JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Chemical and Spectroscopic Analysis of Lignin in Isolated Flax Fibers

W. H. MORRISON, III,* D. S. HIMMELSBACH, D. E. AKIN, AND J. D. EVANS†

R. B. Russell Agricultural Research Center, USDA, ARS, P. O. Box 5677, Athens, Georgia 30604

The chemistry of pure flax fibers, free of contaminating nonfiber components, has not been determined. Fibers from the center sections of the stem of seed and fiber flax (*Linum usitatissium L.*), which had been retted after soaking in water and removal of the epidermis by hand, underwent chemical and spectroscopic analysis. Wet chemical analysis showed only trace indications of aromatics and no long chain fatty acids or alcohols in fibers. Pyrolysis mass spectroscopy (PyMS) and pyrolysis gas chromatography mass spectrometry (PyGCMS) showed only trace amounts of aromatic constituents that could be attributed to the presence of lignin. Mid-infrared (Mid-IR) and Raman spectroscopy of these fibers showed no aromatic compounds present. This study suggests that earlier work reporting the presence of lignin ranging from 1 to 4% may be the result of residual shive or epidermis/cuticle material remaining after the retting process which may be responsible for the favorable properties desired by the composites industry.

KEYWORDS: Flax; fiber; cuticle; spectroscopy; Mid-IR; Raman

INTRODUCTION

The production of flax fiber requires a process called retting that involves the degradation of the connective material between the fiber and epidermis and core or shive. Retting is seldom a quantitative process and all analyses of the fiber have been conducted on fiber that is contaminated with bits of cuticle and/or shive. Earlier work (*1*, *2*) has shown that even yarn rated of high quality has cuticle particles remaining attached to the fibers. In a later study (*3*), epidermis was carefully removed from flax stem followed by hand removal of the underlying fibers. Analysis showed that the fiber contained small amounts of aromatic compounds associated with attached shive. Guaiacyl lignin is the main type of lignin, appearing in about twice the concentrations as syringyl lignin. Dihydroxyfatty acids were used as an indicator of the presence of cuticle. Results showed that these fatty acids were almost exclusively associated with the epidermis and little if any associated with the fiber.

The amount of lignin associated with retted fiber has been estimated at between 1 and 4% (*4*−*6*). However, these determinations have been made using colorimetric and/or proximate analysis that can include other chemical entities. Staining with acid phloroglucinol, a general stain for lignin (*7*), suggested that there are compounds that stain and occur sporadically within the fiber and at cell junctures (*8*). Using solid-state ^{13}C NMR, Love et al. (*5*) suggested that these aromatic species are anthocyanins.

In the present study, flax fibers from both seed and fiber flax were carefully isolated to minimize the amount of nonfiber material to allow for a detailed chemical and spectroscopic analysis. Knowledge of the chemistry related to the pure fiber would allow for more accurate assessment of the characteristics important to all areas of flax fiber utilization and establish absolute markers characteristic of the pure fiber that could be used in quality assessment determinations.

MATERIALS AND METHODS

Flax Samples. Flax cultivars Omega (seed flax) and Laura (fiber flax) were grown under identical conditions in Florence, SC as a winter crop (1999−2000). A third cultivar, Ariane (fiber flax), which was grown as a fiber crop (early, harvested May 5, 1999) and also for fiber and seed near Florence, SC (late, harvested May 27, 1999), as a winter crop the previous year. Ten centimeter sections were taken from the center of the plant and placed in water for about 3 h. After the plants were soaked, the stems were removed from the water and the outer layer (OL) which is made up of epidermis and cuticle was removed by hand. The OL-free stems were then incubated at 40 °C for 24 h in a solution of 0.05% Viscozyme L and 18 mM EDTA in the commercial product Mayoquest 200 (*9*), the supernatant was drained off and 10 mL of boiling water added followed by vigorous shaking similar to in-vitro retting and preparation for Frieds test analysis (*10*). The freed fibers were removed and washed an additional three times with 10 mL portions of boiling water. The cleaned fibers were then dried under vacuum.

Pyrolysis Mass spectrometry (PyMS) and Pyrolysis Gas Chromatography Mass Spectrometry (PyGCMS). Samples were ground in a Spex 5100 mixer mill. In-source pyrolysis mass spectrometry (PyMS) was performed on a Finnigan Polaris Q equipped with a direct exposure probe (rhenium loop). Analysis conditions were: ionization energy, 20 eV; mass range 50−350; scan time 1 s; temperature rise, ca. 10 °C s^{-1} to 675 °C; ion source temperature 200 °C. Finely ground samples were analyzed by preparing a suspension of the sample in distilled water using a glass mortar and pestle. A small amount of the

* Corresponding author. Phone: 706-546-3361. Fax: 706-546-3607. E-mail: whmorrison@prodigy.net.

† Present address: South Central Poultry Research Laboratory, P.O. Box 5367, Mississippi State, University, MS 39759.

10.1021/jf020885t
Published on Web 03/21/2003

suspension was placed on the loop and water evaporated under vacuum. Each sample was run in triplicate.

Pyrolysis GCMS was performed using a CDS Pyroprobe 2000 mounted on the injector of a Finnigan Polaris Q GCMS. Quartz tubes containing the samples were placed in the probe coil and heated at rate of 50 °C ms^{-1} to 675 °C and held for 10 s. the GC column used was a DB 5 ms (30 m × 0.25 mm i.d.) with a hold for 2 min at 50 °C and programmed at a rate of 4 °C min^{-1} to 275 °C. Compounds were identified by comparison with published spectra or that of the authentic compound.

Statistical Analysis. Using the Finnigan software, ASCII mass lists were derived from each pyrolysis mass spectrometry scan by selecting the main pyrolyis peak in the total ion current spectra and subtracting the baseline signal prior to and after the main peak. Each mass list was imported into Excel 2000 for Windows 98 and zeros added for the occasional missing data for individual masses, and mass lists truncated to the range of 50−300 mass units. Individual mass lists were first normalized using the total intensities for the 250 masses. The normalized mass lists were imported into Unscrambler 7.6 and a principal component analysis with cross validation conducted.

Chemical Analysis. Samples were treated with 4 M NaOH at 170 °C for 2 h, prepared as previously described (*11*) and analyzed for phenolics, waxes, and cutin material by gas−liquid chromatographic (GLC) analysis of the *N*,*O*,bis(trimethylsilyl)trifluoroacetamide (BSTFA) silyl ethers. Product identification was conducted by gas chromatography−mass spectrometry (GCMS) on a Finnigan Polaris Q with a 0.25 mm id × 30 m DB-5 column. Mass spectra were taken at 70 eV.

Mid-IR Spectroscopy. For mid-IR spectroscopy, infrared spectra were obtained utilizing a DuraScope (SensIR Technologies, Danbury, CT) attenuated total reflectance (ATR) device in a Magna 850 (Thermo Nicolet, Madison, WI) Fourier transform infrared (FT-IR) bench. The bench was equipped with a KBr beam splitter and a DTGS detector. Mats of flax fiber were placed on the surface of the diamond ATR crystal. Pressure was applied to the sample using a transparent rod until "wetting" of the sample on the crystal, as observed on the video monitor of the device, was assured (rating load of 5). Interferograms were collected at a resolution of 8 cm^{-1} over 4384 scan points at a scan velocity of 0.3165 cm/s to cover the range of 4000−650 cm^{-1}. The bench aperture was set at 150 (∼15 mm), the gain at 1.0 and 128 scans were co-added. After Happ-Genzel apodization function, the data were Fourier transformed and displayed with Mertz phase correction in the absorbance mode against the background of the clean diamond crystal (collected under identical conditions with no sample and no pressure applied). The final spectra were displayed without ATR correction.

Raman Spectroscopy. For Raman spectroscopy, Raman spectra were obtained using a Nicolet 950 FT-Raman spectrometer (Thermo Nicolet, Madison, WI) that was equipped with a 1.064 μm Nd:YAG laser source, a liquid N_2-cooled Ge detector, a notch rejection filter for Rayleigh scatter and a CaF_2 beam splitter. Mats of flax fiber were secured in a spring held sample holder and spectra were obtained employing a lens to defocus the excitation laser beam at the sample using 180° reflective geometry with a laser power of 500 mW (12 amps). Interferograms were collected with a resolution of 8 cm^{-1} over 8480 scan points covering the Raman shift range of 3994 to −2 cm^{-1}. The bench aperture was set at 22.28, the gain at 64, and 512 scans were co-added. After Happ-Genzel apodization, the data were Fourier transformed and displayed as power spectra without white light correction. The final spectra were baseline corrected at 16 points and normalized to an intensity of 1.0 for the most intense band.

RESULTS AND DISCUSSION

Our earlier study showed that even after hand separation of cuticle, fiber, and shive, dihydroxy fatty acids (cuticle markers), aromatics (lignin markers), and long chain fatty acids (wax markers) remained on the fibers and were inversely related to the degree of retting (*3*). To obtain the flax fiber free of epidermis and shive, the stems were soaked in water and the epidermis was carefully removed. The epidermis-free stems were then enzyme-retted using a pectinase-rich mixture with other

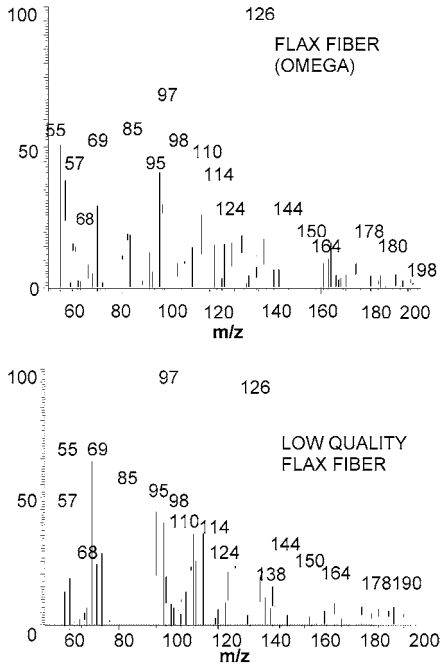

Figure 1. Pyrolysis mass spectra of flax fibers (Omega) and low quality flax fiber.

polysaccharidases, i.e., (Viscozyme) shown to provide efficient retting. Inclusion of EDTA (from Mayoquest 200) assured removal of Ca^{2+}-pectins, facilitating separation of fibers (*9*). Initially, three stains were used to survey the degree of purity of the isolated fibers. Oil red was used to give an indication of the amount of remaining cuticular material, and ruthenium red (0.02% aqueous solution) was used as an indication of pectin. Oil red showed no cuticular material remaining attached to the fibers and ruthenium red showed a slight coloration suggesting only a trace of pectin remaining after repeated washing with boiling water. The third stain, acid phloroglucinol, was then used to indicate the presence of aromatics generally associated with lignin. There was only slight staining sporadically at cell junctures.

Chemical analysis using 4 N NaOH at 170 °C to degrade the sample has been used to characterize the lignin associated with plant fibers. Earlier work (*3*) on cuticle-free, unretted fibers showed significant amounts of lignin markers associated with the fibers primarily with guaiacyl lignin. These aromatics were probably associated with attached shive particles. Analysis of the cuticle-free, retted fiber treated with 4 N NaOH followed by GLC and GLMS analysis of the silyl ethers showed no detectable aromatics.

Pyrolysis mass spectrometry has also been used to evaluate the syringyl/guaiacyl rations in plants cell walls. Due to the greater stability of the aromatics components compared to the carbohydrate fragments, aromatics are easier to detect in lower amounts using this approach. The pyrolysis mass spectra of the fibers in this study are all similar, and an example of the cuticle-free, retted fiber is shown in **Figure 1** along with a retted fiber judged to be of low quality (*1*). The markers at *m*/*z* 138, 150, 152, 164, 166, 178, 180 indicate some trace amounts of guaiacyl lignin. However, the spectrum is primarily that of cellulose as indicated by markers *m*/*z*: 57, 58, 60, 73, 85, 86, 96, 98, 100, 110, 112, 114, 126, and 144 (*11*). Statistical treatment of the PyMS data using principal component analysis (PCA) can be used to identify subtle differences in the samples related to chemical differences between the samples that may be important in end use quality. As shown in **Figure 2**, there is no grouping

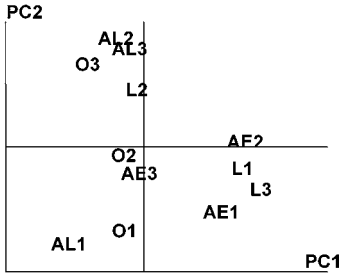

Figure 2. Principle component analysis (PCA) of the PyMS data for triplicate samples of isolated flax fibers from Ariane early harvest (AE); Ariane late harvest (AL); Omega (O): and Laura (L). The first principal component explains 46% of the variance while the second principal component explains 30% of the variance.

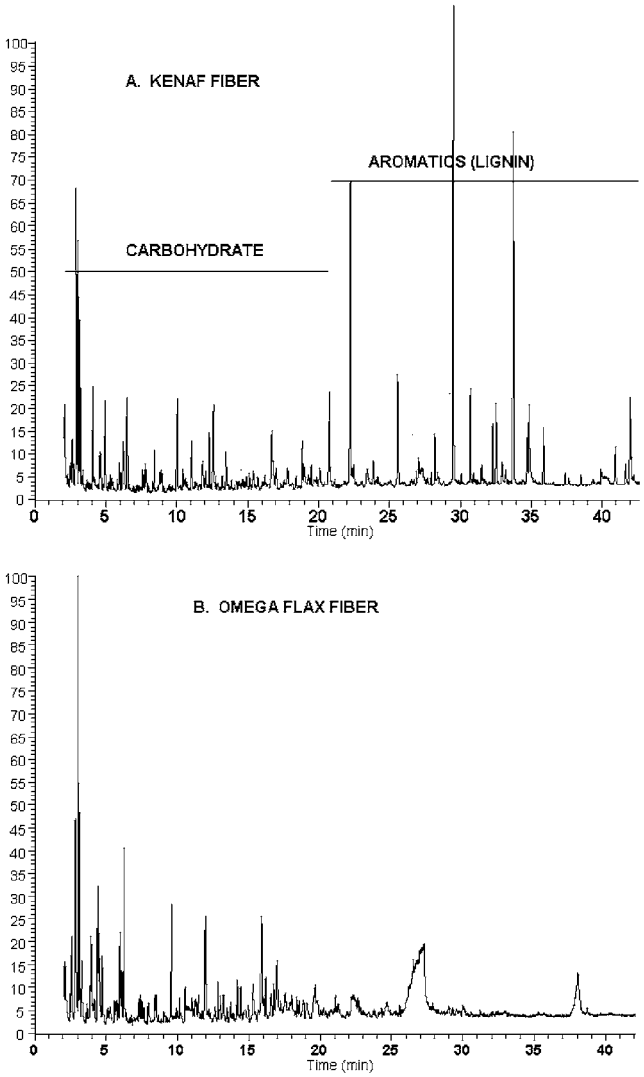

Figure 3. Total ion current chromatogram from PyGCMS of (A) kenaf fiber and (B) flax fiber.

of these fibers that would allow differentiation based on chemical makeup as was seen in earlier work on graded flax fibers and yarns.

PyGCMS can be used to analyze a biopolymer and provide information on the molecular units that make up the parent polymer. The pyrogram of these isolated flax fibers show no aromatic compounds typically found in lignocellulosic materials, a result that is in agreement with the other experimental data. An example of the pyrogram of kenaf fiber (**Figure 3A**) which

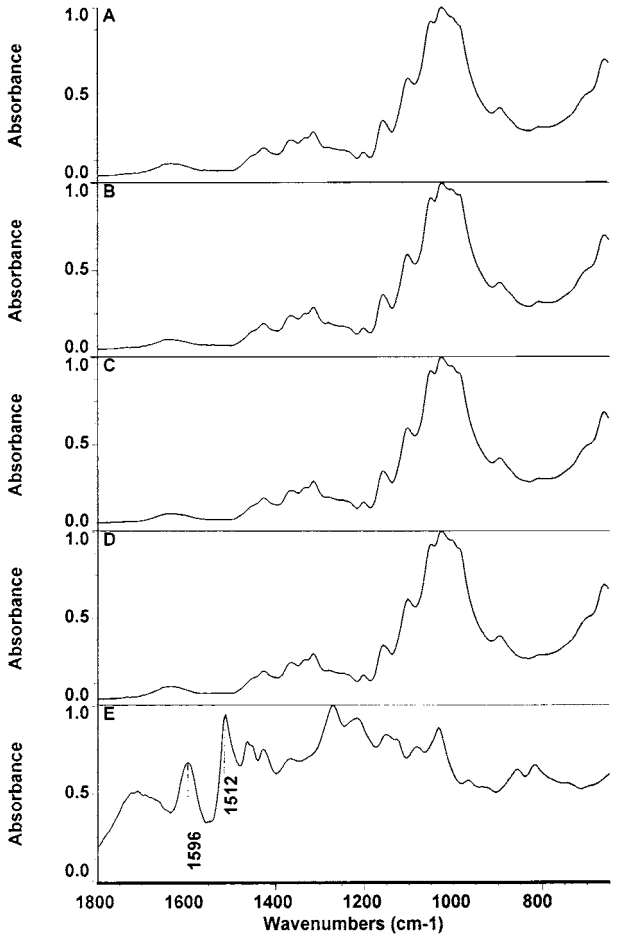

Figure 4. Mid-IR spectra of (A) Laura, (B) Omega, (C) Ariane late, (D) Ariane early, (E) AT special Kraft Lignin (mixed softwoods) (aromatic absorptions at 1596 and 1512 cm^{-1} for lignin spectrum, only the flax fibers show strong absorbance near 1000 cm^{-1} for carbohydrates).

contains lignin (*12*) is shown with the isolated flax fiber (**Figure 3B**) to illustrate the lack of lignin markers in flax.

Spectroscopic analysis of the fibers using Mid-IR and RAMAN spectroscopy is shown in **Figures 4** and **5**, respectively. **Figure 4** shows the Mid-IR spectra of the four flax fibers (panels A−D) and a Kraft lignin (panel 4E). Fiber spectra show no absorption at 1596 and 1512 cm^{-1} that are characteristic of the Kraft lignin. The RAMAN spectra shown in **Figure 5A−D** of flax fiber and an unretted flax fiber that has been hand scutched (**Figure 5E**) illustrate the same point. There is no absorption at 1604 cm^{-1} in the clean flax fiber characteristic of the presence of aromatics.

Reported lignin content varies widely among investigators with ranges from 0.37% for unretted material to 4.6% for water-retted flax (*4*−*6*). The methods used for these determinations are not specific for lignin and the values more likely indicate the presence of residual shive attached to the fibers. Love et al. (*5*) demonstrated that the lignin content in flax determined by the Klason method and the acetyl bromide method gave values of 3.2 and 2.4%, respectively, for retted, scutched, and hackled fiber. Using solid-state ^{13}CNMR the aromatic material was suggested to be an anthocyanin and lignin representing only 0.9%.

This study has shown that flax fiber that has been carefully prepared to remove residual epidermis and shive or core contain none of the compounds associated with these plant tissues. Earlier work (*13*, *14*) has demonstrated the presence of ether-linked phenolic acids associated with residual hemicellulose.

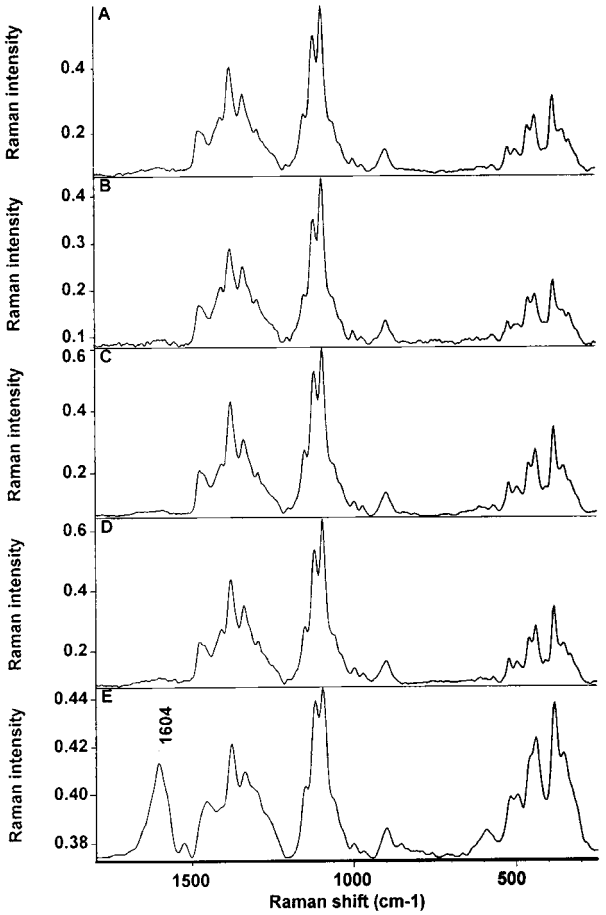

Figure 5. RAMAN spectra of (A) Laura, (B) Omega, (C) Ariane late, (D) Ariane early, (E) raw flax fiber, hand schutched with cuticle and wax (Raman scatter at 1604 cm^{-1} indicated on the unretted fiber spectrum).

Since retting and subsequent processing leaves small amounts of epidermis and core or shive attached to the fibers, these constituents may be the origin of the reported aromatic constituents and act as sites for cross linking with other reagents in the production of composites.

To our knowledge, the chemistry of pure flax fibers has not been determined. The results presented are important in identifying absolute markers associated with fibers, therefore establishing a basis for rapid analysis for fiber contaminants and the chemistry associated with flax fiber.

LITERATURE CITED

(1) Morrison, W. H.; Archibald, D. D. Analysis of Graded Flax Fiber and Yarn by Pyrolysis Mass Spectrometry and Pyrolysis Gas Chromatography Mass Spectrometry. *J. Agric. Food Chem*. **1998**, *46*, 1870−1876.

(2) Morrison, W. H.; Akin, D. E., Himmelsbach, D. S.; Gamble, G. R. Chemical, Microscopic, and Instrumental Analysis of Grades Flax Fiber and Yarn. *J. Sci. Food Agric*. **1999**, *79*, 3−10.

(3) Morrison, W. H.; Akin, D. E. Chemical Composition of Components Comprising Bast Tissue in Flax. *J. Agric. Food Chem*. **2001**, *49*, 2333−2338.

(4) McDougall, G. J.; Morrison, I. M.; Stewart, D.; Weyers, J. D. B.; Hillman, J. R. Plant Fibers: Botany, Chemistry and Processing for Industrial Use. *J. Sci. Food Agric*. **1993**, *62*, 1−20.

(5) Love, G. D.; Snape, C. E.; Jarvis, M. C., Morrison, I. M. Determination of Phenolic Structure in Flax by Solid-State ^{13}C NMR, *Phytochemistry* **1994**, *35*, 489−491.

(6) Sharma, H. S. S.; Faughey, G.; Lyons, G. Comparison of Physical, Chemical, and Thermal Characteristics of Water-, Dew, and Enzyme-Retted Flax Fiber. *J. Appl. Polym. Sci*. **1999**, *74*, 139−143.

(7) Sarkanen, K. V.; Ludwig, C. H. Definition and Nomenclature. In *Lignins: Occurrence, Formation, Structure and Reactions*, Sarkanen; K. V. and Ludwig, C. H., Eds.; Wiley-Interscience: New York, USA, 1971; pp 1−18.

(8) Akin, D. E.; Gamble, G. R.; Morrison, W. H.; Rigsby, L. L.; Dodd, R. B. Chemical and Structural Analysis of Fiber and Core Tissue from Flax. *J. Sci. Food Agric*. **1996**, *72*, 155−165.

(9) Akin, D. E.; Foulk, J. A.; Dodd, R. B. Influence on Flax Fiber of Components in Enzyme-Retting Formulations. *Text. Res. J*. **2002**, *72*, 510−514.

(10) Henriksson, G.; Akin, D. E.; Rigsby, L. L.; Patel, N.; Eriksson, K−E. L. Influence of Chelating Agents and Mechanical Pretreatment on Enzymatic Retting of Flax. *Text. Res. J*. **1997**, 829−836.

(11) Arendonk, Van, J. J. C. M.; Niemann, J. G.; Boon, J. J. The Effects of Enzymatic Removal of Protein Food From Plant Leaf Material as Studies by Pyrolysis-Mass Spectrometry: Detection of Additional Protein Marker Fragment Ions. *J. Anal. Appl. Pyrolysis* **1997**, *42*, 33−51.

(12) Morrison, W. H.; Akin, D. E.; Ramaswamy, G.; Baldwin, E. Evaluating Chemically Retted Kenaf Using Chemical, Histochemical and Microspectrophotometric Analysis. *Text. Res. J*. **1996**, *66*, 651−565.

(13) Lozovaya, V.; Gorshkova, T. A.; Yablokova, E.; V., Rumyantseve, N. I.; Valieve, A., Ulanov, A.; Widhelm, J. K. Cold Alkali Can Extract Phenolic Acids That Are Ether Linked to Cell Wall Components in Dicotyledonous Plants (Buckwheat, Soybean and Flax). *Phytochemistry* **1999**, *90*, 395−400.

(14) Gorshkova, T. A.; Salnikov, V. V.; Pogodina, N. M.; Chemikosova, S. B.; Yablokova, E. V.; Ulanov, A. V.; Agreeve, M. V.; Van Dam, J. E. G.; Lozovaya, V. V. Composition and Distribution of Cell Wall Phenolic Compounds in Flax (Linium usitatissium L.) Stem Tissue. *Ann. Bot.* **2000**, *85*, 477−486.

Received for review August 13, 2002. Revised manuscript received January 28, 2003. Accepted February 6, 2003.

JF020885T