluble fractions released from the fibers of apical regions clearly indicated that these polysaccharides would deposit mainly during wall thickening. To the best of our knowledge, the predominance of glucomannans in hemp fibers has not been reported elsewhere. Such polysaccharides have been already described in alkali extracts of flax fibers (*15*, *41*, *42*) but were mentioned sporadically among water-soluble components (*43*, *44*). In hemp, our data underscored that glucomannans of mature fibers (basal regions, stages 3 and 4) are found not only in alkali fractions but also in water extracts. Hence, water-residual fibers showed some partial detachment of the secondary walls, suggesting a distribution of water-soluble glucomannans at the interface of the secondary and primary wall−middle lamella component. A more comprehensive view on glucomannans structure and implication in the wall architecture would provide useful and relevant insights into the field of hemp fiber processing and isolation.

Additionally, more research would be required to evaluate the whole contribution of hemicellulosic polysaccharides on the wall network with respect to the content and supramolecular structure of cellulose. Particularly, the physical/chemical constraints imposed on the lignification process by the presence of high amounts of structural polysaccharides could be compared to the situation encountered in flax. Hence, the hemp bast fiber may represent an interesting system, in addition to flax, as a model for investigating weakly lignified, cellulose-rich secondary cell walls.

ABBREVIATIONS USED

FA, ferulic acid; H, *p*-hydroxyphenyl; G, guaiacyl; S, syringyl; CWR, cell wall residue.

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LITERATURE CITED

(1) Eichhorn, S. J.; Baillie, C. A.; Zafeiropoulos, N.; Mwaikambo, L. Y.; Ansell, M. P.; Dufresne, A.; Entwistle, K. M.; Herrera-Franco, P. J.; Escamilla, G. C.; Groom, L.; Hughes, M.; Hill, C.; Rials, T. G.; Wild, P. M. Current international research into cellulosic fibres and composites. *J. Mater. Sci.* **2001**, *36*, 2107−2131.

(2) Baley, C. Analysis of flax fibres tensile behaviour and analysis of the tensile stiffness increase. *Composites, Part A* **2002**, *33*, 939−948.

(3) Nechwatal, A.; Mieck, K.-P.; Reussmann, T. Developments in the characterization of natural fibre properties and in the use of natural fibres for composites. *Compos. Sci. Technol.* **2003**, *63*, 1273−1279.

(4) McDougall, G.; Morrison, I.; Stewart, D.; Weyers, J.; Hillman, J. Plant fibres: botany, chemistry and processing for industrial use. *J. Sci. Food Agric.* **1993**, *62*, 1−20.

(5) Rahman, M. Morphology of the fibres of jute, flax, and hemp as seen under a scanning electron microscope. *Indian J. Agric. Sci.* **1979**, *49*, 483−487.

(6) Van Der Werf, H. M. G.; Van Der Veen, J. E. H.; Bouma, A. T. M.; Ten Cate, M. Quality of hemp (*Cannabis sativa* L.) stems as a raw material for paper. *Ind. Crops Prod.* **1994**, *2*, 219−227.

(7) Gorshkova, T. A.; Salnikov, V. V.; Pogodina, M.; Chemikosova, S. B.; Yablokova, E. V.; Ulanov, A. V.; M. V., A.; Van Dam, J. E. G.; Lozovaya, V. V. Composition and distribution of cell wall phenolic compounds in flax (*Linum usitatissimum*) stem tissues. *Ann. Bot.* **2000**, *85*, 477−486.

(8) Morvan, C.; Andeme-Onzighi, C.; Girault, R.; Himmelsbach, D. S.; Driouich, A.; Akin, D. E. Building flax fibres: more than one brick in the walls. *Plant Physiol. Biochem.* **2003**, *41*, 935−944.

(9) Day, A.; Ruel, K.; Neutelings, G.; Crônier, D.; David, H.; Hawkins, S.; Chabbert, B. Lignification in the flax stem: evidence for an unusual lignin in bast fibers. *Planta* **2005**, *1*, 3−4.

(10) Roland, J.; Mosiniak, M.; Roland, D. Dynamique du positionnement de la cellulose dans les parois des fibres textiles du lin (*Linum usitatissimum*). *Acta Bot. Gallica* **1995**, *5*, 463−484.

(11) Müller, M.; Cyihak, C.; Burghammer, M.; Riekel, C. Combined X-ray microbeam small-angle scattering and fibre diffraction experiments on single native cellulose fibres. *J. Appl. Crystallogr.* **2000**, *33*, 817−819.

(12) Focher, B.; Palma, M. T.; Canetti, M.; Torri, G.; Cosentino, C.; Gastaldi, G. Structural differences between non-wood plant celluloses: evidence from solid-state NMR, vibrational spectroscopy, and X-ray diffractometry. *Ind. Crops Prod.* **2001**, *13*, 193−208.

(13) Girault, R.; Bert, F.; Rihouey, C.; Jauneau, A.; Morvan, C.; Jarvis, M. Galactans and cellulose in flax fibres: putative contributions to the tensile strength. *Int. J. Biol. Macromol.* **1997**, *21*, 1179−188.

(14) Gorshkova, T. A.; Wyatt, S. E.; Salnikov, V. V.; Gibeaut, D. M.; Ibragimov, M. R.; Lozovaya, V. V.; Carpita, N. C. Cell-wall polysaccharides of developing flax plants. *Plant Physiol.* **1996**, *110*, 721−729.

(15) Mooney, C.; Stolle-Smits, T.; Schols, H.; De Jong, E. Analysis of retted and non retted flax fibres by chemical and enzymatic means. *J. Biotechnol.* **2001**, *89*, 205−216.

(16) Keller, A.; Leupin, M.; Mediavilla, V.; Wintermantel, E. Influence of growth stage of industrial hemp on chemical and physical properties of the fibres. *Ind. Crops Prod.* **2001**, *13*, 35−48.

(17) Toonen, M. A. J.; Maliepaard, C.; Reijmers, T. H.; van der Voet, H.; Mastebroek, D.; van den Broeck, H. C.; Ebskamp, M. J. M.; Kessler, W.; Kessler, R. W. Predicting the chemical composition of fibre and core fraction of hemp (*Cannabis sativa* L.). *Euphytica* **2004**, *140*, 39−45.
(18) Bonatti, P. M.; Ferrari, C.; Focher, B.; Grippo, C.; Torri, G.; Cosentino, C. Histochemical and supramolecular studies in determining quality of hemp fibres for textile applications. *Euphytica* **2004**, *140*, 55−64.
(19) Vignon, M.; Garciajaldon, C. Structural features of the pectic polysaccharides isolated from retted hemp bast fibres. *Carbohydr. Res. 296* **1996**, 249−260.
(20) Mediavilla, V.; Jonquera, M.; Schmid-Slembrouk, I.; Soldati, A. Decimal code for growth stages of hemp (*Cannabinus sativa* L.). *J. Int. Hemp Assoc.* **1998**, *5*, 67−74.
(21) Clifford, M. N. Specificity of acidic phloroglucinol reagents. *J. Chromatogr.* **1974**, *94*, 321−324.
(22) Meshitsuka, G.; Nakano, J. Studies on the mechanism of lignin color reaction XIII: Mäule color reaction 9. *Mokuzai Gakkaishi* **1979**, *25*, 588−594.
(23) Seaman, J.; Moore, W.; Mitchell, R.; Millet, M. Techniques for determination of wood constituents by quantitative paper chromatography. *Tappi J.* **1954**, *37*, 336−343.
(24) Englyst, H. N.; Cummings, J. H. Simplified method for the measurement of total non starch polysaccharides by gas−liquid chromatography of constituent sugars as alditol acetates. *Analyst* **1984**, *109*, 937−942.
(25) Hamm, M.; Debeire, P.; Monties, B.; Chabbert, B. Changes in the cell wall network during the thermal dehydration of alfalfa stems. *J. Agric. Food Chem.* **2002**, *50*, 1897−1903.
(26) De Ruiter, G.; Schols, H.; Voragen, A.; Rombouts, F. Carbohydrate analysis of water-soluble uronic acid-containing polysaccharides with high-performance anion-exchange chromatography using methanolysis combined with TFA hydrolysis is superior to four other methods. *Anal. Biochem.* **1992**, *207*, 176−185.
(27) Iiyama, K.; Wallis, A. F. A. Determination of lignin in herbaceous plants by an improved acetyl bromide procedure. *J. Sci. Food Agric.* **1990**, *51*, 145−161.
(28) Lapierre, C.; Monties, B.; Rolando, R. Thioacidolysis of poplar lignins: identification of monomeric syringyl products and characterization of guaiacyl-syringyl rich fractions. *Holzforschung* **1986**, *40*, 113−119.
(29) Billa, E.; Tollier, M. T.; Monties, B. Characterisation of the monomeric composition of in situ wheat straw lignins by alkaline nitrobenzene oxidation: effect of temperature and reaction time. *J. Sci. Food Agric.* **1996**, *72*, 250−256.
(30) Beaugrand, J.; Crônier, D.; Debeire, P.; Chabbert, B. Arabinoxylan and hydroxycinnamate content of wheat bran in relation to endoxylanase susceptibility. *J. Cereal Sci.* **2004**, *40*, 223−230.
(31) Iiyama, K.; Lam, T. B. T.; Stone, B. A. Phenolic acid bridges between polysaccharides and lignin in wheat internodes. *Phytochemistry* **1990**, *29*, 733−737.
(32) Mediavilla, V.; Leupin, M.; Keller, A. Influence of the growth stage of industrial hemp on the yield formation in relation to certain fibre quality traits. *Ind. Crops Prod.* **2001**, *13*, 49−56.
(33) Yokoyama, T.; Kadla, J. F.; Chang, H.-M. Microanalytical method for the characterization of fiber components and morphology of woody plants. *J. Agric. Food Chem.* **2002**, *50*, 1040−4044.
(34) Comprehensive model of the lignified plant cell wall. In *Forage Cell Wall Structure and Digestibility*; Jung, H. G., Buxton, D. R., Hatfield, R. D., Ralph, J., Eds.; ASA, CSSA, SSSA: Madison, WI, 1993; pp 247−270.
(35) Oxidation. In *Lignins: Occurrence, Formation, Structure and Reactions*; Sarkanen, K. V., Ludwig, C. H., Eds.; Wiley-Interscience: New York, 1971; pp 433−485.
(36) Jurasek, L. Morphology of computer-modeled lignin structure: fractal dimensions, orientation and porosity. *J. Pulp Pap. Sci.* **1996**, *22*, J376−J380.
(37) Touzel, J. P.; Chabbert, B.; Monties, B.; Debeire, P.; Cathala, B. Synthesis and characterization of dehydrogenation polymers in *Gluconacetobacter xylinus* cellulose and cellulose/pectin composite. *J. Agric. Food Chem.* **2003**, *51*, 981−986.
(38) Gorshkova, T.; Carpita, N.; Chemikosova, S.; Kuzmina, G.; Kozhevnikov, A.; Lozovaya, V. Galactans are a dynamic component of flax cell walls. *Russ. J. Plant Physiol.* **1998**, *45*, 234−239.
(39) Gorshkova, T. A.; Chemikosova, S. B.; Sal'nikov, V. V.; Pavlencheva, N. V.; Gur'janov, O. P.; Stolle-Smits, T.; van Dam, J. E. G. Occurrence of cell-specific galactan is coinciding with bast fiber developmental transition in flax. *Ind. Crops Prod.* **2004**, *19*, 217−224.
(40) Girault, R.; His, I.; Andeme-Onzighi, C.; Driouich, A.; Morvan, C. Identification and partial characterization of proteins and proteins and proteoglucans encrusting the secondary cell walls of flax fibres. *Planta* **2000**, *211*, 256−264.
(41) McDougall, G. J. Isolation and partial characterisation of the non-cellulosic polysaccharides of flax fibre. *Carbohydr. Res.* **1993**, *241*, 227−236.
(42) Vanhazendonk, J. M.; Reinerink, E. J. M.; Dewaard, P.; Vandam, J. E. G. Structural analysis of acetylated hemicellulose polysaccharides from fibre flax (*Linum usitatissimum* L). *Carbohydr. Res.* **1996**, *291*, 141−154.
(43) Goubet, F.; Bourlard, T.; Girault, R.; Alexandre, C.; Vendevelde, M. C.; Morvan, C. Structural features of galactans from flax fibres. *Carbohydr. Polym.* **1995**, *27*, 221−227.
(44) Jacobs, A.; Palm, M.; Zacchi, G.; Dahlman, O. Isolation and characterization of water-soluble hemicelluloses from flax shive. *Carbohydr. Res.* **2003**, *338*, 1869−1876.

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