REVIEW

FT-Raman Spectroscopy and Its Applications in Cereal Science

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ABSTRACT

We present a brief introduction to FT-Raman spectroscopy and examples of its use in cereal science for readers not familiar with Raman spectroscopy. We illustrate the use of FT-Raman spectroscopy as an analytical tool to measure the degree of chemical modification of chemically modified starches and as a technique to determine conformational and structural changes in plant proteins under different environmental conditions. We briefly describe other applications of Raman spectroscopy in cereal science.

FT-Raman spectroscopy has been finding more use and applications in cereal science in the past few years. The purpose of this review is to provide a brief introduction to FT-Raman spectroscopy and some of its recent applications to cereal science for readers not familiar with Raman spectroscopy. Therefore, this review is not intended to be comprehensive, nor will it give a detailed description of the technique. Readers are referred to other recent reviews for more indepth coverage of the spectroscopic technique and a more complete survey of the relevant literature (Goral and Zichy 1990; Ozaki et al 1992; Austin et al 1993; Wang and Van Wart 1993; Gerrard 1994; Li-Chan et al 1994; Mathies 1995; Peticolas 1995; Spiro and Czernuszewicz 1995; Li-Chan 1996).

In this review, we shall give a very brief description of FT-Raman spectroscopy and its relationship to the more commonly used infrared spectroscopy method. We will present some applications of FT-Raman spectroscopy in cereal science such as the measurement of the degree of chemical modification of chemically modified starches and the elucidation of conformational and structural changes induced on plant proteins by different environmental conditions relevant to processing cereals. We shall also mention some other recently reported applications of FT-Raman spectroscopy to cereal science.

BACKGROUND

Both Raman and infrared spectroscopy are based on the discrete vibrational transitions that take place in the ground electronic state of molecules. Raman scattering involves excitation of a molecule with a photon of light (usually from a laser source) that is then scattered with a change in energy from the excitation photon by the vibrational transition energy of the molecule (this process is shown in the left-hand part of Fig. 1A). Infrared absorption spectroscopy typically involves absorption of an infrared photon of light from a lower vibrational energy level to a higher vibrational energy level (this process is shown in the right-hand portion of Fig. 1A). These vibrational transitions correspond to the different stretching, bending, wagging, deformation, and other types of vibrational motions of the molecule. Figure 1B depicts a simple schematic of the three vibrational modes of the water molecule (the symmetric O-H stretch, the H-O-H bend, and the asymmetric O-H stretch vibrations). The Raman spectrum consists of a plot of the intensity of scattered light as a function of wavenumber (cm^{-1}) shift from the laser excitation wavelength (this is called the Raman shift, $\Delta\nu$ in cm^{-1}). The vibrational Raman bands observed provide information about the vibrational motions of the molecule similar to that of an infrared spectrum consisting of the absorption of energy as a function of frequency. Raman scattering depends on changes in the polarizability of functional groups as the molecule vibrates, and infrared absorption depends on changes in the intrinsic dipole moment with molecular vibrations. Therefore, Raman and infrared spectra appear different from one another and provide complementary information about the vibrations of the molecule. Typically, nonpolar groups like C=C, C-C, and S-S are more intense in Raman spectra, and polar groups like O-H, C=O, C-O, and N-H are more intense in infrared spectra.

Water is a strongly polar molecule and has very intense infrared absorption bands. This makes it difficult to obtain infrared spectra in aqueous samples. In contrast, water has a very weak Raman signal and this makes it much easier to obtain Raman spectra in aqueous samples. Thus, Raman spectroscopy can be more routinely applied to study biological systems such as food that contain significant amounts of water. A second major advantage of Raman spectroscopy is that it does not require the samples to be optically clear, and spectra can readily be obtained for opaque samples. The Raman band intensity depends linearly on the amount of substance (Long 1977). This makes it easy to correlate the amount of substance present to the Raman signal intensity, and Raman spectra are an attractive tool to measure the degree of chemical changes that have taken place. One disadvantage of Raman spectroscopy is that Raman scattering is inherently weak and it was not until lasers were used for excitation that it became more routinely used and developed (Long 1977). Visible and ultraviolet laser excitation wavelengths typically used for normal Raman and resonance Raman spectroscopy can sometimes lead to interference from fluorescence from the sample or impurities in the sample. This fluorescence background can sometimes completely obscure the Raman signal of interest. The recent development of Fourier-Transform (FT) Raman spectrometers that employ a 1,064-nm near-infrared excitation laser helps to minimize potential fluorescence problems because the near-infrared excitation wavelength does not usually have enough energy to excite most electronic excited states. Fourier-Transform instruments make use of a Michelson interferometer and enable the spectrum of interest to be collected much faster and with higher signal-to-noise compared with conventional single channel scanning spectroscopy instruments (Hendra et al 1991). FT-Raman spectrometers were first made practical in 1986, and continuing development since then has seen explosive growth in their use and applications to a variety of areas of science and industry. FT-Raman spectroscopy has found great utility in the polymer, pharmaceutical, and food industries as a quantitative analytical technique and for use in other applications (Davies et al 1990; Jackson et al 1990; Deely et al 1991; Hendra et al 1991; Jones and Wesley 1991; Sadeghi-Jorabchi et al 1991; Tseng et al 1994; Wang et al 1997; Phillips et al 1998, 1999a, 1999b, 2000; Ma et al 2000).

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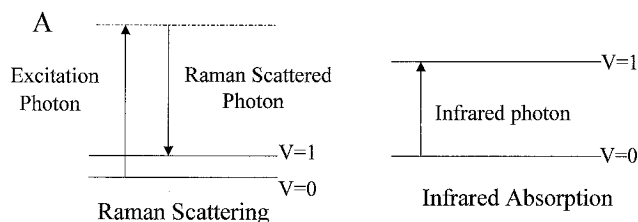
The different substances that contribute to a Raman spectrum of a particular sample can usually be easily differentiated because each of the substances will have a signature Raman spectrum composed of sharp well-resolved vibrational bands characteristic of the chemical functional groups present in the sample. This can also help minimize interference from residual compounds and impurities. Near-infrared spectroscopy (NIR) has found utility as an analytical and quality control tool in various cereal science applications because water, protein, starch, and fat have some functional groups that absorb in the 400–1,700-nm region (Delwiche et al 1996; Windham et al 1997; Wesley et al 1998). However, only a limited number of chemical functional groups absorb in the near-infrared region, and the chemical structures of interest may not be easily accessible with NIR spectroscopy, whereas Raman and infrared vibrational spectroscopies can probe a much larger range of chemical functional groups. NIR spectra are typically composed of relatively broad spectral features that may not be well resolved, whereas Raman and infrared spectra display much higher spectral resolution so that many different chemical features may be more easily discerned. The relatively well-resolved Raman and infrared vibrational bands are sensitive to the chemical and physical environment of the chemical group associated with the molecular vibration. Therefore, the Raman and infrared band positions and intensities provide a very sensitive chemical and structural probe. Raman (and infrared) spectroscopy can provide substantially more chemical structural and conformational information than NIR spectroscopy and may be more suitable for applications where this type of information is needed or cannot be easily extracted from NIR spectroscopy. We shall present some typical applications of FT-Raman spectroscopy in cereal chemistry to measure chemical and conformational changes that have occurred in starch and protein samples. We shall also briefly mention other recently reported applications of Raman spectroscopy in cereal chemistry.



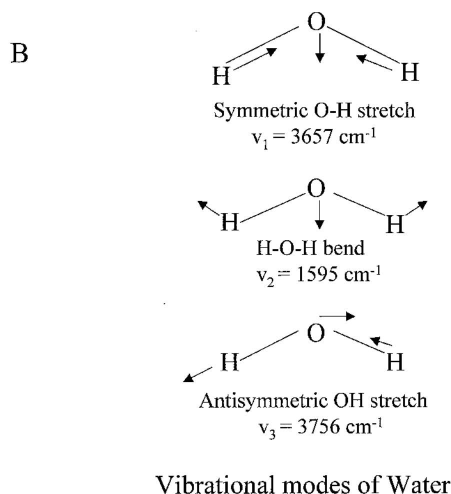


Fig. 1. (**A**) Schematic energy level diagram of Raman scattering process (left) and infrared absorption process (right). (**B**) Vibrational motions associated with symmetric O-H stretch, H-O-H bend, and asymmetric O-H stretch vibrational modes of water.

APPLICATIONS IN CEREAL CHEMISTRY

Method for Collection and Analysis of FT-Raman Spectra

The FT-Raman apparatus and methods are similar to those previously described (Phillips et al 1998, 1999a; Ma et al 2000). Raman spectra were obtained using a FT-Raman spectrometer (Bio-Rad, Cambridge, MA) with 1,064-nm excitation. FT-Raman spectra can usually be obtained for samples in almost any physical state (dry powder, semiliquid, liquid, and amorphous films) and from quantities as small as a few milligrams. Samples (≈5.0–0.1 g) of the chemically modified starches or oat globulin were placed into glass capillary tubes. FT-Raman spectra were recorded for the glass tube by itself and the glass tube with the sample. The FT-Raman spectrum of the sample was found by subtracting the spectrum of the glass tube from the spectrum of the glass tube plus sample. The Raman scattered signal was collected using a backscattering geometry and ≈100 mW of 1,064-nm light was used to excite the sample. The FT-Raman signal was collected for ≈3–10 min for each spectrum with a resolution of 2–8 cm^{-1}, depending on the particular sample being studied. Because the Raman bands of silica from the capillary tube were very small and usually did not overlap with any Raman bands of interest, the Raman spectra were used directly for the area and least squares calculations of the Raman intensities. This helped to reduce any errors that would be introduced from spectral subtractions.

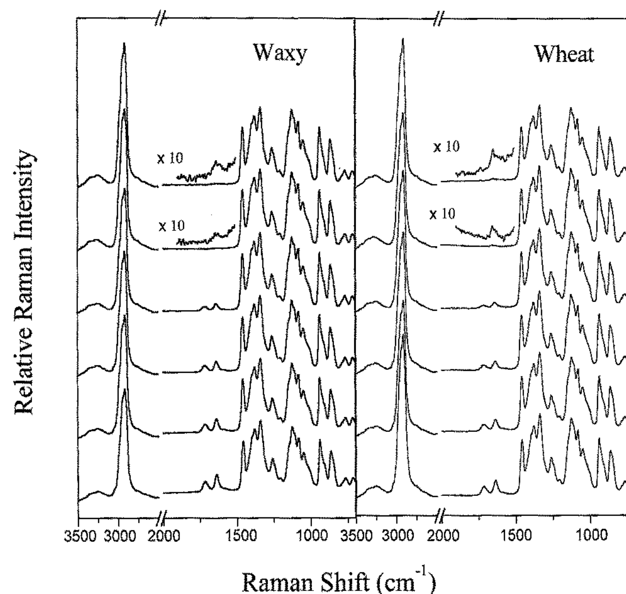


Fig. 2. FT-Raman spectra of waxy maize and wheat starches with varying degrees of substitution (DS) of maleate and a control (native) starch sample. Maleate C=O and C=C signature Raman bands in the 1,600–1,760 cm^{-1} region grow substantially in intensity as DS increases.

TABLE I
Parameters for Linear Regression Analysis of the Calibration Curves for the Raman Intensity Ratio vs. Degree of Substitution (DS) of Maleate in Modified Starches[a,b]

Method	*B*	*A*	*r*	LOD
Area				
Waxy maize	6.8741	–0.0006	0.9978	0.0047
Wheat	6.6537	–0.0054	0.9973	0.0044
CLS				
Waxy maize	18.436	0.0253	0.9957	0.0047
Wheat	22.134	0.0019	0.9943	0.0065

[a] CLS = classical least squares treatment, LOD = limit of detection.
[b] $Y = A + B \times X$ where Y = ratio of the Raman intensity of 1,600–1,760 cm^{-1} region C=O and C=C stretch bands and 941 cm^{-1} region C-C stretch bands determined using either the area method or the CLS method. B = slope of the linear regression curve, A = intercept of the linear regression curve, X = DDS of maleate. n = 5 for all samples.

Application to Chemically Modified Starches

We have recently reported the application of FT-Raman spectroscopy to determine the degree of substitution (DS) of several chemical modifications of starches including acetylated (Phillips et al 1998, 1999a), cationic modified (Phillips et al 1999b), succinylated (Phillips et al 1999c, 2000), maleinated (Phillips et al 2001), and benzylated (Phillips et al 2001) starches. We shall focus on maleinated starches for a typical example. Figure 2 displays typical FT-Raman spectra for pure waxy maize and wheat and their maleinated starches. The Raman bands at ≈900 cm^{-1} are due mainly to C-C stretches and in the 2,800–3,000 cm^{-1} region due to C-H stretches. Three new Raman bands appear in the FT-Raman spectra of the maleinated starch spectra shown in Fig. 2 and correlate with the DS of the maleinate chemical modification of the starch. The new Raman bands at 1,641, 1,657, and 3,046 cm^{-1} likely correspond to the nominal C=O stretch, the C=C stretch, and the O-H stretch vibrational modes associated with the maleate chemical modification.

Two different methods were used to analyze the Raman spectra and examine the correlation of the Raman band intensities of the C=O and C=C stretch Raman bands to the DS of maleate in chemically modified starches. The first analysis method employed integrated Raman band intensities (1,600–1,760 cm^{-1}) to obtain the total Raman intensity of the C=O and C=C stretch Raman bands. The C-C stretch Raman bands of the parent starch (810–975 cm^{-1} region) were integrated for use as an internal standard between different spectra. The ratio of the integrated areas of the C=O and C=C stretch Raman bands to the integrated areas of the C-C stretch bands (represented by I_{1641}/I_{941}) versus the DS of maleate determined from the standard titrimetric method were plotted (Fig. 2). The linear regression analysis of the plot in Fig. 3 is summarized in Table I. Although the small amylose and amylopectin Raman bands (Phillips et al 1999d) are overlapped with the C=O and C=C stretch Raman bands, they do not affect the linearity of the correlation to the amount of maleate substitution. The small background due to the amylose (1,657 cm^{-1}) and amylo-

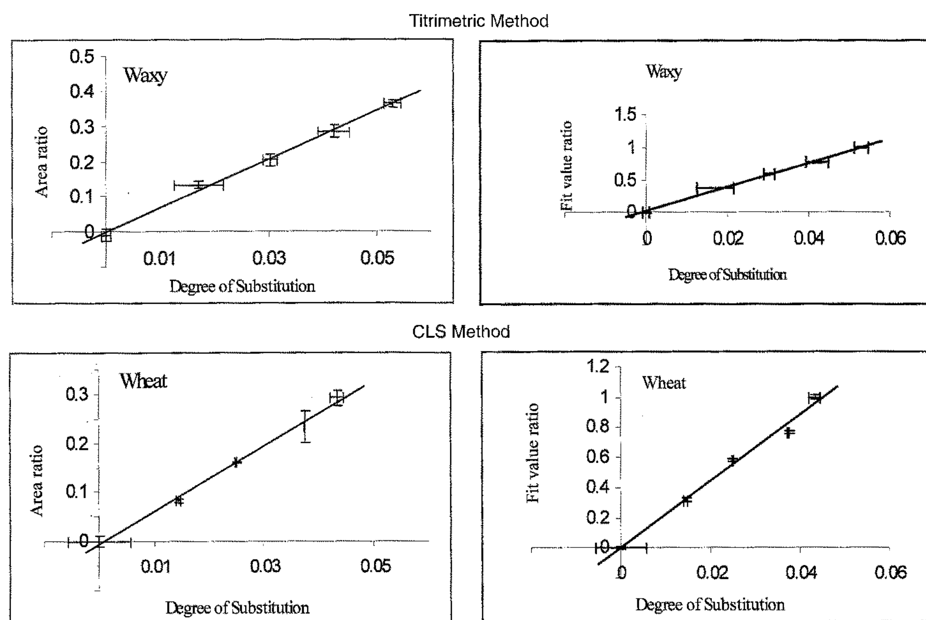


Fig. 3. (**Top**) Ratio of integrated areas of C=O and C=C stretch Raman bands in the 1,600–1,760 cm^{-1} region to integrated areas of C-C stretch bands in the 810–975 cm^{-1} region (I_{1641}/I_{941}) vs. degree of substitution (DS) of maleate determined from titrimetric method. (**Bottom**) Ratio of intensities determined by classical least squares (CLS) method of C=O and C=C stretch Raman bands in the 1,600–1,760 cm^{-1} region to those of C-C stretch bands in the 810–975 cm^{-1} region (I_{1641}/I_{941}) vs. DS of maleate determined from titrimetric method. Error bars of data points are ±1 standard deviation of Raman (y-axis) and titration (x-axis) measurements. Linear regression parameters for plots are in Table I.

TABLE II
Degree of Substitution (DS) of Maleate for Waxy Maize Samples with Unknown DS of Maleate Using Raman Spectra and Calibration Curves[a]

Sample	DS of Maleate[b]	Area[c] Ratio I_{1641}/I_{941}	DS Determined by Area Calibration Curve	RE = (DS – Area DS)/DS
Raman spectra and calibration curves				
Waxy maize				
Sample U1	0.0361 ± 0.0042	0.2583 ± 0.0064	0.0377	–0.0429
Sample U2	0.0167 ± 0.0033	0.1106 ± 0.0021	0.0162	0.0308
Sample U3	0.0096 ± 0.0017	0.0621 ± 0.0009	0.0091	0.0493
Sample	**DS of Maleate[b]**	**CLS[d] Ratio I_{1641}/I_{941}**	**DS Determined by CLS Calibration Curve**	**RE = (DS – CLS DS)/DS**
Raman spectra and Raman CLS calibration curves				
Waxy maize				
Sample U1	0.0361 ± 0.0042	0.7045 ± 0.0011	0.0368	–0.0201
Sample U2	0.0167 ± 0.0033	0.3235 ± 0.0067	0.0162	0.0305
Sample U3	0.0096 ± 0.0017	0.2111 ± 0.0052	0.0101	–0.0504

[a] Comparison to separate results using standard wet chemistry technique are used to find relative error of spectroscopic methods. CLS = classical least squares treatment. RE = relative error.
[b] Determined using titration method (Phillips et al 2001).
[c] Determined using integrated area measurements for C=O and C=C stretch Raman bands in the 1,600–1,760 cm^{-1} region to the those of C-C stretch bands in the 810–975 cm^{-1} region (represented by I_{1641}/I_{941}).
[d] Determined using the CLS method described in text to find intensities for C=O and C=C stretch Raman bands in the 1,600–1,760 cm^{-1} region to those of the C=C stretch bands in the 810–975 cm^{-1} region (represented by I_{1641}/I_{941}).

pectin (1,637 cm^{-1}) Raman bands gives rise to somewhat different y-intercepts with the slope changing with amylose content in the linear regression plots (Fig. 3) and parameters (Table I). This indicates that one may need different Raman calibration curves for starches with noticeably different amylose contents to achieve the best results for the determination of the DS of chemically modified starch samples with unknown amounts of modification.

A second method of analysis to develop Raman calibration curves was performed using a classical least squares (CLS) treatment of the Raman spectra (employing four components incorporated into the reference set for the calculations). These components included the ensemble averages of the control (0% DS) and the highest DS samples and a set of constant values and a ramp were included to account for the uneven background contribution to the sample spectra from potential fluorescence. The calculations were made for the 1,600–1,760 cm^{-1} region of the C=O and C=C stretch bands and the 810–975 cm^{-1} region of the internal standard C-C stretch bands and the least squares values of each component was determined for the samples (using the CLS calculations). These values should lie between 0 and 1 and signify the fraction of each component that contributed to the sample spectrum. [We note that we assume that the components are mutually independent of each other.] The sample spectrum can be reconstructed by linear combinations of the four components and their least squares values and the ratio of least squares value for the starch reference to the control should correct for any variations during data acquisition. In an ideal case, the values obtained from the CLS calculation should result in 0 for the pure starch and 1 for the highest DS sample examined for each type of starch. Table I lists the results of the CLS calculations and their linear regression results of the least squares values versus the DS of maleate (also plotted in Fig. 3).

Inspection of Table I reveals that both methods exhibit a very high degree of linearity for their Raman marker band intensities with the DS of maleate ($r > 0.99$ for all of the types of starches examined in our study). The limit of detection of (LOD) was defined as three times the y-intercept divided by the slope of the calibration curve. The LOD is used to represent the sensitivity of an analytical method and is given in Table I for each of the Raman calibration curves. Their values lie in the range of 0.0015 to 0.0066. The Raman calibration curves can be used to determine the DS of maleate in unknown starch samples.

To test the calibration curves, we determined the DS of maleate for a set of waxy maize samples with unknown amounts of substitution using the calibration curves for the two Raman-based methods for validation purposes (Table II). The actual DS was determined using the standard wet chemistry method (Wurzburg 1964) for the unknown waxy maize samples (also shown in Table II). The Raman and wet chemistry measurements were done in triplicate. Examination of Table II indicates that the relative error (RE) between the standard wet chemistry technique value and those found from the Raman calibration curves of Table II and the Raman spectroscopic measurements are ≤0.05. This demonstrates that the Raman spectroscopic measurements and their calibration curves can be used with confidence to find the DS of maleate for chemically modified starches with an accuracy similar to that of the standard wet chemistry method commonly used for that purpose. We have found similar results for other chemically modified starches, and FT-Raman spectroscopy should find many more applications as an analytical tool in cereal chemistry.

Application to Study Oat Globulin Conformation

Oat globulin is an oligomeric protein with quaternary structure very similar to that of soy 11S globulin (glycinin), a heat-coagulable protein. It is made up of six pairs of acidic and basic polypeptides, with each acidic and basic polypeptides linked by disulfide bonds to form a subunit. The six subunits are linked by noncovalent forces

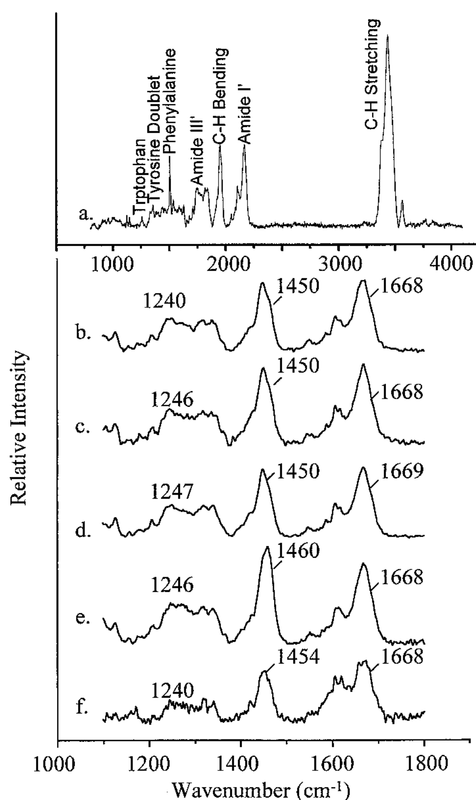


Fig. 4. Raman spectra of oat globulin dispersion (≈10%) under various buffer conditions: (a) and (b) distilled water, (c) 40 m*M* SDS, (d) 5% ME, (e) 40% EG, (f) 6*M* urea. (Adapted from Ma et al 2000.)

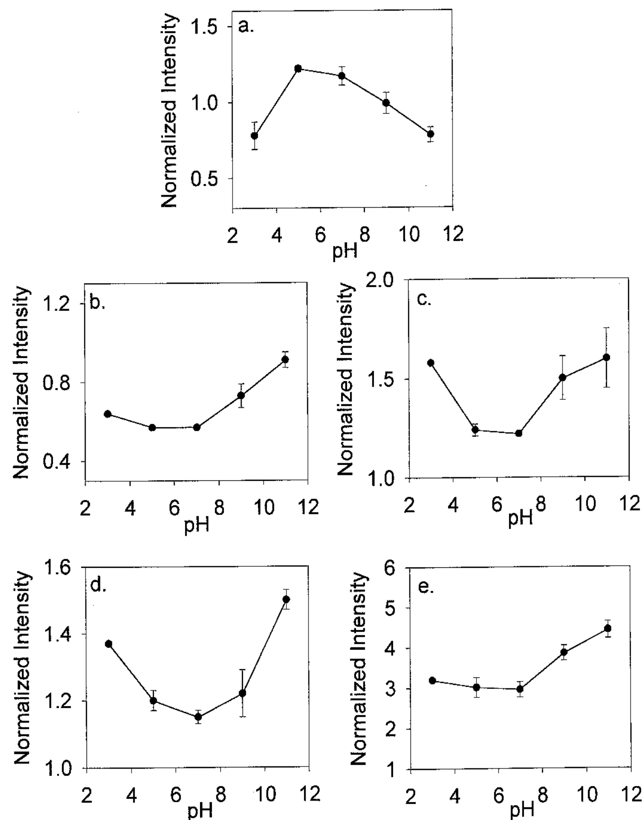


Fig. 5. Effect of pH on normalized intensity of several regions in Raman spectrum of oat globulin dispersions (or pellets): (a) Tyrosine, (b) Amide III', (c) Amide I', (d) C-H bend, (e) C-H stretch. Error bars represent standard deviations of means. (Adapted from Ma et al 2000.)

to form a hexamer (Derbyshire et al 1976; Brinegar and Peterson 1982; Neilsen 1985). A detailed understanding of the structure-function relationship in oat globulin is essential in predicting and controlling the functional performance of oat protein in fabricated foods. Studies of protein conformation under different buffer conditions can provide valuable information for improving specific functional performance of protein ingredients such as gelation and emulsification. The conformation of oat globulin has been studied by differential scanning calorimetry (DSC) (Harwalkar and Ma 1987) and UV and fluorescence spectrophotometry (Ma and Harwalkar 1988a). However, changes in the secondary structures cannot be assessed by these techniques.

The applicability of Raman spectroscopy to both solid and liquid samples makes it a useful tool to investigate in situ protein structural changes during denaturation and aggregation-gelation (Li-Chan et al 1994; Li-Chan 1996). However, fluorescence due to phenolic compounds continues to be a major problem in Raman spectroscopic analysis of plant materials, including vegetable proteins, when visible laser excitation is used. This can be solved by the use of FT-Raman spectroscopy (Schrader et al 1991; Li-Chan et al 1994). Despite the advantages of the FT-Raman instrument, there are few reports on its use in studying proteins from either animal and plant sources. We studied the changes in the oat globulin conformation under the influence of different environmental conditions and heating.

Figure 4a shows a typical Raman spectrum of 10% oat globulin dispersion in distilled water, and the assignment of some major bands. Figure 5 shows the effect of pH on normalized intensity of several Raman bands of oat globulin dispersions. Extreme pH levels led to decreases in the tyrosine doublet band intensity, I_{850}/I_{830} (Fig. 5a) indicating increased "buriedness" or participation of the tyrosine phenolic groups as hydrogen bond donors (Li-Chan 1996). The intensities of the amide III' (Fig. 5b), amide I' (Fig. 5c) and C-H banding (Fig. 5d) and stretching (Fig. 5e) bands were all increased by highly acidic (pH 3) and alkaline (pH 9–11) pH levels, suggesting protein denaturation. These results are consistent with previous DSC data (Harwalkar and Ma 1987) which showed that the denaturation temperature (T_d), an index of thermal stability, and enthalpy, an index of the amount of ordered structure, were affected by pH. Most proteins are stable over a certain pH range, normally near their isoelectric pH where repulsive forces are low and the proteins remain in their native state. At pH levels far from the isoelectric point, large net charges are induced and proteins will be partially unfolded due to intramolecular side-chain charge repulsion leading to rupture of hydrogen bonds and a breakup of hydrophobic interactions (Morrissey et al 1987).

Figure 4b–f shows the effect of several protein structure perturbants on the Raman spectrum of oat globulin dispersions. SDS, β-mercaptoethanol (ME), and ethylene glycol (EG), all caused a marked shift in the amide III' band to higher frequency, which indicates a transition from β-sheet to random coil conformation. EG (40% v/v) and 6*M* urea also led to shifts in the C-H bending peak. SDS, an anionic detergent, can bind to protein by noncovalent forces to increase the net charge and lead to ionic repulsion and unfolding of polypeptides (Steinhardt 1975). ME is a reducing agent and can break up disulfide linkages and destabilize oligomers such as oat globulin that contain monomers linked by disulfide bonds. Ethylene glycol could lower the dielectric constant of water and weaken the nonpolar interactions between protein molecules, thereby causing destabilization. Urea effectively disrupts the hydrogen-bonded structure of water and facilitates protein unfolding by weakening hydrophobic interactions (Kinsella 1982). The Raman data showed that these reagents caused marked changes in the secondary and tertiary structures of oat globulin. This could be attributed to perturbation of the tertiary and quaternary structures of the oligomeric protein by destabilizing some primary (hydrogen bonds, hydrophobic forces) and secondary (disulfide bonds) chemical forces that stabilize oat globulin conformation. The results are consistent with previous DSC data (Harwalkar and Ma 1987) which showed that in the presence of these perturbants, thermal stability or enthalpy of oat globulin were decreased, indicating conformational changes and partial denaturation of the protein.

Figure 6 shows the changes in the Raman band intensity in several spectral regions when oat globulin was heated at 110°C for different periods of time. There was an initial decrease of the amide III' (Fig. 6a) and amide I' (Fig. 6b) regions in the first 30 min, followed by marked rises in peak intensity at 60 min, suggesting protein denaturation. Similar trends were observed in the C-H bending (Fig. 6c) and stretching (Fig. 6d) vibrations, again indicating protein unfolding when oat globulin was heated for extended periods of time. Heating of oat globulin near T_d (110°C) led to progressive decreases in the enthalpy, indicating protein denaturation (Ma and Harwalkar 1988b). Heating at 110°C also caused a progressive red shift in the UV absorption spectra and a blue shift in the fluorescence emission spectra, again suggesting protein unfolding (Ma and Harwalkar 1988a). The Raman data are in agreement with these results, although the DSC and the UV/fluorescence spectroscopic data showed more rapid protein unfolding. This suggests that Raman spectroscopy is more sensitive to secondary structural changes while DSC and UV/fluorescence spectroscopy are sensitive to changes in tertiary and quaternary structure. During thermal denaturation of oat globulin, alteration in the tertiary and quaternary structure may take place before changes in secondary structure.

Other Applications in Cereal Chemistry

There have been several reports of using FT-Raman spectroscopy to measure the degree of amylose content in starches from several botanical sources (Phillips et al 1999d; Barton II et al 2000). This application holds future promise of correlating amylose content with other characteristics of starches and their use in various processes. FT-Raman spectroscopy is also beginning to find applications as a process monitoring tool in cereal chemistry. A FT-Raman method for monitoring starch gelatinization and enzyme catalyzed starch hydrolysis was recently detailed (Schuster et al 2000). We are also currently extending the use of our Raman calibration curves from determining the degree of chemical modification of starches, to online monitoring applications for industrial

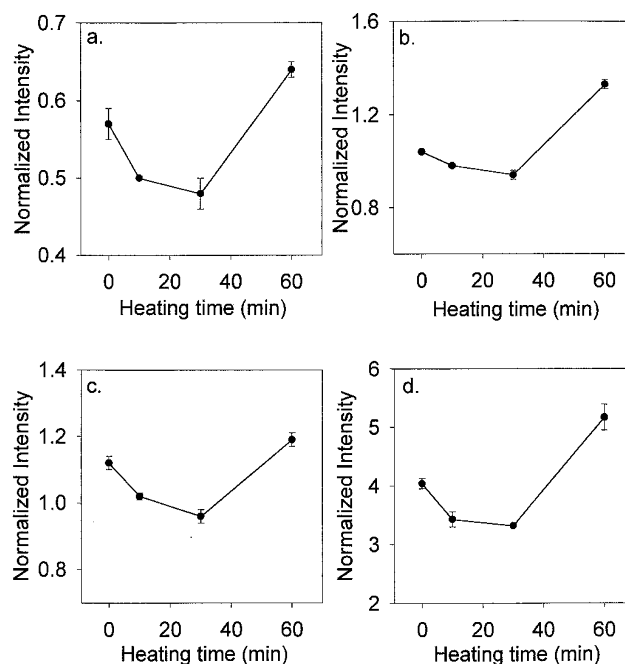


Fig. 6. Effect of heating (110°C) on normalized intensity of several regions in Raman spectrum of oat globulin (freeze-dried powder): (a) Amide III', (b) Amide I', (c) C-H bend, (d) C-H stretch. Error bars represent standard deviations of means. (Adapted from Ma et al 2000.)

quality control during the starch chemical modification process. The use of FT-Raman spectroscopy for online monitoring in many different types of applications in cereal science holds much promise, and we will be likely to see more development in the future.

Raman microscopy has also been applied to applications in cereal science. The spatial distribution of protein and phenolic components in wheat grain was recently elucidated using confocal Raman microscopy in a set of elegant experiments (Piot et al 2000). This Raman technique also holds much promise for future development and application in various areas of cereal science.

Practical Considerations

FT-Raman spectrometers are available from a number of major analytical instrument companies (Bruker, Perkin-Elmer, Nicolet, Bio-Rad, and others). These commercial instruments have similar overall specifications and are based on similar technology. FT-Raman spectrometers may be purchased as stand-alone instruments or may, in some cases, be added on to an existing FT-IR instrument (this option could save money and allow the user to collect both Raman and infrared spectra for different applications). Before purchasing an FT-Raman spectrometer, it is useful to collect spectra under the same conditions on different instruments for the type of samples that one may be interested in obtaining Raman spectra. This helps to choose an instrument that works best for the application and type of samples one wants to acquire Raman spectra. It is sometimes necessary to acquire Raman spectra as a function of the laser excitation power because one may need to avoid "cooking" the sample and possibly introducing spectral changes that are not related to the sample or process that one is attempting to record.

CONCLUSIONS

We have presented a brief layman's introduction to FT-Raman spectroscopy and several applications in cereal science. The use of FT-Raman spectroscopy as an analytical tool to measure the degree of chemical modification of chemically modified starches was described. FT-Raman spectroscopy is also a powerful technique in determining conformational and structural changes in a plant protein. We briefly described some practical considerations in using FT-Raman spectroscopy as well as other applications of Raman spectroscopy in cereal science. FT-Raman (and Raman) spectroscopy is a very powerful tool to elucidate chemical and conformational features (and changes in chemical and conformational structure caused by environmental changes or other processes) in cereal science that are not easily obtained from other commonly used spectroscopic methods. Thus, FT-Raman spectroscopy promises to find many new applications in the future in cereal science.

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