# Fourier-Transform Infrared and Raman Spectroscopic Study of Biochemical and Chemical Treatments of Oak Wood (*Quercus rubra*) and Barley (*Hordeum vulgare*) Straw

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FT-IR and Raman spectroscopies were used to investigate the changes in composition and structure of oak wood and barley straw that had been subject to chemical and biochemical treatments. The samples were also analyzed gravimetrically for residual neutral sugar composition and lignin and uronic acid content. The spectroscopic techniques provided complementary information. Changes in the relative proportions of crystalline and amorphous cellulose accompanying (bio)chemical treatment were best reflected in the Raman and DRIFT spectra, respectively. Delignification of both tissues produces bands in the Raman spectra consistent with the lignin oxidation. Treatment of both types of raw material with aqueous acid produced highly colored residues resulting in Raman spectra of limited use due to problems with fluorescence. However, the DRIFT spectra of these tissues did not suffer this problem and provided information on the behavior of lignin (hydrolysis and repolymerization) and the noncellulosic polysaccharides (hydrolysis) in acid conditions. The decreased fluorescence in the Raman spectra of barley straw after alkali extraction is suggested to be due to the removal of the covalently bound cinnamic acids.

**Keywords:** *Fourier transform; infrared; Raman; fiber; cellulose; lignin; noncellulosic polysaccharide; fluorescence*

## INTRODUCTION

The intractability of plant cell walls means that traditional methods of analysis rely heavily on the use of harsh chemicals and derivatization, thereby altering the native cell wall structure and possibly generating artifacts. Several nondestructive spectroscopic techniques have been tried, with limited success, to elucidate structural and compositional information. These include $^{13}C$ CP/MAS NMR (Cyr *et al.*, 1988; Jarvis and Apperley, 1990), near-IR (Reeves, 1988), photoacoustic IR (Gould, 1982), and diffuse reflectance FT-IR (DRIFT) (McGinnis, 1985; Faix, 1986; Michell *et al.*, 1991). DRIFT spectroscopy has proved to be successful as a rapid method for estimating the lignin content of woods (Schultz and Burns, 1990) and pulps (Berben *et al.*, 1987). It has only been within the past decade that FT-Raman spectroscopy has emerged as a useful tool for the plant chemist (Agarwal and Atalla, 1986; Atalla, 1987; Kenton and Rubinovitz, 1990; Evans, 1991; Atalla *et al.*, 1992).

Although the Raman frequency shift and the vibrational frequency of a certain vibration in a molecule are identical, the selection rules for Raman and IR spectroscopies are not. A molecule absorbs IR radiation only when the permanent electric dipole changes during the molecular vibration, whereas Raman spectroscopy is a light scattering process and is due to the oscillation of the induced dipole moment or the molecular polarizability. This can be summarized for IR as more polar bonds give greater intensity and for Raman as less polar bonds (and therefore more polarizable) give greater scattering. Consequently, symmetric vibrations usually give rise to strong Raman bands, nonsymmetric ones being weak and sometimes unobservable. Nonsymmetric vibrations are stronger in the IR spectrum. For example, carbon–carbon double bonds are weak in the IR and strong in the Raman; carbonyl groups are strong in the IR and weak in the Raman.

Until recently, the use of Raman spectroscopy in the study of woods and biological materials has, in general, been limited due to the problem of fluorescence. This has been partly overcome by the shift to lower energy laser sources, principally by neodium:YAG (Nd:YAG) lasing at 1064 nm (Evans, 1991). Of course, at this wavelength the use of interferometry and FT methods can be employed, enabling efficient radiation collection and considerable convenience in the rapid collection of data with the further advantage of trivial sampling. It can be seen, therefore, that a combination of DRIFT and FT-Raman spectroscopies should provide a more complete and detailed view of plant cell wall structure and composition.

Two different types of plant cell walls were studied; woody (oak wood) and nonwoody (barley straw). Both are extremely important raw materials. Oak wood is a hard wood and such woods are used in both the building and pulp and paper industries. During processing it is exposed to a range of chemical and biochemical treatments in the form of preservatives, biodeterioration, and chemical modification (pulping). Barley straw has predominantly been used as a feed for ruminants. However, increased awareness concerning global deforestation has meant that alternatives to wood as a raw material for pulp and paper are being sought. Europe is currently overproducing cereals (Ministry of Agriculture, Forestry and Fisheries, 1994), which means that there is a surfeit of straw. Since, in the United Kingdom at least, the straw cannot be simply burned in the field, another method of disposal or use must be found.

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Rather than destroy a valuable raw material, using it for pulp and paper production would be more environmentally and economically beneficial.

For both woody and nonwoody tissues the changes in cell wall structure and composition accompanying processing largely dictate the end use performance. The ability to accurately monitor these changes is, therefore, vital. Here we report on the efficacy of DRIFT and Raman spectroscopies as techniques for studying changes in plant cell wall structure and composition following (bio)chemical treatments.

## EXPERIMENTAL PROCEDURES

All chemicals used were of Analar grade and purchased from Merck/BDH (Glasgow, Scotland). Celluclast, a bacterial cellulase (Novo-Nordisk, Copenhagen, Denmark), was partially purified according to the method of Fry (1987). Mature oak wood chips and barley straw were hammer-milled to pass a 1 mm sieve and then washed with distilled water ($dH_2O$) until free of color and carbohydrate (Dubois *et al.*, 1956). The suspensions were filtered and the residues oven-dried at 50 °C for 24 h. The dried residues were Soxhlet extracted with freshly distilled diethyl ether for 8 h and then air-dried for 16 h. These residues were used for all subsequent extractions.

**Treatments.** *Cellulase.* The raw fiber (10.0 g) was suspended in 500 mL of 2% Celluclast in 20 mM sodium acetate, pH 4.5. The suspension was stirred at 30 °C for 24 h and then filtered. The residue was washed with $dH_2O$ until the washings were free of carbohydrate and then freeze-dried.

*Delignification.* Delignification was carried out according to the acidic sodium chlorite method of Wise *et al.* (1946). Delignifications were achieved with the addition of 2 and 4 equiv of reagents, respectively.

*0.1, 1.0, 4.0 M NaOH/0.1, 1.0 M $H_2SO_4$.* The raw fiber (10.0 g) was suspended in 500 mL of 0.1 M NaOH and stirred at room temperature for 18 h. The mixture was filtered and the residue washed with $dH_2O$ until the washings were of neutral pH. The residue was then freeze-dried. The same procedure was used for treatment with 1.0/4.0 M NaOH and 0.1/1.0 M $H_2SO_4$.

*Acid Detergent.* The raw fiber (10.0 g) was suspended in 500 mL of 2% cetyltrimethylammonium bromide (CTAB) in 0.5 M $H_2SO_4$. The suspension was heated to reflux for 4 h and, once cooled, filtered and the residue washed with $dH_2O$ until the washings were of neutral pH. The residue was then freeze-dried.

**Analyses.** Residues were hydrolyzed with 72% sulfuric acid as described by Saeman *et al.* (1954) and the monosaccharides analyzed as their peracetylated alditol derivatives on a HP 5980 II gas chromatograph using a 25 m × 0.53 mm capillary column with a 0.5 μm BP-225 bonded phase (SGE Ltd., U.K.). Lignin was determined using the acetyl bromide method as described by Morrison (1972). Uronic acid was determined according to the method of Filisetti-Cozzi and Carpita (1991).

**Spectroscopy.** *DRIFT.* DRIFT spectra were acquired on a Bruker IFS 66 FT-IR spectrometer (Bruker Spectrospin, Coventry, U.K.) using a diffuse reflectance cell (Graseby Specac, U.K.). The background was finely ground KBr. Three hundred scans were accumulated prior to Fourier transformation. All spectra were recorded at 4 $cm^{-1}$ resolution and vector-normalized after Fourier transformation.

*Raman.* Raman spectra were recorded using a Perkin-Elmer 1700 series Raman/IR spectrometer (Perkin-Elmer Ltd., Beaconsfield, U.K.), incorporating a Spectron Model 301 cw Nd:YAG laser operating at 1064 nm, and an InGsAs detector. Samples were hand-pressed into pellets, and spectra were recorded using 180° backscattering at 250 mW, 4 $cm^{-1}$ resolution, and 300 scans.

## RESULTS AND DISCUSSION

**Oak Wood.** The DRIFT and Raman spectra of untreated oak wood are shown in Figure 1a,c, respectively. Each contains a distinctly different pattern of absorbances. For example, the DRIFT spectrum contains distinct ester carbonyl absorbances at 1740 and 1260 $cm^{-1}$ (Faix, 1992), whereas in the Raman spectrum they are present with much reduced intensities. The lignin absorbances in the DRIFT spectrum, at 1595 and 1510 $cm^{-1}$, are of similar intensity, whereas in the Raman spectrum the former peak is very intense while the latter is reduced to little more than a bump. The absorbance at 1595 $cm^{-1}$ is reported to be associated with an aromatic ring stretch that is strongly associated with the aromatic C–O stretching mode (Sarkanen *et al.*, 1967). The large intensity of this absorbance is in agreement with the type of lignin present in oak wood. Oak wood is a hardwood, the lignins of which are composed of both syringyl and guaiacyl aromatic rings (ratio 1:1 to 1:5), whereas softwoods contain almost exclusively guaiacyl rings (Monties, 1989; Sjoström, 1993). This additional proportion of methoxyl groups from the syringyl rings enhances the 1595 $cm^{-1}$ absorbance in the Raman spectrum. Although the absorbance at 1510 $cm^{-1}$ is also reported to be a ring stretch vibration (Faix, 1992), it is virtually absent in the Raman spectrum, suggesting that it is associated with a polar, or asymmetric, ring stretch.

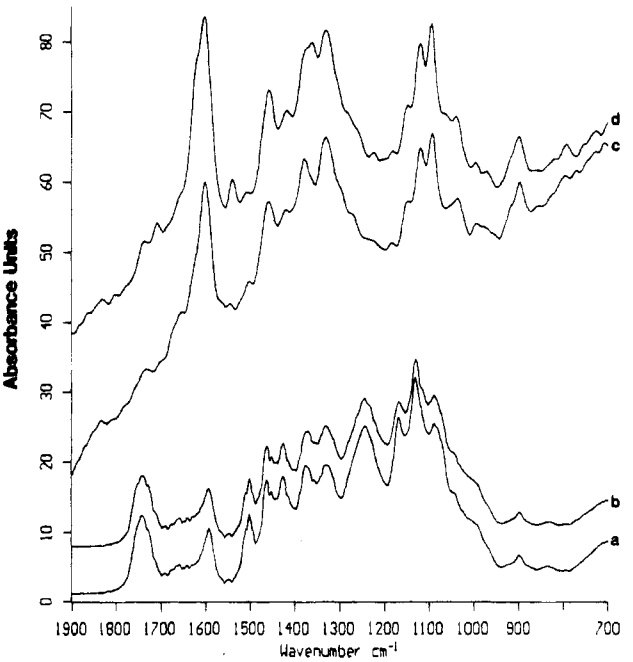


**Figure 1.** DRIFT (a, b) and Raman (c, d) spectra of untreated and cellulase-treated oak wood, respectively.

The cellulose-related absorbances in the Raman and DRIFT spectra are seen at 1162, 1130, 1098, and 900 $cm^{-1}$ (Agarwal and Atalla, 1987; Atalla *et al.*, 1992; Gilbert *et al.*, 1993). It has been reported that the absorbance at 900 $cm^{-1}$ is associated with the antisymmetric out-of-phase ring stretch of amorphous cellulose (Michell, 1990). Although not quite as evident as for the lignin absorbances, the inverse relationship is still clearly seen.

The remaining absorbances are associated with the C–H (1500–1300) $cm^{-1}$ and C–O (1300–900 $cm^{-1}$) vibrations of polysaccharides and lignin side chains and methoxyls. The C–H absorbances are more clearly resolved in the DRIFT spectrum, while the ether absorbances are masked by those of C=O (1260 $cm^{-1}$) and O–H (1280–1220 $cm^{-1}$).

Treatment of oak wood with cellulase had minimal effect on the residual composition (Table 1) and was limited to a reduction of residual anhydroglucose by 2.4%. In the corresponding DRIFT spectra (Figure 1b),

**Table 1. Residual Neutral Sugar Composition and Uronic Acid and Lignin Contents of Untreated and Treated Oak Wood and Barley Straw Residues**

| treatment | Ara[a] | Xyl | Man | Gal | Glc | lignin[b] | uronic acid[b] | yield[c] |
|---|---|---|---|---|---|---|---|---|
| | | | | Oak | | | | |
| untreated | 2.2 | 28.6 | 1.2 | 1.4 | 66.6 | 20.5 | 0.6 | 100.0 |
| cellulase | 3.1 | 30.5 | 0.9 | 1.3 | 64.2 | 22.0 | 1.1 | 97.0 |
| delig (2 equiv) | 2.6 | 24.6 | 0.9 | 4.0 | 67.9 | 16.3 | 0.9 | 86.6 |
| delig (4 equiv) | 2.7 | 18.8 | 1.1 | 3.3 | 74.1 | 10.5 | 0.8 | 79.7 |
| 0.1 M $H_2SO_4$ | nd[d] | 23.1 | 1.1 | 2.9 | 72.8 | 21.2 | nd | 93.4 |
| 1.0 M $H_2SO_4$ | 0.7 | 17.3 | 1.2 | nd | 80.8 | 25.0 | nd | 88.3 |
| acid detergent | 1.1 | 18.2 | 1.1 | nd | 79.6 | 19.5 | 0.6 | 77.4 |
| 0.1 M NaOH | 3.6 | 34.0 | 2.1 | 4.8 | 55.5 | 22.0 | 0.6 | 94.3 |
| 1.0 M NaOH | 3.0 | 24.0 | 1.0 | 2.5 | 69.5 | 21.0 | 0.4 | 89.5 |
| 4.0 M NaOH | 2.4 | 11.9 | 0.6 | 3.0 | 82.1 | 20.7 | 0.2 | 75.5 |
| | | | | Barley Straw | | | | |
| untreated | 8.8 | 31.1 | 1.7 | 1.6 | 56.3 | 16.5 | nd | 100.0 |
| cellulase | 9.8 | 31.8 | 2.2 | 2.0 | 54.3 | 15.5 | nd | 98.0 |
| delig (2 equiv) | 10.7 | 34.6 | nd | 0.6 | 54.1 | 8.0 | nd | 89.5 |
| delig (4 equiv) | 10.0 | 31.6 | nd | nd | 58.4 | 0.4 | nd | 80.1 |
| 0.1 M $H_2SO_4$ | 5.0 | 29.8 | 0.4 | 0.4 | 64.4 | 16.8 | nd | 93.0 |
| 1.0 M $H_2SO_4$ | 3.4 | 17.5 | 1.3 | nd | 77.8 | 17.4 | nd | 81.3 |
| acid detergent | 5.2 | 17.6 | 1.6 | nd | 75.6 | 11.2 | nd | 76.4 |
| 0.1 M NaOH | 9.7 | 31.6 | 1.2 | 2.0 | 55.5 | 11.5 | nd | 94.0 |
| 1.0 M NaOH | 7.6 | 21.6 | 1.6 | 2.3 | 66.9 | 9.4 | nd | 81.8 |
| 4.0 M NaOH | 12.1 | 14.2 | 1.4 | 1.2 | 71.7 | 8.4 | nd | 63.4 |

[a] These values are expressed as percent of the total neutral sugars and are a mean of duplicates. The error is ±0.4%. [b] These values are expressed as percent dry weight and are the mean of triplicates. The errors are ±0.4% (lignin) and ±0.2% (uronic acid). [c] The yields are expressed as a percent of the original material. [d] nd, not determined.

a slight reduction in intensity and broadening of the absorbance at 900 $cm^{-1}$ is seen. This increase in the proportion of crystalline cellulose is seen in the Raman spectrum as an increase in the relative intensity of the peak at 1098 $cm^{-1}$. The reduction in cellulose produces a relative increase in the lignin content, which is reflected in both the DRIFT and Raman spectra by increased intensities of the absorbances at 1595 and 1220 $cm^{-1}$ (aryl ether C–O stretch), respectively.

Not surprisingly, the removal of some cellulose has affected the cell wall polysaccharide interactions. This is seen in the DRIFT and Raman spectra (Figure 1b,d) as a broadening of the C–H stretch absorbance around 1380 $cm^{-1}$ with a shift of the maximum to 1370 $cm^{-1}$.

Treatment of oak wood with increasing concentrations of the acidic sodium chlorite produced significant changes in both the residual composition (Table 1) and spectra (Figure 2). The addition of 2 and 4 equiv of reagent reduced the residual lignin content of 20.5% to 16.2 and 10.5%, respectively. In the corresponding DRIFT (Figure 2b,c) and Raman (Figure 2e,f) spectra this is seen as a reduction in intensity at 1595 and 1510 $cm^{-1}$.

Prolonged treatment with acidic sodium chlorite has been reported to remove noncellulosic polysaccharides (NCPs) as well as lignin (Morrison, 1975). The analytical data confirm the removal of NCPs is largely restricted to a reduction in residual xylose and, to a lesser extent, galactose. The corresponding relative increase in the proportion of cellulose accounts for the increase in intensity of the bands at 1130 and 1098 $cm^{-1}$ in the Raman spectra (Figure 3e,f).

Another feature of delignification is the increase in intensity of the general carbonyl absorbances at 1780–1640 and 1260 $cm^{-1}$. In the DRIFT spectra the absorbances become broader. Also, in the Raman spectra, the bands, although weak, increase in intensity and become resolved into peaks at 1730, 1670, and 1640 $cm^{-1}$ representing conjugated esters, aldehydes, and ketones, respectively (Evans, 1991; Kemp, 1991). This

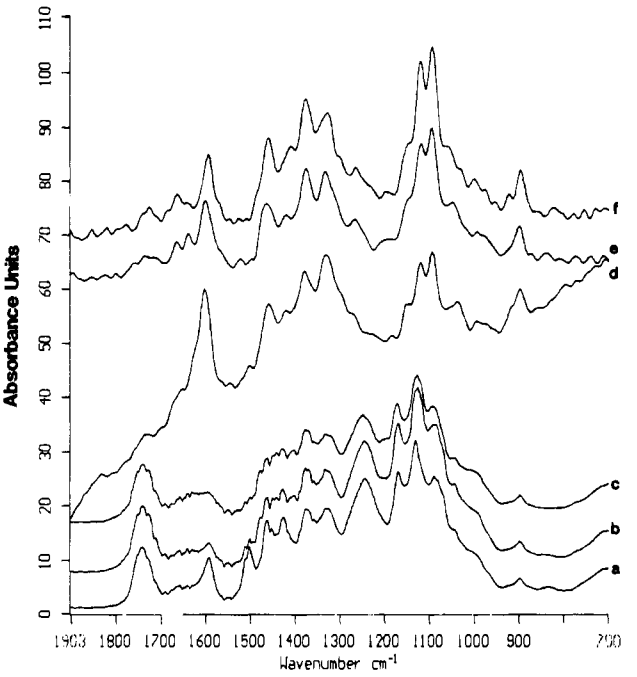


**Figure 2.** DRIFT (a, b, and c) and Raman (d, e, and f) spectra of untreated oak wood and of oak wood after treatment with 2 and 4 equiv of acidic sodium chlorite, respectively.

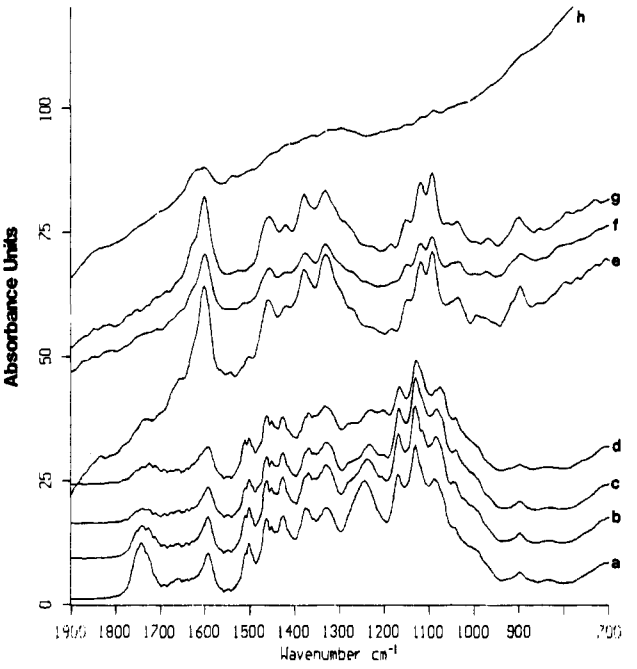


**Figure 3.** DRIFT (a, b, c, and d) and Raman (e, f, g, and h) spectra of untreated oak wood and of oak wood after treatment with 0.1 M $H_2SO_4$, acid detergent and 1.0 M $H_2SO_4$, respectively.

is in agreement with the proposed mode of action of sodium chlorite in acid, whereby chlorine and the chloronium ion ($Cl^+$) react with lignin to form carbonyl-bearing moieties (Adler, 1977; Gierer, 1986).

Treatment of oak wood with increasing concentrations of $H_2SO_4$ produced a progressive darkening of the already brown residue due to the acid-promoted lignin polymerization and chromophore formation (Stewart and Morrison, 1993). The further polymerization of lignin produces a dark polyconjugated macromolecule which fluoresces in the Raman, giving poorly resolved spectra (Figure 3e,g). If the sample is highly colored, energy is absorbed and sample deterioration (burning) occurs (Maddams and Royaud, 1990). There is virtually no definition in the Raman spectrum after 1.0 M $H_2$-

$SO_4$ treatment. However, sample color does not cause problems in the acquisition of DRIFT spectra (Figure 3a–d).

The most obvious feature after treatment with 0.1 M $H_2SO_4$ is a reduction in the ester absorbances (1740 and 1260 $cm^{-1}$). This, in conjunction with a reduction in the xylose content of almost 20% and a complete removal of uronic acid, suggests that the principal effect has been the removal of xylans with their associated sugar residues. Since 4-*O*-methylglucuronoxylan is reported to constitute 15–30% of hardwood dry matter (Pettersen, 1984), it seems probable that this is the polymer extracted by treatment with 0.1 M $H_2SO_4$. The reduction in arabinose may be due to the extraction of middle lamella and primary wall pectins, which are reported to contain arabinose (Timell, 1965). The uronic acid level of the untreated residue was somewhat lower than that reported by Fengel and Wegner (1984).

Both residual lignin and cellulose levels were affected by acid treatment. Extraction with 0.1 and 1.0 M $H_2SO_4$ increased the residual lignin contents by 3 and 22%, respectively. However, treatment with 0.5 M $H_2SO_4$ containing 2% CTAB, a cationic detergent, reduced the residual lignin content by 5%. This apparent anomaly is due to the behavior of lignin in acid: a balance between the kinetics of hydrolysis (depolymerization) and polymerization reactions. As the acid concentration is increased, polymerization predominates, producing a more tightly cross-linked lignin which, in conjunction with the concurrent NCP hydrolysis, results in a relative increase in residual lignin content. However, the introduction of a detergent allows the hydrolyzed lignin fragments to remain solubilized in a detergent micelle and excluded from repolymerization, giving a net reduction in residual lignin content. This is in agreement with the acid detergent studies of Hatfield *et al.* (1994) and is reflected in the corresponding DRIFT spectra (Figure 3c) as a reduction in absorbance at 1595 and 1510 $cm^{-1}$.

The DRIFT spectra also suggest that the relative proportion of cellulose has increased. Treatment with increasing acid concentration is accompanied by a decrease in the absorbance at 900 $cm^{-1}$, suggesting the hydrolysis of amorphous cellulose. This removal is not reflected in the residual neutral sugar content (RNSC), although it may have been masked by the loss of residual xylose (as glucuronoxylan).

Extraction with alkali produced more defined Raman spectra (Figure 4) than those obtained after treatment with acid. These, and the DRIFT spectra, suggest that the main effect of alkali extraction was removal of glucuronoxylan. The NCP-associated carbonyl absorbances at 1740 and 1260 $cm^{-1}$ are greatly reduced in intensity after treatment with 0.1 M NaOH, reflecting the lability of esters to alkali (Stewart and Morrison, 1993). Increasing the NaOH concentration removes these absorbances. In his FT-IR study of hardwood and softwood pulping, Michell (1988) also reported such losses in absorbance. This is reflected in the RNSC as a progressive loss in residual xylose and uronic acid (Table 1).

The Raman, and to a lesser extent, DRIFT spectra of the alkali-treated residues (Figure 4) show an increasing intensity of the band at 1660 $cm^{-1}$. This is due to the reaction of lignin and NaOH to produce benzylic carbonyl compounds (Gierer, 1990; Evans, 1991). Although carbonyl vibrations are not normally seen in the Raman, benzylic carbonyls have a reduced dipole, as a result of aromatic ring conjugation; therefore, the vibration is present. The other carbonyl absorbances in the DRIFT

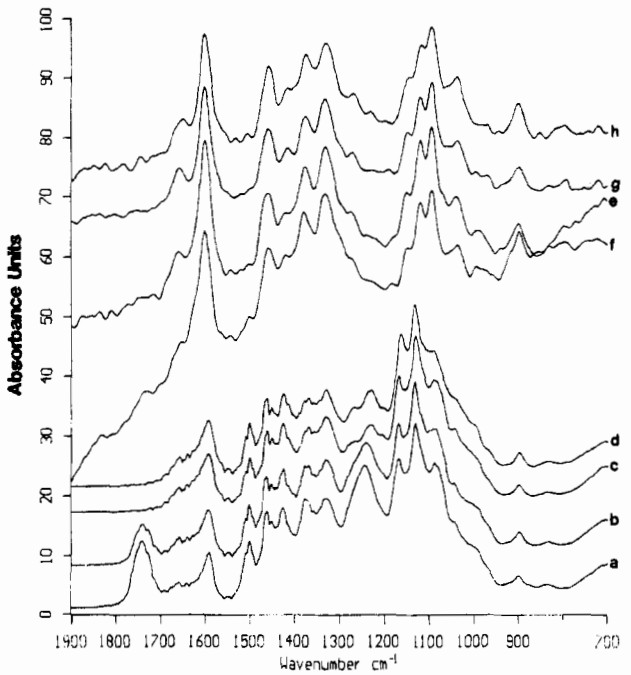


**Figure 4.** DRIFT (a, b, c, and d) and Raman (e, f, g, and h) spectra of untreated oak wood and of oak wood after treatment with 0.1, 1.0, and 4.0 M NaOH, respectively.

spectra are probably due to polysaccharide alkaline degradation products (Simkovic *et al.*, 1986; Niemelä, 1987).

Both types of spectra show evidence of a reduction in the proportion of crystalline cellulose after treatment with 4.0 M NaOH. The absorbances over the region 1200–1090 $cm^{-1}$ are reduced in intensity and, in the Raman spectrum, the peaks are poorly resolved. In the DRIFT spectrum, the amorphous cellulose peak (900 $cm^{-1}$) has increased in intensity. Similar changes in the proportion of amorphous and crystalline cellulose accompanying treatment with high concentrations of NaOH have been reported by McKenzie and Higgins (1958) and Horii (1988).

**Barley Straw.** The lignin and uronic acid contents and RNSC of the treated straws are shown in Table 1.

The Raman spectrum of the untreated barley straw (Figure 5c) is poorly resolved, again due to fluorescence. However, the fluorescence is much more prevalent than that encountered in the spectrum of the more highly colored, untreated oak wood sample (Figure 1c). This may due to the presence of the substituted cinnamic acids, particularly ferulic and *p*-coumaric, which are fully conjugated compounds. It has been suggested that, in Graminaea at least, the substituted cinnamic acids form both ester and ether linkages between lignin and NCPs (Scalbert *et al.*, 1985; Lam *et al.*, 1992, 1994; Helm and Ralph, 1993; Ralph *et al.*, 1994). Regardless of the type of linkage, covalent attachment to the lignin aromatic system would further increase the conjugation, and hence fluorescence, thereby reducing the resolution in the Raman spectrum. The substituted cinnamic acids have been reported to be present in woody species but only in trace amounts (Shimada *et al.*, 1971). Despite the quality of the Raman spectrum, several absorbances can be distinguished. The lignin absorbance at 1595 $cm^{-1}$ is very intense, while the corresponding absorbance at 1510 $cm^{-1}$, as in the oak wood spectrum, is virtually absent. The principal cellulose absorbance (1098 $cm^{-1}$), although evident, is weak.

The DRIFT spectrum of the untreated barley straw contains several intense and well-resolved absorbances

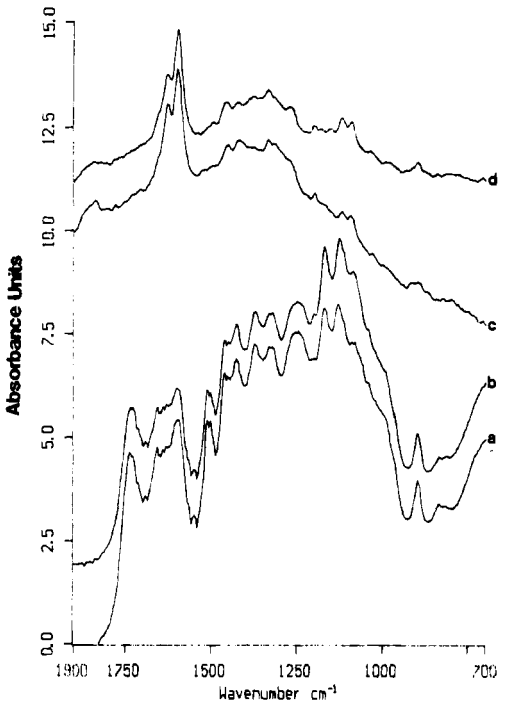


**Figure 5.** DRIFT (a, b) and Raman (c, d) spectra of untreated and cellulase-treated barley straw, respectively.

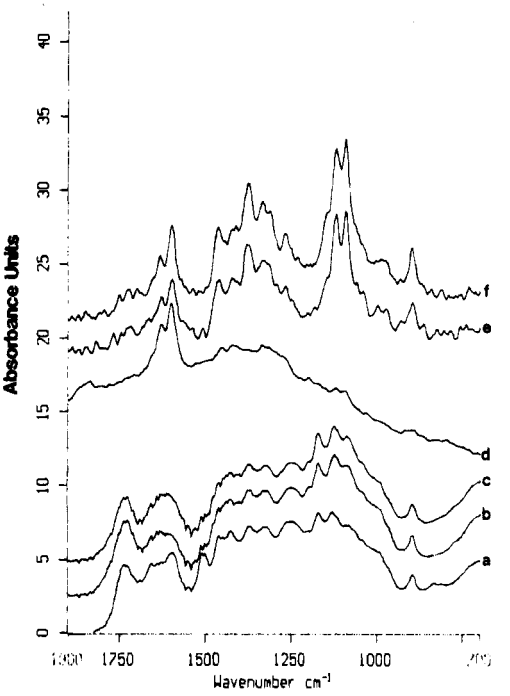


**Figure 6.** DRIFT (a, b, and c) and Raman (d, e, and f) spectra of untreated barley straw and of barley straw after treatment with 2 and 4 equiv of acidic sodium chlorite, respectively.

representing the major constituent polymers: NCP (ester linkages) (1740 and 1260 $cm^{-1}$), lignin (1595 and 1510 $cm^{-1}$), and cellulose (1130, 1098 and 900 $cm^{-1}$). Within the region 1680–1610 $cm^{-1}$ there is a greater range and intensity of absorbances than are seen in the corresponding oak wood spectrum. These absorbances are almost certainly due to the presence of aldehydic compounds. Hartley and Keene (1984) reported that a range of aldehydic phenols, linked via the phenolic group to NCP, were present in the cell walls of Graminaea. Such moieties have not, to the authors' knowledge, been reported to be present in woods.

Cellulase treatment of barley straw reduced the residual anhydroglucose content by approximately 4% (Table 1). Unlike the corresponding oak wood spectrum (Figure 1d), this reduction in cellulose is not seen in the Raman spectrum (Figure 5d). However, there is a slight increase in the crystalline cellulose peak at 1098 $cm^{-1}$ in the DRIFT spectrum (Figure 5b), but this may only be as a result of improved spectral resolution.

Delignification of barley straw with 2 and 4 equiv of acidic sodium chlorite reduced the residual lignin contents by 52 and 98%, respectively. This is reflected in the DRIFT and, to a lesser extent, Raman spectra as a reduction in the intensity of the bands at 1595 and 1510 $cm^{-1}$ (Figure 6b,d). Similar reductions in lignin accompanying acid chlorite delignification were reported in the CP/MAS NMR and DRIFT studies of forage lignins by Reeves and Schmidt (1993) and Reeves (1993), respectively. A concomitant increase in the proportion of crystalline cellulose is seen as an increase in the band at 1098 $cm^{-1}$ (Figure 6e,f) and a flattening of the band at 900 $cm^{-1}$ (Figure 6b,c). As with oak wood delignification, the benzylic carbonyl band at 1660 $cm^{-1}$ in the Raman spectra increases with increasing delignification.

Acid treatment again caused problems with fluorescence (Figure 7) to such an extent that the spectrum of the barley straw treated with 1.0 M $H_2SO_4$ (not shown) was totally unresolved. The Raman spectra of the other acid-treated straws were only slightly more distinct but of little use.

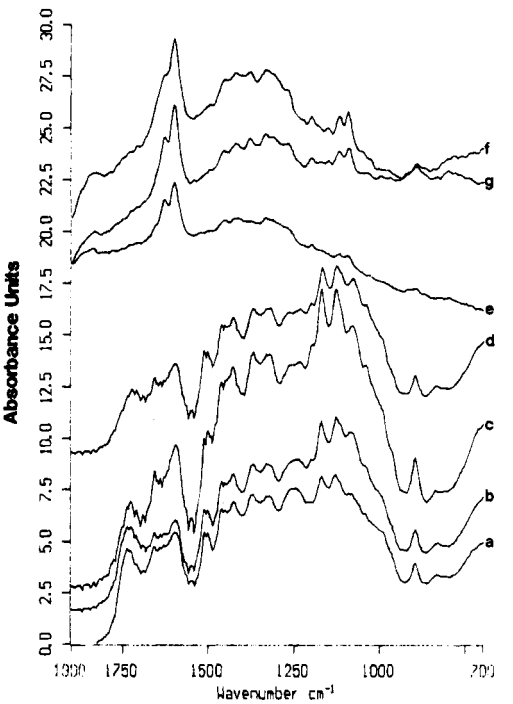


**Figure 7.** DRIFT (a, b, c, and d) and Raman (e, f, g, and h) spectra of untreated barley straw and of barley straw after treatment with 0.1 M $H_2SO_4$, acid detergent and 1.0 M $H_2SO_4$, respectively.

The DRIFT spectra of the acid-treated straws proved to be much more informative (Figure 7b–d). Treatment with increasing acid concentration resulted in a progressive reduction in the carbonyl absorbances (1740 and 1260 $cm^{-1}$). It has been reported previously that furanosides are more acid labile than pyranosides (Ferrier and Collins, 1972) and that the $\beta(1-4)$ xylopyranose chains and arabinofuranosyl side chains in Graminaea are acetylated (Chesson *et al.*, 1983). These arabinofuranosyl residues are acid labile (Fry 1987) and are removed during treatment with $H_2SO_4$ of low molarity. As the molarity increases, the more resistant xylopyranoside backbone is hydrolyzed. This is cor-

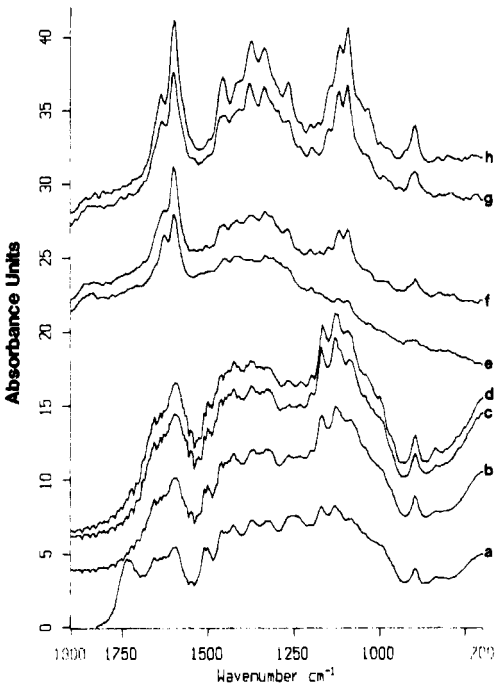


**Figure 8.** DRIFT (a, b, c, and d) and Raman (e, f, g, and h) spectra of untreated barley straw and of barley straw after treatment with 0.1, 1.0, and 4.0 M NaOH, respectively.

roborated by the RNSC data. Treatment with 0.1 M $H_2SO_4$ removes approximately 50% of the arabinose. However, increasing the acid concentration to 0.5 and 1.0 M causes a dramatic drop in the amount of residual xylose.

Significantly, after treatment with 0.1 M $H_2SO_4$, the residual arabinose content does not decrease to zero with increasing acid concentration. This may be due to the fact that at least some of the arabinose residues are proposed to participate in lignin–carbohydrate linkages (Chesson *et al.*, 1983; Stewart and Morrison, 1993). These residues will be inaccessible to reagents and therefore resistant to hydrolysis by acid.

Acid detergent treatment of barley straw, like oak wood, produced a reduction in lignin content. This is reflected in the DRIFT spectrum (Figure 3c) as a reduction in absorbance at 1595 $cm^{-1}$ and is consistent with the acid detergent treatment of forages reported by Reeves (1993).

Treatment with alkali resulted in significant changes in both the composition and spectra of the residues. The most obvious feature of treatment with 0.1 M NaOH is the removal of the ester carbonyl absorbance (Figure 8, 1740 and 1260 $cm^{-1}$). This is due to ester hydrolysis rather than polysaccharide removal (Table 1). Although the RSNC of the untreated and 0.1 M NaOH treated residues are comparable, the residual lignin content was reduced by 30%. This is seen in the DRIFT spectra as a reduction in the lignin absorbance at 1510 $cm^{-1}$ rather than 1595 $cm^{-1}$ since the latter is masked by the carboxylate C–O stretch at 1600 $cm^{-1}$. The combined loss of lignin and noncellulosic polysaccharides accompanying alkali extraction is in agreement with the results reported by Cyr *et al.* (1988) in their CP/MAS NMR study of chemical treatments of woods and forages, including barley straw. The absorbance at 1600 $cm^{-1}$ increases in intensity with increasing alkali concentration due to the alkaline degradation of both NCP and cellulose (Simkovic *et al.*, 1986; Niemelä, 1987). As the alkali concentration increases, the lignin and NCP contents decrease.

A feature of increasing alkali concentration is the beneficial effect on the resolution of the Raman spectra. Since a contribution to the fluorescence was earlier suggested to come from the cinnamic acids, progressive alkali extraction would remove these compounds and their lignin conjugates, thereby increasing resolution. This increased resolution allows a more valid analysis of the Raman spectra to be undertaken. Both the crystalline (1098 $cm^{-1}$) and amorphous (900 $cm^{-1}$) cellulose bands increase in intensity with increasing alkali concentration. This is due to a combination of two processes. First, the hydroxide ion disrupts the internal hydrogen bonds of the crystalline cellulose microfibril, thereby reducing crystallinity (Nevell, 1985). Second, the relative amounts of both types of cellulose are increased since the NCPs are extracted preferentially by alkali (Sjöström, 1993).

**Conclusion.** Both spectral techniques have been shown to be useful in the analysis of plant fibers and cell walls. Their use in isolation provided general structural and compositional information of a typical woody and a typical annual plant, oak wood and barley, respectively, after (bio)chemical treatments. More accurate assignments and interpretation of treatments were made by reference to the compositional data obtained by wet chemical methods and to similar studies using both FT-IR and CP/MAS NMR spectroscopies.

Some changes in the cell wall structure were reflected in only the spectroscopic data. For example, changes in the relative proportions of crystalline and amorphous cellulose accompanying (bio)chemical treatment were best reflected in the Raman and DRIFT spectra, respectively.

An example of the complementary nature of the spectroscopic techniques was highlighted by acidic sodium chlorite delignification. The DRIFT spectra of the treated residues showed broad, generalized carbonyl absorbances, suggesting unresolved lignin oxidation. The Raman spectra, however, contain carbonyl bands, which suggested that oxidation of the lignin side chain was occurring at the benzylic position.

The Raman technique did have limitations, especially in samples containing colored (oak wood) or (barley straw) conjugated systems. These limitations were not apparent in the DRIFT spectra.

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