# Near-Infrared Fourier-Transform Raman Spectroscopy of Flax (*Linum usitatissimum* L.) Stems

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Samples of flax (*Linum usitatissimum* L.) stem and its anatomical parts were studied by near-infrared Fourier transform Raman (NIR-FT-Raman) spectroscopy to determine if the major chemical components of each could be detected by this method. The Raman spectra of reference compounds from relatively pure materials served as models for the chemical components. Bands for cellulose were greatest in the fibers. Hemicellulosic polysaccharides were observed to be prevalent in bast tissue and fibers. Weak signals for pectins were observed in the bast, cuticle/epidermis, fibers, and stem. Bands for aromatic rings were detectable in all materials. Bands from waxes/fatty acid esters were detectable in the cuticle/epidermal tissue. The results indicated that NIR-FT-Raman could be used detect the major chemical components in flax in situ and provide a simple, rapid, and noninvasive assessment of their relative amounts and location within the tissues of the flax plant.

## INTRODUCTION

Currently, there is considerable renewed interest in North America (Stephens, 1996) and Europe (Kozlowski, 1996) in the use of fibers from flax (*Linum usitatissimum* L.) for textile and industrial purposes. With this interest comes interest in improving the quality of the fibers and the methods by which they can be obtained (van Dam et al., 1994). This requires increased knowledge of the chemistry and structure of the plant (Focher, 1992; McDougall et al., 1993).

We have recently explored several laboratory spectroscopic methods for the chemical and structural analysis of the flax stem and the core and fiber fractions (Akin et al., 1996). These methods have included solid-state $^{13}C$ NMR, mid-infrared spectroscopy, and UV microspectrophotometry. All of these methods provide very specific information about select components of flax materials, but no single technique has been able to provide rapid definitive information on all of the major chemical components of these materials.

Since the development of near-infrared Fourier-transform Raman (NIR-FT-Raman) spectroscopy (Hirschfeld and Chase, 1986; Chase, 1986), the technique has been increasingly used to assess agricultural materials (Kenton and Rubinovitz, 1990; Evans, 1991; Atalla et al., 1992; Séné et al., 1994; Stewart et al., 1995). The advantages of FT-Raman are excellent frequency precision and, with NIR excitation, no sample preparation and reduced fluorescence interference. NIR-FT-Raman has the advantage over mid-infrared (MIR) spectroscopy in not requiring the sample atmosphere to be purged to reduce the interfering bands from water vapor and $CO_2$. Like MIR spectroscopy, Raman spectroscopy gives bands related to fundamental vibrations

that can provide "fingerprints" of components that can be used for characterization. This is not the case with NIR absorption spectroscopy, in which bands arise from overtone and combination bands of fundamental vibrations from constituents. These bands typically overlap each other and generally prohibit clear identification of individual components. In addition, NIR spectroscopy requires almost total reliance on chemometric methods (Martens and Naes, 1987). Unlike MIR and NIR spectroscopy, which depend on changes in the dipole moment, Raman spectroscopy depends on the change in polarizability or deformability of the electron cloud (Colthup, 1990) that accompanies the vibrations. Raman spectra are generally characterized by fewer and sharper bands. Identifiable bands may often be easier to find in Raman spectra of complex samples, such as with biological materials (Séné et al., 1994). However, even with the best analysis system, it is difficult to identify specific components in a complex matrix. Our work here explores the possibility that NIR-FT-Raman spectroscopy meets that requirement and evaluates its potential for use in the quality assessment of flax fiber and other flax-based materials as well as in the assessment of the processing methods used to isolate these materials.

## MATERIALS AND METHODS

**Flax Samples.** Ariane cultivar flax was grown from the end of April 1994 for 90 days under well-managed conditions in The Netherlands and supplied by Van de Bilt zaden en vlas, b.v., Sluiskil, The Netherlands. For the initial analysis of flax stems, the bottom 7.5 cm and the seed head (at the branch point) were removed and the remainder of the plant stem was cut into 7–8 cm equal length sections. For all subsequent analyses only the center 7–15 cm of the plant material was utilized. Bast [cuticular, epidermal, parenchyma (cortex) tissues and phloem fibers], core (xylem tissue), cuticle/epidermis, and fibers were manually separated from whole flax stems. Bast was manually pulled from the core tissue, and

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the fiber was peeled from the bast. The peeled fiber was then hand cleaned to remove as much of the surrounding cortex tissue as possible in an attempt to have only fibers. Hereafter, these bast fibers will be referred to simply as "fibers". Cuticle/epidermal tissue was carefully scraped with a razor blade from the surface of the stem, and any detectable fibers were removed while viewed under a dissecting microscope. Since the cuticle and epidermal tissues could not be physically separated, cuticular waxes and fatty acid esters were extracted from the surface of flax stems with hexane by Soxhlet extraction [as described elsewhere (Gamble et al., 1997)].

**Reference Samples.** The carbohydrate reference samples utilized were as follows: cellulose, as microcrystalline cellulose PH-105, obtained from FMC Corp. (Princeton, NJ); citric pectin, as methyl α-D-galacturonate, from Eastman Organic Chemicals (Rochchester, NY); 1,5-α-L-arabinan from sugar beets, pectic galactan from lupin, glucomannan from konjac, and xyloglucan from tamarind, all obtained from Megazyme (Warriewood, Australia); rhamnogalacturonan I from sycamore cell culture obtained from the Complex Carbohydrate Research Center (Athens, GA); xylan obtained from Koch Light Labs Ltd. (Bucks, England); and poly(α-D-galacturonic acid) from oranges, obtained from Sigma (St. Louis, MO). Calcium pectate was prepared by dispersing poly(α-D-galacturonic acid) in deionized water at 50 °C (0.5 mg/mL) and titration to pH 7.0 with 3 mM Ca(OH)$_2$. After cooling to room temperature, the resulting precipitate was centrifuged (1 h at 2000 rpm), the supernatant decanted off, and the residue dried overnight in a vacuum oven at 40 °C. The phenolic reference samples were syringaldehyde obtained from Aldrich Chemical Co. (St. Louis, MO), ferulic acid from K&K Laboratories (Plainview, NY), and lignin, as ball-milled enzyme-treated lignin from Eastern cottonwood (*Populus deltoides*), supplied by the National Renewable Energy Laboratory (Golden, CO). The wax/fatty acid ester reference sample was stearyl arachidate (low melting solid) obtained from Nu-Chek-Prep, Inc. (Philadelphia, PA). All reference samples were powdered solids, except as noted.

**Spectra.** All spectra were obtained using a Nicolet 950 FT-Raman spectrometer (Nicolet Instrument Corp., Madison, WI) that was equipped with a 1.064 $\mu$m Nd:YAG laser source, an LN$_2$-cooled Ge detector, a notch rejection filter for 1.064 $\mu$m, and a CaF$_2$ beam splitter. The Raman spectra of all samples were collected using 180° reflective geometry. All reference samples were hand pressed into a 3 mm gold die and were obtained by employing a lens to defocus the excitation laser beam at the sample. The spectra of flax stems and parts were obtained by using a focused excitation laser beam after they were positioned in a spring-loaded, vise-style, solid sample holder. Six repetitions of each flax sample at different positions along the length were obtained. All spectra were collected over the entire Raman shift range of 3990 to $-3006$ cm$^{-1}$ (default range of the spectrometer) and corrected using a white light background spectrum of KBr. The reference sample spectra, with the exception of the calcium salt of poly-(galacturonic acid), were collected with a resolution of 4 cm$^{-1}$ over 16K data points and averaged over 512 scans using the maximum amount of laser output power possible without charring the sample (250 mW$-1$ W). The spectrum of the calcium salt of poly(α-D-galacturonic acid) was collected with a resolution of 8 cm$^{-1}$ and 2048 scans at 50 mW of laser power. This sample required the use of lower laser power, to prevent charring, and thus required more scans to achieve a signal/noise ratio comparable to that of the other reference samples. The spectra of the flax materials were collected with a resolution of 16 cm$^{-1}$ over 4K data points, zero-filled to 8K, and averaged over 256 scans using 630 mW of laser output power for each sample.

**Software/Data Processing.** All spectra were collected, corrected, and Fourier transformed with Happ-Genzel apodization using Omnic version 3.1 (Nicolet Instrument Corp.). Spectral files of reference samples and averaged flax spectra were imported into GRAMS/32 (Galactic Industries Corp., Salem, NH), where the files were truncated to the range of 3200$-200$ cm$^{-1}$ prior to use. Baseline removal was conducted
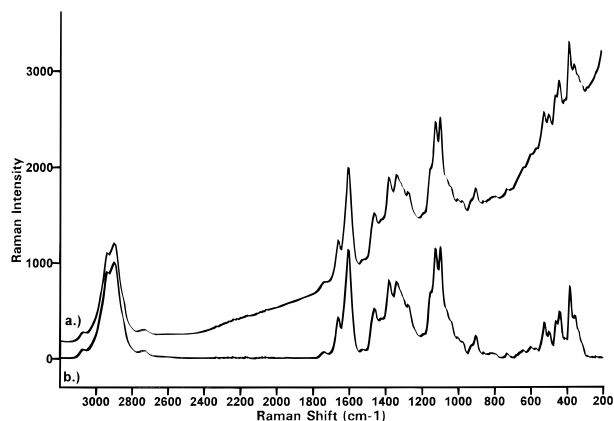


**Figure 1.** Averaged NIR-FT-Raman spectra of intact whole stem of Ariane flax: (a) original and (b) baseline removed with BAS routine of RAZOR program based on a 10 cm$^{-1}$ Lorentzian peak shape as a reference.

using the BAS subroutine contained in the program RAZOR, designed for GRAMS/386 (version 2.0, Spectrum Square Associates, Inc., Ithaca, NY). BAS identifies peaks using a maximum likelihood/Bayesian peak-picker and connects baseline segments with a straight line under the peaks. The settings used were as follows: noise statistics, normal; peak threshold, S/N = 3; window peak, 10 cm$^{-1}$ full-width at half-height Lorenztian; and baseline sensitivity, 1. This procedure was automated with a locally developed program written in Array Basic (Galactic Industries Corp.). Each baseline was further corrected in the region 2400$-2000$ cm$^{-1}$ by a linear multipoint method to remove the residual effects of water vapor that occur in this region. The resulting spectra were then normalized to unit area in GRAMS/32 by dividing each spectrum by its total integrated area.

## RESULTS AND DISCUSSION

Figure 1 shows the spectral results obtained on a typical flax sample, after correction against KBr, before (Figure 1a) and after baseline corrections (Figure 1b). The spectrum chosen was that of the averaged set of spectra taken at the center of each 7.5 cm segment along the entire length of an intact flax stem (minus the root or first 7.5 cm and minus the top, starting at the branch point for the seed head). The left limit of these spectra was chosen as 3200 cm$^{-1}$ to avoid any effects of the distortion of the spectrum that occur at higher Raman shift due to the correction against the white light spectrum of KBr and interference due to water vapor. This correction was found necessary to maintain the accuracy of Raman scattering intensities throughout the spectrum and to eliminate instrumental effects. In general, the scattering intensities of the corrected spectra are lower than those of the uncorrected spectra and at Raman shifts >1600 cm$^{-1}$ are further reduced relative to those greater than this position. The right limit of the spectra was set at 200 cm$^{-1}$, just beyond the point at which no more signals from the sample were observed and where the baseline started to rise sharply due to the laser excitation frequency.

Figure 2a shows the difference between Raman spectra of the midsections taken from two different flax stems. This indicates that sampling several stems would be necessary to characterize a particular bundle of flax. Figure 2b shows the difference between the Raman spectra obtained from the midsection of the flax stem and that of the average of seven spectra taken over the entire length of the flax stem, minus the seed head
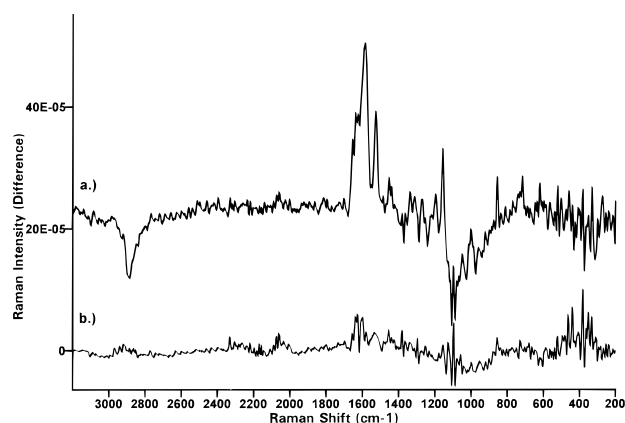
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**Figure 2.** NIR-FT-Raman difference spectra between (a) center section of two different flax stems from a bundle of stems and (b) center section of flax stem minus average over whole stem.

and root. The small differences between these two spectra indicate that the spectrum of the midsection fairly well approximates the average over the entire stem. Thus, subsequent flax sample materials were taken only from the center section of the flax stems. Clearly, the difference between stems is of greater concern in analysis than is the taking the midsection to represent a whole individual stem.

Interpretation of the complex spectra of flax stem and its parts was first attempted by the use of literature assignments of component spectra. However, few applicable spectra could be found under any consistent instrumental conditions, let alone those obtained using NIR-FT-Raman. Thus, a spectral library was built for this study utilizing available reference materials or ones that could be obtained with only minor modifications of available materials. Figures 3 and 4 show the normalized Raman spectra of the polysaccharide reference materials that were chosen to represent the major carbohydrate components found in flax. Figure 3 shows the Raman spectra of cellulose and the hemicellulosic polysaccharide reference materials. Cellulose, the primary carbohydrate component in flax fiber (McDougall, 1993), is represented here by microcrystalline cellulose (Figure 3a). The hemicellulosic polysaccharides are represented by glucomannan, xyloglucan, xylan, and arabinan (Figure 3b–e, respectively). These are the major alkaline extractable polysaccharides from the cell walls of flax (McDougall, 1993; Gorshkova et al., 1996). Both glucomannan and xylan may be acetylated (van Hazendonk et al., 1996). Here, the glucomannan is presented in its acetylated form to provide an acetylated example. Arabinan has been found in both the hemicellulosic and pectic fractions of developing flax plants (Gorshkova et al., 1996) and is included here with the spectra of the hemicellulosic polysaccharides from a structural standpoint. Arabinan should also be considered with the pectic carbohydrates, on the basis of its source of isolation.

All of these polysaccharides show Raman bands in the same general regions as cellulose, but with varying intensities. These regions have been assigned for cellulose as the C—H stretch region, from 3000 to 2800 cm$^{-1}$; the OH/CH deformation and CH/CH$_2$ wag region, from ca. 1500–1200 cm$^{-1}$; the C—O stretch and ring mode region, from ca. 1200–950 cm$^{-1}$; and the side-group deformation region for COH, CCH, and OCH, from 950 to 700 cm$^{-1}$, which includes the band from 910

to 890 cm$^{-1}$ due to HCC and HCO bending at the C-6 position (Blackwell et al., 1970; Parker, 1983; Mathlouthi and Koenig, 1986; Wiley and Atalla, 1987a). Below about 700 cm$^{-1}$, vibrations there are distinctive for many polysaccharides, and these are often sensitive to the dihedral angles at the glycosidic linkages. Since all of these vibrations appear to arise from coupled modes of heavy atoms, C—C and C—O stretching, normal-coordinate analysis has been required for their assignment (Mathlouthi and Koenig, 1986; Wiley and Atalla, 1987a). Cellulose displays notable distinctive maxima at 1095 and 379 cm$^{-1}$ (vertical dashed lines in Figure 3). The band at 379 cm$^{-1}$ appears to be unique for crystalline type I (native) cellulose and has been assigned to heavy atom stretching, mostly CCO (Mathlouthi and Koenig, 1986; Wiley and Atalla, 1987a). The band for the C—H stretch in cellulose is strong but is not particularly diagnostic for cellulose in the flax matrix. All of the hemicellulosic polysaccharides considered here have stronger bands for C—H stretch and HCC and HCO bending at C-6, with nearly the same band centers, ca. 2895 and 890 cm$^{-1}$ (vertical long/short dashed lines in Figure 3), except in the case of arabinan. These bands are shifted in the case of arabinan, probably due to the change from the β-D-pyranoside to the α-L-furanoside configuration. The more intense 890 cm$^{-1}$ signal for hemicellulosic polysaccharides may be due to the less ordered or less crystalline nature of these polymers, as has been observed in mid-infrared spectra for cellulose type II (Michell, 1991). This band has been noted to be weak in *Valonia* cellulose, which has large crystallites, and stronger in ramie and bacterial cellulose, which have smaller crystallites (Wiley and Atalla, 1987b). All of the hemicellulosic polysaccharides display an additional medium to strong intensity band in the 515–470 cm$^{-1}$ region (hatched area in Figure 3), which appears to be diagnostic for them. The acetylated glucomannan spectrum (Figure 3b) displays an additional signal at 1728 cm$^{-1}$, outside the regions mentioned, which is due to the carbonyl of the acetyl group (Parker, 1983). This peak appears to be sufficiently separated from other carbonyl bands to be diagnostic for acetylated hemicellulosic polysaccharides. The arabinan spectrum shows a broad band with a maximum at 1608 cm$^{-1}$ that is most likely due to the carbonyl signal from the residual galacturonate (6%, according to Megazyme's analysis) associated with this sample.

The existence of multiple forms of pectin in flax required a detailed spectroscopic investigation of specific model compounds for flax pectin. The cortex cell walls of mature flax have been reported to be enriched in methylated pectins and homogalacturonans (Morvan et al., 1988). Pectin occurs as partially esterified (1→4) linked α-D-galacturonic acid residues that are occasionally (1→2) branched with L-rhamnose residues. Non-methyl-esterified galacturonans are free to complex with metal ions and do so by preferentially aggregating with Ca$^{2+}$ forming gels (Rees et al., 1982). A neutral galactan, in addition to the arabinan already discussed, is also found in flax and is typically isolated in the pectic fraction. In the strictest sense, this galactan is not a pectin (Stephen, 1983). It appears to be primarily found in the phloem cell walls of mature flax as a linear β(1→4) galactan and has been found with a lower molecular weight rhamnogalacturonan, type I, that may serve as the backbone to which the galactan is attached (Davis et al., 1990). These
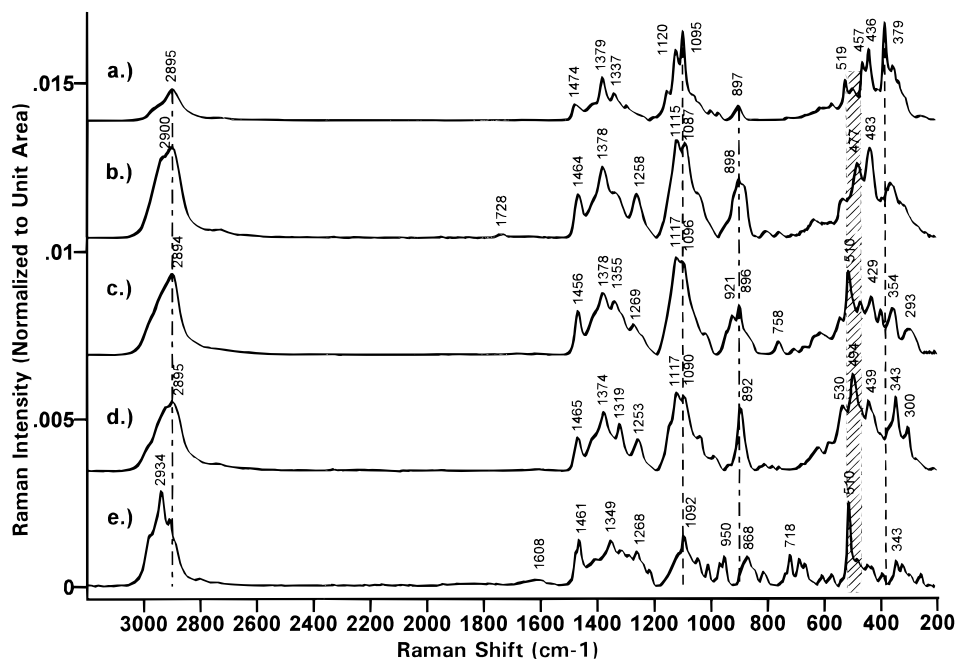
**Figure 3.** Normalized NIR-FT-Raman spectra of neutral polysaccharides that have been found in flax: (a) cellulose (microcrystalline), (b) acetylated glucomannan, (c) xylan, (d) xyloglucan, and (e) arabinan.
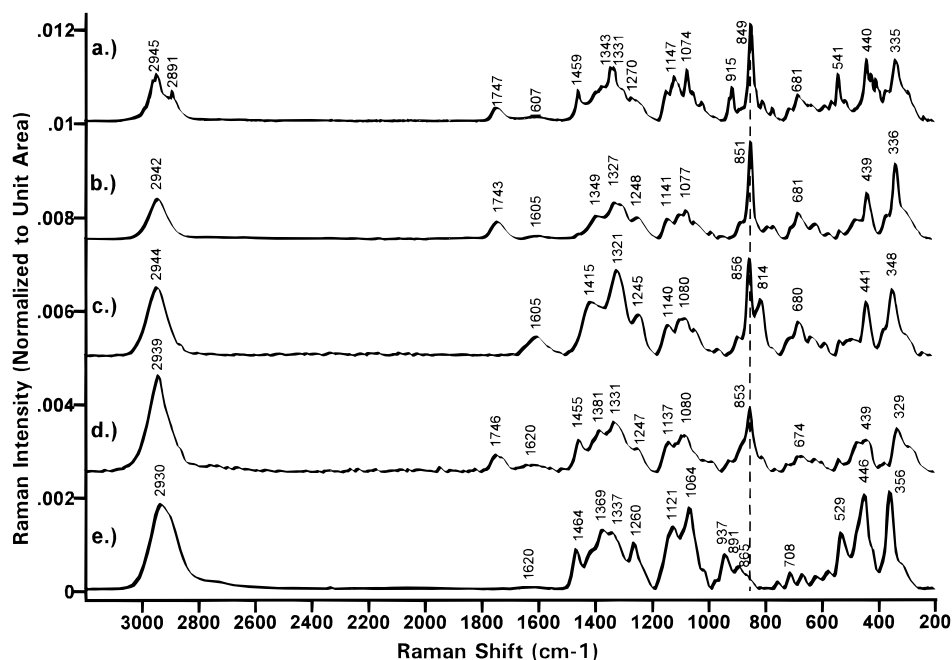


**Figure 4.** Normalized NIR-FT-Raman spectra of pectic polysaccharides that have been found in flax: (a) methyl α-D-polygalacturonate, (b) α-D-polygalacturonic acid, (c) calcium pectate (dried gel), (d) rhamnogalacturonan I, and (e) pectic galactan.

structures are represented by the reference samples: calcium pectate, pectic galactan, methyl α-D-polygalacturonate, poly(α-D-galacturonic acid), and rhamnogalacturonan I.

Figure 4 shows the normalized Raman spectra of these pectic or pectin-associated polysaccharide reference materials. All of these spectra, except the galactan (Figure 4e), show an intense signal ca. 850 cm$^{-1}$ that has been assigned to the COC antisymmetric stretch of the glycosidic linkage in acidic pectins (Séné et al., 1994). The galactan displays only a weak shoulder at 865 cm$^{-1}$. This finding is consistent with the fact that this pectic galactan, obtained by alkaline treatment of lupin fiber, contains only ≈5.4% galacturonic acid (by

Megazyme's gas−liquid chromatographic analysis). It appears from this spectrum that this level of galacturonic acid is nearing the limit of detection for NIR-FT-Raman. There is still some signal in the broad carbonyl band centered ca. 1620 cm$^{-1}$ that suggests that the galacturonic acid may be present as a salt in this sample. The conversion to the calcium salt (Figure 4c) from the acid (Figure 4b) gives rise to a shift from the C=O stretch at 1743 cm$^{-1}$ to an asymmetric OCO stretch at 1605 cm$^{-1}$ and a symmetric OCO at 1415 cm$^{-1}$ (Colthup et al., 1990). In addition, in calcium pectate the signal ca. 850 cm$^{-1}$ is reduced in intensity and a new signal appears at 814 cm$^{-1}$. The C=O stretch, observed at 1747 cm$^{-1}$, for the methyl ester of

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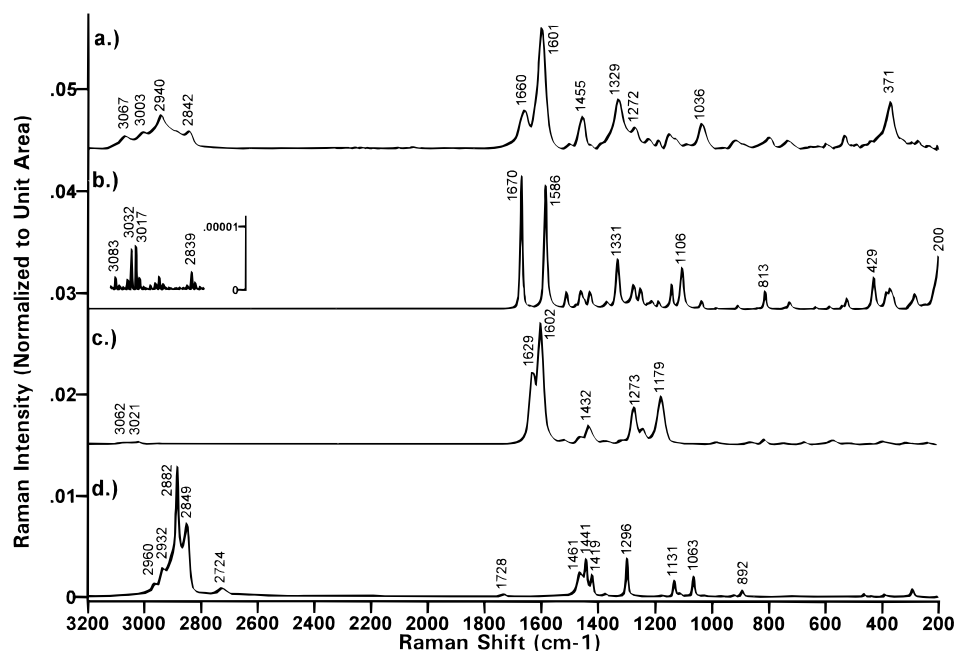*J. Agric. Food Chem.,* Vol. 46, No. 3, 1998 **995**



**Figure 5.** Normalized NIR-FT-Raman spectra of phenolic compounds and a waxy component that may be present in flax: (a) lignin, (b) syringaldehyde, (c) ferulic acid, and (e) stearyl arachidate (low melting solid).

poly($\alpha$-D-galacturonic acid) (Figure 4a) shows little shift from that of the parent acid (Figure 4b). This is as expected, since there is little change in the polarizability in going from the acid to the ester. The methyl ester can be distinguished from the other materials by the presence of bands due to the methyl group: the C−H stretch at 2891 cm$^{-1}$ and the C−H bend at 1459 cm$^{-1}$. In general, the intensity of the C−H stretch in the galacturonates is on the order of that found in cellulose, although shifted to ca. 2943 cm$^{-1}$ on the average, but the C−O stretch at ca. 1100 cm$^{-1}$ is much weaker. The intensity of the C−H stretch for the galactan is more on the order of the hemicellulosic polysaccharides but shifts to around 2930−2939 cm$^{-1}$. Thus, the C−H stretch vibrations for these compounds are not especially diagnostic.

The final reference materials considered were those of aromatic compounds and a fatty acid ester whose Raman spectra are shown in Figure 5. The major types of aromatic compounds that have been detected in these flax samples (Akin et al., 1996) produce extremely sensitive diagnostic signals in the Raman. The most intense and characteristic signal for aromatics is that due to the aromatic ring stretch that occurs at ca. 1600 cm$^{-1}$. In lignin (Figure 5a) this band occurs at 1601 cm$^{-1}$ with an accompanying band, 1660−1650 cm$^{-1}$, which has been assigned to ring-conjugated C=C stretch in the propanoic side chain when the $\gamma$ position is an alcohol, to C=O for the $\gamma$ aldehyde, or to a ring-conjugated keto C=O (Atalla and Agarwal, 1997; Evans, 1991). In syringaldehyde (Figure 5b) the aromatic ring stretch occurs at 1586 cm$^{-1}$ and is accompanied by the HC=O stretch at 1670 cm$^{-1}$. The Raman spectrum of ferulic acid (Figure 5c) displays the aromatic ring stretch at 1602 cm$^{-1}$, almost at the same position as in lignin and has an accompanying signal at 1629 cm$^{-1}$ due to the C=C in the propanoic side chain (Séné et al., 1994). All of these aromatic compounds also display aromatic C−H stretch bands in the region 3100−3000 (Parker, 1983; Evans, 1991). The bands occurring between 3080 and 3050 cm$^{-1}$ are particularly diagnostic

for lignin or aromatic moieties with reduced side chains. The enhancement of other bands due to conjugation causes the aromatic C−H bands to appear relatively weak. Thus, these bands are barely observable in spectra of syringaldehyde and ferulic acid. Figure 5d shows the spectrum of stearyl arachidate (a model for wax). It displays two noticeably intense sharp bands in the C−H stretch region at 2882 and 2849 cm$^{-1}$ due to the CH$_2$ asymmetric and symmetric C−H stretches, respectively; an ester C=O stretch at 1728 cm$^{-1}$; several resolved CH$_2$ deformations ca. 1440 cm$^{-1}$; a single CH$_2$ twist at 1296; a definitive pattern of C−C stretches between 1150 and 1050 cm$^{-1}$; and C−C skeletal stretches 920−890 cm$^{-1}$ (Parker, 1983).

Although the assignments for all of these model compounds are not nearly complete and it is beyond the scope of this work to do so, they suffice to point out the major features that are expected in the Raman spectra of flax stem and its anatomical parts. It is also necessary to note that, although the emphasis here has been on the most diagnostic signals for each model component in the flax matrix, some bands may still be obscured by those of other components. Also, the enhancement factors for some components are much greater than those for other components that may be in much higher concentration. Some feel for this may be gained by comparison of other bands in the reference compounds to an aliphatic C−H band. For example, the height ratio of the band at 1095 cm$^{-1}$ to that at 2894 cm$^{-1}$ in the microcrystalline cellulose spectrum (Figure 3a) is 1.4:1.0, whereas the ratio of the band at 1670 cm$^{-1}$ to the band at 2839 cm$^{-1}$ is 900:1 in syringaldehyde (Figure 5b). In addition, there is the matrix effect itself and the facts that isolation of components usually causes their chemical and/or physical modification and that the reference materials were not obtained from flax (for practical reasons). With these caveats in mind, the spectra of flax stems and their parts may still be compared on a relative basis.

Figure 6 shows the Raman spectra obtained on flax stem, core, bast tissue, and fiber. Comparison of the
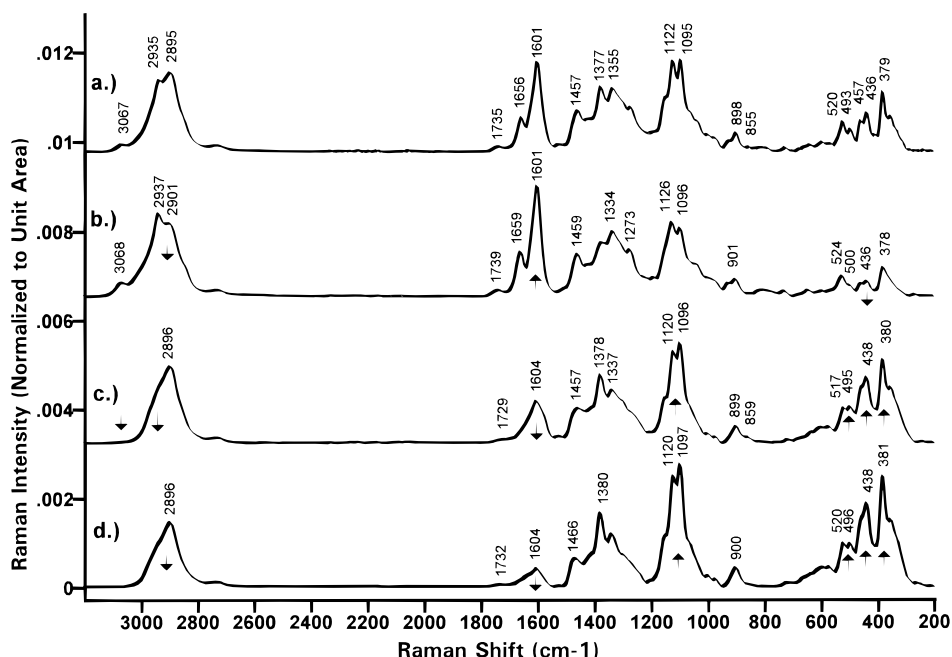
**Figure 6.** Averaged normalized NIR-FT-Raman normalized spectra of (a) whole stem, (b) core, (c) bast, and (d) fibers. Arrows in spectra b–d show increase (↑) or decrease (↓) of intensity of vibrations relative to spectrum a.

spectrum of the entire stem (Figure 6a) to that of the core (Figure 6b) shows there is a decrease in the intensity of bands due to aliphatic C–H stretch ca. 2900 cm$^{-1}$, C–O stretch ca. 1100 cm$^{-1}$, and all bands in the 500–300 cm$^{-1}$ region and an increase in bands due in the aromatic C–H stretch ca. 3070 cm$^{-1}$, aliphatic C–H stretch ca. 2940 cm$^{-1}$, C=O stretch ca. 1660 cm$^{-1}$, and aromatic ring stretch at 1601 cm$^{-1}$ (note arrows). These observations are consistent with polymeric lignin (see Figure 5a) being primarily present in the core tissue of flax. The signal at ca. 2940 cm$^{-1}$ is probably due to the C–H stretch arising from the propanyl side chain in lignin. Other, less dramatic, differences are also observable. The signal at 855 cm$^{-1}$, just detectable in the spectrum of the stem, is absent from that of the core tissue. This difference most likely indicates that there is essentially no true pectic material present in the core tissue. Also, the band at 1735 cm$^{-1}$ is of about the same intensity in the stem and core, indicating that they contain about the same amount of acetylated components. This suggests that the hemicellulose content is these two materials is about the same. This is supported by the fact that the intensity of the band at 901 cm$^{-1}$ is the same, even though the cellulose content is obviously reduced. This is not evident from the band at 500 cm$^{-1}$, which is typically associated with hemicellulosic polysaccharides, but is reduced in intensity due to the reduction of the underlying bands due to cellulose in this region. The spectrum of the bast tissue (Figure 6c) shows a dramatic decrease in the intensity of bands that can be associated with aromatics at ca. 3070, 2940, 1660, and 1600 cm$^{-1}$, relative to the stem and core tissue. Considering the relative strength intensities in the Raman spectrum of the lignin model compound, the reduction in intensity of bands at ca. 3070 and 1660 cm$^{-1}$ would appear to indicate that very little lignin, if any, exists in the bast tissue. However, the presence of monomers or oligomers that may be breakdown residues, like syringaldehyde or ferulic acid and other aromatics such as tannins and pigments, plus possible changes in the degree of lignin polymerization

cannot be ruled out. In fact, gas–liquid chromatographic results (Akin et al., 1996) indicate that aromatic constituents are present in the bast tissue. In addition, preliminary HPLC results (Gamble et al., 1998) indicate there are >28 different aromatic constituents in the bast tissue of flax. The spectrum of the bast tissue also indicates large increases in the bands at ca. 1120 and 1095 cm$^{-1}$ and in the 550–300 cm$^{-1}$ region, indicating large increases in cellulose in the bast tissue relative to the core. A hemicellulosic component remains in the bast as indicated by the intensity of the bands at 1729, 899, and 495 cm$^{-1}$. The presence of acidic pectin is also evident by the signal observed at 859 cm$^{-1}$, and calcium pectate is a probable contributor to the band at 1604 cm$^{-1}$, as was shown in Figure 4c. All of these observations are consistent with previously proposed dicot cell wall models (Carpita, 1990). The Raman spectrum of the fiber (Figure 6d), essentially free from cuticular and epidermal tissue, appears to be primarily that of cellulose (Figure 3a). However, notable signal intensities occur at 1732, 1604, and 496 cm$^{-1}$ that still provide evidence for the presence of acetylated hemicellulosic polysaccharides. The signal at 1604 cm$^{-1}$ may be due to residual low molecular weight phenolic pigments (Love et al., 1994), tannins, and/or small amounts of lignin that are lodged in the cell corners between some fiber bundles (Akin et al., 1996) and amy also be due to residual calcium pectate. This indicates that there are aromatics or pectins and hemicellulosic polysaccharides associated with these fibers that cannot be physically removed.

Figure 7a shows the Raman spectrum of the cuticular/epidermal tissue that was carefully scraped away from the stem so as to be as fiber-free as possible. The most intense signal in this spectrum occurs at 1604 cm$^{-1}$ and thus indicates the presence of aromatics and probably calcium pectate. Light microscopic investigation of this material, after staining with phloroglucinol–HCl, indicated patches of red-stained regions, suggesting that lignin may be present (Wardrop, 1971). Some of this aromatic signal probably arose from scraping across
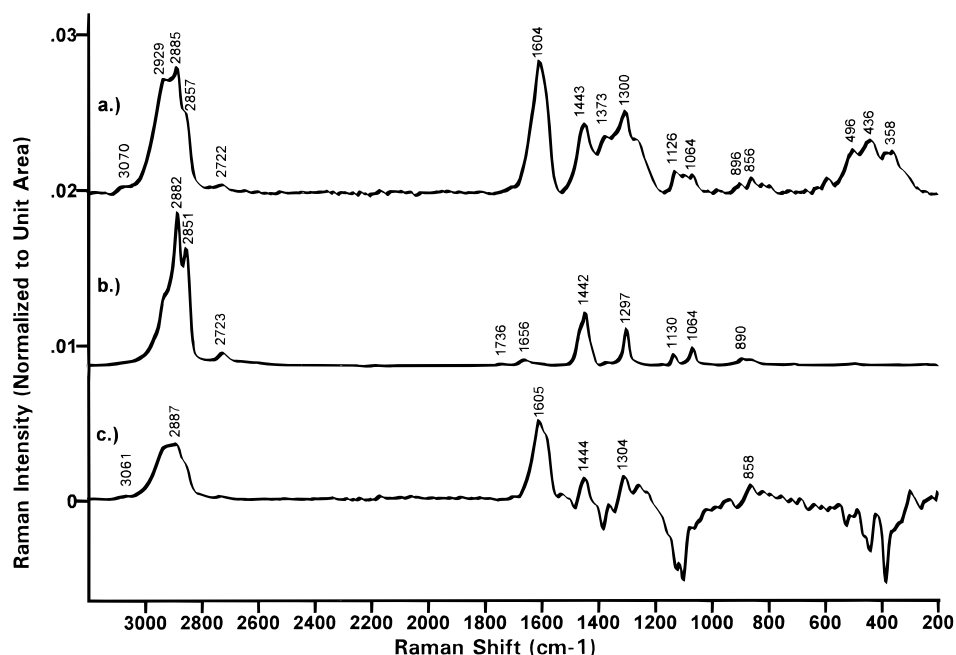
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**Figure 7.** Averaged normalized NIR-FT-Raman spectra of (a) cuticular/epidermal tissue from the surface of the bast, (b) hexane extract bast, and (c) difference spectrum of fiber and bast tissue.

**Table 1. Diagnostic Raman Bands for Major Chemical Components of Flax Stems and Primary Location**

| component | Raman shift$^{a,b}$ (cm$^{-1}$) | primary location(s) (tissue type) |
|---|---|---|
| cellulose | 1120 sn, 1095 sn, 900 w, 380 s | fibers |
| hemicellulosic polysaccharides | 1730 w,$^{c}$ 900 m, 515−470 m | stem and core |
| lignin | 3070 w, 2940 m, 1660 m, 1600 vs | core |
| pectins (acidic) | 1745 m, 850 s | cuticle/epidermis |
| pectate (salt) | 1605 mb, 855 s, 815 m | cuticle/epidermis |
| pectate (methyl) | 2891 m, 1745 m, 1460 m, 850 s, 541 m | cuticle/epidermis |
| waxes or fatty acids/esters (saturated) | 2880−2890 vsn, 2845−2850 mn, 1725 vw,$^{d}$ 1420−1460 m, 1300 m, 1130 m, 1060 m | cuticle/epidermis |

$^{a}$Band maxima rounded to nearest 5 cm$^{-1}$. $^{b}$Nature of bands: b, broad; s, strong; m, medium; n, narrow; v, very; w, weak. $^{c}$Present if acetylated. $^{d}$Absent from alcohols and weaker in acids/esters as hydrocarbon chain lengthens.

lignified midveins that remain from leaves that were initially attached to the stem and thus does not necessarily indicate that lignin is in the cuticle/epidermal tissue. There is no discernible band ca. 1660 cm$^{-1}$ for lignin, but it may be obscured by the plethora of possible flavonoid structures (Dubois and Harborne, 1975), phenolic oligomers, and other non-lignin aromatics that when mixed together give a broad aromatic band. The other intense bands in this spectrum are the C−H stretches at 2929 and 2885 cm$^{-1}$. These are most likely due to waxes and fatty acids/esters. The hexane extract of the stem surface gave the spectrum shown in Figure 7b. This spectrum strongly resembles that of stearyl arachidate (Figure 5d). It appears to be primarily composed of saturated waxes, but it does contain some unsaturated component as evidenced by the signal at 1656 cm$^{-1}$ (Figure 7b). In an attempt to further isolate bands due to the cuticular/epidermal tissue, the spectrum of the fibers (Figure 6d) was subtracted (1:1) from that of the entire bast tissue (Figure 6c). This difference spectrum (Figure 7c) confirmed the existence of waxes/ fatty acids esters in the bast by the bands observed at 2887, 1444, and 1304 cm$^{-1}$ and again indicated the presence of aromatics by the bands at 3061 and 1605 cm$^{-1}$. The subtraction also accentuated the band at 858 cm$^{-1}$, suggesting the presence of uronic acids. There was a general absence of bands due to cellulose (negative bands in Figure 7c).

Table 1 is a summary of the results, giving only the diagnostic Raman vibrations for each of the major chemical components of flax stems and only the primary location where they can be found. The nature of the Raman bands (i.e., line shape and relative intensity) is also given to help in their recognition. The presence of a component can only be established if all the diagnostic bands can be observed in the sample. Although much more work needs to be done to exhaustively account for all of the bands observed in the Raman spectra flax, the information herein should provide a sufficient basis for the employment of NIR-FT-Raman spectroscopy as a quality assessment tool for the evaluation of flax materials. Thus, the focus of our future work in this area will be to use it to assess the chemical nature and (therefore) the quality of flax fibers and other materials produced by various treatment methods.

CONCLUSION

NIR-FT-Raman spectroscopy has been demonstrated to be a rapid, simple means of assessing the chemical nature of flax stems and the location of compounds within specific tissue types. All of the major chemical components of flax can be detected, but with various degrees of sensitivity. This method of analysis should prove exceptionally useful for the chemical identification and quality assessment of a multitude of flax-based materials.

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## ABBREVIATIONS USED

NIR, near-infrared; FT, Fourier-transform; MIR, mid-infrared.

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