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REVIEW ARTICLE

Secondary structure of food proteins by Fourier transform spectroscopy in the mid-infrared region

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Abstract Fourier transform spectroscopy in the mid-infrared (400–5,000 cm^{-1}) (FT-IR) is being recognized as a powerful tool for analyzing chemical composition of food, with special concern to molecular architecture of food proteins. Unlike other spectroscopic techniques, it provides high-quality spectra with very small amount of protein, in various environments irrespective of the molecular mass. The fraction of peptide bonds in α-helical, β-pleated sheet, turns and aperiodic conformations can be accurately estimated by analysis of the amide I band (1,600–1,700 cm^{-1}) in the mid-IR region. In addition, FT-IR measurement of secondary structure highlights the mechanism of protein aggregation and stability, making this technique of strategic importance in the food proteomic field. Examples of applications of FT-IR spectroscopy in the study of structural features of food proteins critical of nutritional and technological performance are discussed.

Keywords FT-IR · Infrared spectroscopy · Food proteins · Secondary structure

Principles of Fourier transform-infrared (FT-IR) spectroscopy

Infrared spectroscopy is a vibrational spectroscopic technique that exploits the principle that molecules have specific discrete energy levels corresponding to frequencies at which they rotate or vibrate. Vibrational spectra may be obtained either by infrared absorption (IR) or Raman scattering spectroscopy, that provide complementary information.

Infrared spectra result from the absorption of energy by chemical bonds, primarily stretching and bending motions. In particular, mid-infrared spectroscopy regards the absorption of radiation of wavelenghts from 2 to 25 μm (400–5,000 wavenumbers). The absorption arises from transitions between vibrational and rotational states of the molecule and occurs when the transition causes a change in the dipole moment (Barth 2007).

Characteristic groups of atoms give rise to vibrational bands centered at typical frequencies regardless of the molecule in which they are found. Because these resonant frequencies are determined by the shape of the molecular potential energy surfaces, by the masses of the atoms and the associated vibronic coupling, the technique can be used for the structural and chemical characterization of very complex mixtures.

The precise peak frequency of these bands depends on inter- and intramolecular effects, including peptide-bond angles and hydrogen-bonding patterns. Thus, vibrational spectra can be used to estimate the secondary structure of proteins, providing insights into structural properties critical of biological role (Barth 2007).

The intensity of the absorption is proportional to the concentration of the absorbing species. Therefore, as with other optical spectroscopies, the Lambert–Beer relationship is the basis of quantitative analysis:

$$A = -\log\left(\frac{I}{I_0}\right) = \varepsilon c d \quad (1)$$

where A is the absorbance, I is the light intensity measured after passing through the sample, I_0 is the incident light

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intensity, ε is the molar (decadic) extinction coefficient, c is the chromofore concentration and d is the light pathlength through the sample. When well resolved bands can be identified as originating from specific components, the Lambert–Beer law can be applied directly.

Modern infrared spectrometers are, in most cases, Fourier transform spectrometers. The central part of a FT-IR spectrometer is the Michelson interferometer, whose elements are shown in Fig. 1. This technique is based on the pattern of interference of two light beams produced by a broadband source. The beam generated by the source is splitted in two halves, each one covering a different pathlength through the perpendicular arms of an optical setup (see Fig. 1). The beamsplitter is a semireflecting device and is often prepared by depositing a thin film of germanium onto a flat KBr substrate. The phase difference between the two beams is obtained through the displacement of a moving mirror in one of the optical arm that introduces a time delay in one of the beam: once recombined at the detector site, the two beams reveal their phase difference through an interference pattern usually called interferogram. Indeed, the interferogram measured as a function of the time delay can be considered as the Fourier transformation of the intensity spectrum of the source. Commercial interferometers are normally adapted to accomplish for the well-known problems due to discrete sampling of the signal (aliasing), Fourier Transform over limited frequency range (apodization), phase uncertainty and dynamical alignment of the optical components. High-quality instruments are now available for FT-IR spectroscopy in a spectral range as wide as possible: the optical set-up of the interferometer (mainly the source, beamsplitter and detector) can be chosen in order to range from frequencies of few THz up to the near ultraviolet. Moreover, the coupling with new devices as IR microscope, focal plane array detector and the use of no-thermal sources, has currently enlarged the field of FT-IR application.

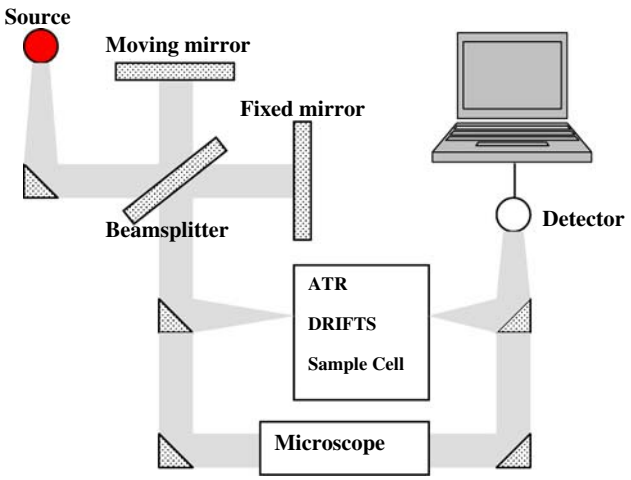

Fig. 1 Scheme of the Michelson interferometer and the experiment set-up. In the sample site an accessory for DRIFT as well as the one for ATR, or device for cell and pellets can be placed. In a different path the infrared radiation is interfaced with an infrared microscope

Absorption measurements in the mid-infrared region (FT-IR)

Measurements in solution

The protein absorption can be determined by the Lambert–Beer relationship, using a cell where a given amount of the sample is dissolved in H_2O. Using the same cell, both a reference spectrum with the empty cell and the spectrum of the solution are acquired in identical condition. In most cases, the reference spectrum is collected with the cell filled with a buffer solution, in order to match the refractive index of the sample and to avoid interference fringes due to multiple internal reflections. In turn, the interference fringes are used to determine the actual thickness of the cell volume. This technique has the advantage to reproduce a suitable environment of the protein, but conversely, it cannot be applied in most cases. Indeed, the strong IR absorbance of H_2O (O–H–O bending mode), centered at 1,640 cm^{-1}, prevents the measure of the amide bands, if the optical path exceeds 5–7 μm. In this condition, the spectrum of liquid water can be numerically deconvolved and the amide bands reconstructed from the whole transmittance spectrum (Haris and Severcan 1999). Practical algorithms for the water spectrum subtraction can be found in Dousseau et al. (1989). However, the small pathlength of the cell makes difficult the filling and the drying of the cell, causing a mismatch of optical path between reference and sample spectra. In most of the available cells, Teflon or mylar spacers are used to set the optical path length, but they need to be replaced whenever the cell is filled with buffer or with sample solution. This procedure does not guarantee for the reproducibility of the optical path length between sample and reference (Kong and Yu 2007). In order to mend these problems, solution of protein in D_2O are often used, since the D–O–D bending absorption is displaced at 1,200 cm^{-1}. However, some studies claim that the amide I band of proteins is strongly affected by the H–D substitution (Kong and Yu 2007), casting a serious warning on this method.

A further problem is the low solubility of many vegetable proteins: for high-quality spectrum of the amide I band a large amount of protein is required, thus producing high viscous samples. In this case, intrinsical inhomogeneity and opacity of the solution may affect the spectrum through diffuse IR radiation, frequency-dependent baseline and lineshape distortions.

FT-IR microscopy

Recently, FT microspectroscopy has yielded new insight in the measure of the transmittance spectrum of biologic samples (Toyran et al. 2007). Cross-section of biological tissue (few micron in thickness) obtained by cryotome can be analyzed by means of an infrared FT microscope with spatial resolution limited at 5–10 μm by diffraction effects. The mid-IR transmittance from selected area of the section collected as a function of the transversal position may be used to realize spectral mapping of the sample.

Measurements in pellets

The absorbance of dried protein can be estimated through the measure of the IR intensity transmitted by a pellet of transparent media mixed with a small amount of the protein in particles (Carbonaro et al. 2008). For the IR region, finely ground KBr salt is often used as a medium because its real refractive index is constant in the whole frequency region and the absorption negligible small. The pellets are obtained by mixing the salt (usually 200 mg) with protein up to a concentration of 1–5% in weight. The mixture is then pressed under vacuum using a force of 10 tons in a suited cell, thus obtaining a self-standing pellet whose thickness is 100–200 μm. A pellet of pure salt used as a reference is identically built.

The intensity $I(\nu)$ of the IR radiation transmitted through the sample pellet and that transmitted by the reference, $I_0(\nu)$, are both collected in order to achieve the optical density $O_d(\nu)$

$$O_d(\nu) = -\ln\left[\frac{I(\nu)}{I_0(\nu)}\right] \tag{2}$$

This method is not suggested for a precise definition of the protein secondary structure, which requires an accurate characterization of the amide lineshapes in order to resolve their fine components (see par. 1.3). Indeed, the resulting lineshape is a convolution of terms due to the intrinsic protein absorption and to the optical properties of the single grains. Moreover, for particles size comparable with the wavelength of the IR radiation, diffusion effects cannot be avoided and the spatial and size distribution of the particles becomes of significance. Therefore, the use of finely ground material is recommended.

Measurements of diffuse reflectance

According to the Kubelka–Munk theory (Kubelka 1948, 1954), a medium of infinite thickness with an absorption coefficient for unity length α and diffusion coefficient s, diffuses a backward radiation R_∞ given by:

$$R_\infty = 1 + \frac{\alpha}{s} - \sqrt{\frac{\alpha^2}{s^2} + 2\frac{\alpha}{s}} \tag{3}$$

Equation 3 is usually written in the form

$$\frac{(1 - R_\infty)^2}{2R_\infty} = \frac{\alpha}{s} = f(R_\infty) \tag{4}$$

where $f(R_\infty)$ is the Kubelka–Munk function (KM) which provides information on both the absorption and diffusion coefficients.

The experimental procedure consists of several points. First, a diffuse spectrum $I_0(\nu)$ of finely ground KBr powder is collected and considered as a reference. A further acquisition of a diffuse spectrum $I_c(\nu)$ is performed once a small concentration c of protein is dispersed in the KBr matrix. The ratio

$$R(\nu) = I_c(\nu)/I_0(\nu) \tag{5}$$

provides an estimate of the diffuse reflectance of the protein itself, and can be handled following Eq. 4. The obtained KM function $f(R)$ directly returns the absorption coefficient α' of the protein:

$$f(R) \propto \alpha'(\nu)c \tag{6}$$

The diffuse reflectance FT-IR spectroscopy (DRIFTS) presents several advantages when compared with the measure of absorption coefficient obtained from pellets: (1) DRIFTS spectra are suitable for samples with high absorption; (2) they are not subjected to baseline distortions due to scattering from grain; (3) samples can be easily prepared and managed using small amounts of protein (typically 1 mg).

This technique cannot provide quantitative measure of the absorption coefficient; however, it is extremely useful for samples whose transmission is difficult to analyze and it provides more accurate information with respect to the most used transmission of pellets. One major problem of the DRIFTS technique resides on the unwanted mixing of diffuse and specular reflectivity: the latter normally increases in correspondence of the sample absorptions thus producing spurious effects on the spectra. Therefore, most of the commercial accessories for DRIFTS collect the diffuse radiation in a limited solid angle that leaves out the specular component, even if it implies a loss of the intensity.

Measurements of attenuated total reflection (ATR)

This method is based on the evanescent radiation field at the interface between two contiguous media. The infrared beam produced from a source experiences multiple internal reflections between the plane sides of a not-absorbing

crystal (usually ZnSe, refractive index 2.24, but also silicon or Germanium crystals). In any single reflection, the quantum-mechanical nature of the radiation produces an evanescent field at the interface between the crystal and outer medium: this radiation can be used to probe the absorption spectrum of the sample (Harrick1967). In more details, the mid-IR radiation is focalized in a ZnSe crystal used as total reflective medium, suitable sized for a number of total internal reflections from 3 to 7. The sample, dissolved in a liquid solution, is placed on the side of the plane crystal, where the evanescent wave penetrates for a depth given by:

$$d(\lambda) = \frac{\lambda}{2\pi n_1 \sqrt{\sin^2 \theta - n_{12}}} \quad (7)$$

being λ the wavelength of the radiation, n_1 the refractive index of the crystal, n_{12} the ratio between liquid and crystal refractive index and θ the incidence angle. A typical value for d are is about 1.5 μm in the mid-IR region. The absorption coefficient as defined in Eq. 1 is then evaluated collecting the spectrum of the liquid solution and that obtained from the liquid buffer as a reference. This method routinely accounts for the reproducibility of the sample and reference thickness, thus allowing a more precise subtraction of the absorption bands due to the reference spectrum. However, because the penetration depth depends on λ, a quantitative analysis of absorption bands over a large region of frequencies requires a careful data correction.

Amide bands I, II and III

Nine normal modes are generated for the amide band of proteins and peptides (Barth 2007). These are called A, B, and I–VII in order of decreasing frequency (see Table 1). The amide bands I (80% C=O stretch, near 1,650 cm^{-1}), II (60% N–H bend and 40% C–N stretch, near 1,550 cm^{-1}), and III (40% C–N stretch, 30% N–H bend, near 1,300 cm^{-1}) are generally employed to study protein structure (Pelton and McLean 2000).

In practice, the amide I band in FT-IR (while both the amide I and III bands in Raman) are primarily used to assign secondary structures to proteins. Identification of particular frequencies with secondary structures has been made by reference to spectra of homopolypeptides and proteins with primarily α-helical or β-sheet structures, theoretical calculations (normal mode analysis), as well as by close comparison with proteins with a well-established three-dimensional structure.

The IR frequencies in the amide I region, diagnostic of protein secondary structures, are reported in Table 2. Intense bands around 1,654 cm^{-1} are observed for α-helical conformed proteins. A strong band between 1,612 and 1,640 cm^{-1} and a weaker band about 1,682 cm^{-1} are commonly observed for antiparallel β-sheets, whereas parallel β-sheets bands are reported at frequencies between 1,626 and 1,640 cm^{-1}. Random coil (RC) structure is generally assigned to the band at 1,645 cm^{-1} (Goormaghtigh et al. 1994; Mantsch and Chapman 1996; Pelton and McLean 2000).

Table 1 Infrared absorption bands from proteins and peptides

Band	Frequency (cm^{-1})	Description
Amide A	3,300	NH stretching
Amide B	3,100	NH stretching
Amide I	1,600–1,690	C=O stretching
Amide II	1,480–1,575	CN stretching; NH bending
Amide III	1,229–1,301	CN stretching; NH bending
Amide IV	625–767	OCN bending
Amide V	640–800	Out-of-plane NH bending
Amide VI	537–606	Out-of-plane C=O bending
Amide VII	200	Skeletal torsion

Table 2 Amide I frequencies assigned to protein secondary structure (cm^{-1} in H_2O)

Secondary structure	Range	Average
A-helix	1,648–1,660	1,654
α-helix turns[a]	1,630	1,630
β-sheet	1,612–1,641	1,625
	1,626–1,640	1,633
	1,670–1,694	1,682
Turns	1,662–1,684	1,673
Random coil	1,640–1,650	1,645

Based on experimental data and assignments available from the literature (Goormaghtigh et al. 1994; Mantsch and Chapman 1996; Pelton and McLean 2000)

[a] According to Murayama and Tomida (2004)

However, amide I absorption is affected by backbone structure. α-Helix band position is shifted down with increasing helix length, whereas short α-helices can produce several bands in the amide I region. Position of bands of parallel and antiparallel β-sheets depends on the number of strands. Antiparallel β-sheet band is also affected by twisting of sheets (Torii and Tasumi 1992).

Some amino acid side chains of globular proteins, especially arginine, glutamine, aspartic and glutamic acids, lysine, tyrosine, histidine and phenylalanine have intense absorption in the amide I spectral region (Venyaminov and Kalnin 1990). The quantitative estimation of amino acid side chain groups is necessary to refine analysis of secondary structure of proteins with a high content of these residues.

In the IR spectra, the amide II band is observed at 1,540–1,550 cm^{-1} and a weaker shoulder at 1,510–1,525 cm^{-1}. Peptides and proteins with an antiparallel β-sheet structure have strong amide II bands between 1,510 and 1,530 cm^{-1}; a parallel β-sheet structure is found at somewhat higher frequencies (1,530–1,550 cm^{-1}) (Pelton and McLean 2000).

The amide III band is normally quite weak and occurs in a region of mixed vibrations (CH bending, tyrosine and phenylalanine ring vibrations) that are not easily correlated to protein secondary structure, especially in protein mixture where this region is usually obscured (Dollinger et al. 1986). Amide III region has been successfully used for determination of α-helix or β-sheets (Fu et al. 1994), as well as of β-turns and RC structural elements of pure proteins (Cai and Singh 1999).

Quantification of percentage of secondary structures

A major difficulty in FT-IR spectral analysis of proteins is that the bands are a complex of several overlapping components representing different structural conformations. Because the width of these components is greater than the separation between the maxima of adjacent peaks it cannot be resolved by simple inspection of the spectra. However, the general upgrading of the experimental procedure and method agree to a more precise definition of the bands suggesting a less critical role for the data analysis. Band deconvolution in the Fourier domain (FSD, Fourier self deconvolution) is the most commonly used band-narrowing method in spectra where band overlap is severe. The use of the second (and higher) derivatives method only works in very specialized applications, because derivatives unlike FSD do not preserve the band areas.

Fourier self-deconvolution provides band-narrowing through multiplication of the Fourier transformed spectrum by a line-shape function and an apodization function, yielding a Fourier transform of narrower bandwidth. According to some studies, a value of 13 cm^{-1} for the full bandwidth at half height (FWHH) and a resolution enhancement factor (K) of 2.4 appear to be adequate for the amide bands (Kauppinen et al. 1981; Byler and Susi 1986). Recently, computationally methods for the estimation of mean bandwidth of overlapping bands of complex spectra have been developed (Lorenz-Fonfria and Padros 2008).

Once the component amide bands have been identified on the basis of the resolution-enhanced and second derivative spectra, quantification can be performed by assuming that the extinction coefficients for the different structural elements are the same for any components. Under this assumption, the intensities are proportional to the amount of each secondary structure. Because the resolution enhancement causes distortion of the bandshapes, enhanced spectra cannot be directly used to quantify secondary structure and a process of adjusting the positions, intensities, and shapes of the component bands to the experimental spectrum is necessary.

To determine the relative amounts of different protein secondary structures (α-helixes, β-sheets, turns, RC) two steps need to be applied. The first one uses the FSD spectrum to identify the amide I component peak frequencies according to the FSD algorithm, as described in Kauppinen et al. (1981). The second step is a multipeak fitting with Gaussian or Lorentzian functions using suitable analysis software to quantify the multicomponent peak areas in the protein amide I bands. The relative amounts of α-helixes, β-sheets, etc., are calculated from the fitted peak areas.

Other techniques have recently been developed for the prediction of secondary structure using the correlation coefficient between the FT-IR bands and the crystallographic data for proteins whose X-ray data are available. Alternative approaches for quantification of secondary structures include multivariate analysis, such as principal component analysis and factor analysis: both procedures are aimed at extracting information on a reduced number of properties from strongly correlated variables. In particular, the factor analysis method consists on the search of a set of eigenspectra which reproduces the original ones within their experimental errors. The set of coefficients which attains to this condition is then used in a linear regression in order to predict the unknown secondary structure of proteins from their spectra (Lee et al. 1990). More recently, neural network procedure (NN) has been successfully adopted for the analysis of the infrared data (Akkas et al. 2007). NN is a nonlinear iterative mapping driving an input vector **x** toward an output vector **y** through out the search of a vector **w** that minimizes an error function (Severcan et al. 2004). When the input **x** are the information from IR spectra and **y** the known secondary structure from literature data, a base parameter vector **w** can be obtained and used to predict the secondary structure of unknown samples. Other approaches for the quantification of secondary structures include partial least square regression methods which build a small basis set composed of a linear combinations of the original calibration spectra (Dousseau and Pezolet 1990).

All these methods, including fitting and narrowing deconvolution, should be approached cautiously. Indeed a combination of full-spectrum techniques, followed by a careful analysis of the number of overlapping bands in the Amide I region, is usually recommended.

Application of FT-IR to food analysis

Unlike near-infrared spectroscopy (NIR), FT-IR methods have been applied to food ingredient analysis in relatively

recent times (Ishiguro et al. 2006; Hashimoto and Kameoka 2008). For many years, mid-IR spectroscopy applications have been mainly limited to qualitative analysis, including identification of unknown compounds or use as detectors for instruments such as gas chromatographs and HPLC (Coleman 1993).

Whereas there has been much involvement of mid-IR spectroscopy in secondary structure analysis of isolated food proteins, their quantification in intact food systems has only been possible after development of FT-IR modern spectrometers.

In fact, it was found later that FT-IR had several advantages compared with NIR spectroscopy (Reeves 1994; Reeves and Zapf 1998). Indeed, FT-IR spectra present fundamental as well as overtone bands, whereas NIR spectra consist of overtones and combination bands that are more difficult to interpret and assign. Currently, considerable effort is being carrying out by spectroscopists to achieve unequivocal interpretation of FT-IR spectra (Roeges 1994) and to extend principles to NIR spectra.

FT-IR is a rapid, sensitive and non-waste generating technique that can be used with no external calibration for qualitative analysis. Moreover, with FT-IR spectroscopy it is possible to monitor changes in the structure and properties of biomolecules such as DNA, RNA, proteins, carbohydrates, lipids in biological tissues and cell, simultaneously (Coleman 1993). A further advantage of FT-IR spectroscopy over other techniques is that spectra can be obtained in a wide range of environments, not only in solution, but also in powders and on surfaces including polymers, metals and bioceramics. Therefore, it can be applied to food in different forms such as dried or liquid, allowing characterization of fresh material, too.

Both qualitative and quantitative data can be obtained. Comparison of specific ingredient content, such as protein and phytate, of different soybean varieties by FT-IR has been reported (Ishiguro et al. 2006).

In comparison with studies on isolated proteins, application of this technique to the study of protein structure at a molecular level inside whole food matrix implies complex mixtures. In this case, it is not possible to assign secondary structure elements to any particular protein. Nonetheless, quite useful information about protein quality and functionality of different foods can be derived (Hashimoto and Kameoka 2008).

Introduction of new devices, notably diamond attenuated total reflectance (ATR) and DRIFT spectroscopy has reduced shortcomings in sample treatment, thus increasing interest and potentiality in FT-IR applications.

A selection of examples of application of FT-IR in food protein characterization are reported in Table 3 and are discussed below.

Table 3 Examples of application of FT-IR to food analysis

Application case	Reference
Structure of animal (milk, meat) proteins	Sawyer and Holt (1993); Fang and Dalgleish (1997, 1998); Lefevre and Subirade (2000); Meersman et al. (2002); Li-Chan (2007)
Structure of plant (legume, cereals) storage proteins	Popineau et al. (1994); Dev et al. (1988); Chehin et al. (1999); Gianibelli et al. (2001); Ma et al. (2001); Shewry and Halford (2002); Ellepola et al. (2005); Wellner et al. (1996); Carbonaro et al. (2008)
Protein and phytate content of soybean varieties	Ishiguro et al. (2006)
Calcium binding to soy protein hydrolysates	Bao et al. (2008)
Interaction between wheat gliadins and dextrin	Secundo and Guerrieri (2005)
Emulsion stabilizing properties of sunflower proteins	Burnett et al. (2002)
Analysis of spray-dried milk protein powders	Kher et al. (2007)
Effect of brine salting on Atlantic salmon	Bocker et al. (2008)
Pressure *vs* thermal unfolding of myoglobin	Meersman et al. (2002)
Radiation-induced changes of hazelnut tissues	Dogan et al. (2007)
Hard-to-cook molecular changes in common bean	Maurer et al. (2004)
Denaturation of meat proteins	Kirschner et al. (2004)
Authentication of meat	Al-Jowder et al. (1997); Kirschner et al. (2004); Wu et al. (2007)
Ripening of soft cheeses	Martin-del-Campo et al. (2007)
Imaging of molecular microstructure of cereal tissues	Yu (2004); Yu et al. (2004)
Heat-induced changes in legume protein structure	Carbonaro and Nucara (2007)
Digestibility properties of legume proteins	Carbonaro (2006b)

Structural studies of food proteins

The secondary structure of several isolated proteins has been determined by different spectroscopic techniques, including FT-IR (Arrondo et al. 1993; Jackson and Mantsch 1995; Kumosinski and Unruh 1996; Barth 2007). Indeed, for many proteins (large proteins or proteins unable to form crystals) FT-IR was the only available spectroscopic technique. For these proteins, secondary structure estimation by FT-IR has been quite straightforward and well-established method alongside X-ray crystallography and NMR (Kumosinski and Unruh 1996). Valuable information about protein-folding, denaturation, protein–protein interaction, enzyme–substrate/inhibitor binding and subunit assembly have been provided, helping in the understanding of protein function (Sawyer and Holt 1993; Arrondo and Goni 1999; Fabian et al. 1999).

FT-IR spectra are, therefore, available for a number of food proteins and food-related proteins, like digestive enzymes (Hashimoto and Kameoka 2008). Either animal (milk, meat) (Troullier et al. 2000; Bhattacharjee et al. 2005; Meersman et al. 2002) or plant (legume, cereals) proteins, including concanavalin A (Arrondo et al. 1988) and storage proteins (Dev et al. 1988; Wellner et al. 1996; Gianibelli et al. 2001; Meng and Ma 2001; Shewry and Halford 2002; Ellepola et al. 2005; Carbonaro et al. 2008) have been analyzed.

The FT-IR spectrum of common bean 7S globulin in the spectral region 1,000–1,750 cm^{-1} is shown as an example in Fig. 2. The absorption at 1,100 cm^{-1} (band A) is attributed to C–O and C–C stretching, and also to CCH, COH and HCO deformational vibrations (Naumann 2001). The band around 1,420 cm^{-1} (band B) falls in the region of C–H bending modes (Bhattacharjee et al. 2005). Both bands arise mainly from carbohydrates, in particular structural carbohydrates of cellulosic origin, coextracted with 7S globulin (Pietrzak and Miller 2005). Quantification of different percentage of secondary structure by analysis of amide I band of the spectrum has indicated a major component at 1,638 cm^{-1}, assigned to β-sheet modes. This contribution accounted for 30% of the total, in fair agreement with X-ray crystallographic data, indicating that β-sheet is the dominant structure of 7S globulin.

Good correspondence between percentage of secondary structure calculated by FT-IR (Carbonaro et al. 2008) and X-ray crystallographic results has been found for β-lactoglobulin, too (37 vs. 40% for β-sheet and 13 vs. 15% for α-helix, by FT-IR and X-ray, respectively). As Fig. 3 shows, the 3D structure of bovine β-lactoglobulin is characterized by extensive β-conformation and very little

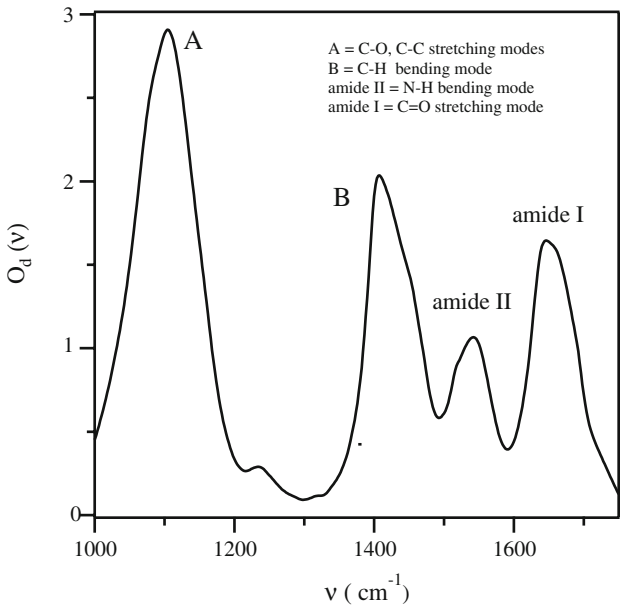

Fig. 2 FT-IR spectrum of 7S globulin (1% in KBr pellet). From Carbonaro et al. 2008

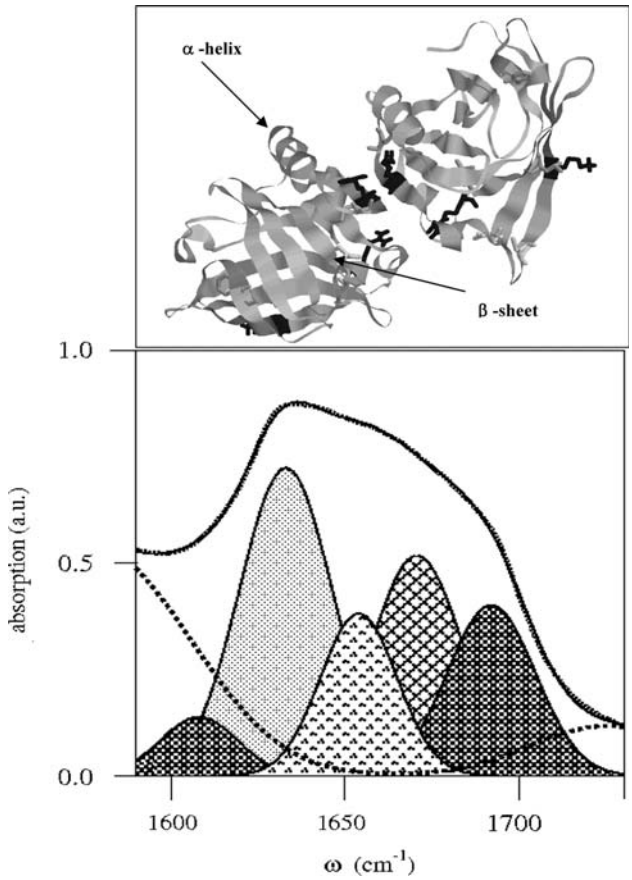

Fig. 3 3D structure of bovine β-lactoglobulin (*top panel*). Spectral deconvolution of the amide I band of β-lactoglobulin measured by DRIFTS (*bottom panel*). The *light gray* Gaussian represents the β-sheet component, the *dotted one* the α-helix. The two *dark gray* and the shaded Gaussians are contributions from aggregates and β-turn, respectively (Carbonaro et al. unpublished)

α-helix. In the bottom panel of Fig. 3, the single contributions of the amide I band are shown.

A reliable evaluation of the relative spectral weight of the amide I spectrum has been demonstrated to be realized with not more than five components (Carbonaro et al. 2008). A comparison with available FT-IR data on secondary structure quantification of 7S globulins provides conflicting results. It is worth noting that the peak frequency can be affected by measurement conditions and analysis procedure. Data reported in the literature are often based on a number of components in the amide I band higher than 5, likely accounting for the differences in percentage of secondary structures noticed.

FT-IR in food technology

Structural studies also aimed at the elucidation of functional properties of food proteins. FT-IR information has been valuable not only in the case of major milk whey proteins, β-lactoglobulin and α-lactalbumin (Fang and Dalgleish 1997, 1998; Lefevre and Subirade 2000; Li-Chan 2007), but also of lentil lectin (Chehin et al. 1999), oat, rice, soy globulins (Ma et al. 2001, Mills et al. 2003; Ellepola et al. 2005), wheat gliadins and glutenins (Popineau et al. 1994; Wellner et al. 1996; Mangavel et al. 2001).

The details of specific interactions between food proteins and other molecules commonly employed in the food industry (polysaccharides, lipids, metals) have been elucidated by FT-IR. In particular, using ATR-FT/IR conformational changes that occur upon thermal treatment of wheat gliadins in the absence and in the presence of dextrins have been investigated (Secundo and Guerrieri 2005).

Mechanism of calcium binding by soy protein hydrolysates obtained by different proteases has been compared by FT-IR (Bao et al. 2008).

Secondary structural changes that occur upon adsorption of 2S albumins of sunflower seed at the oil/water interface have been analyzed. Molecular changes underlying the different capacity of sunflower albumins in stabilizing emulsion have been described (Burnett et al. 2002).

FT-IR spectroscopy has proved to be useful in detecting molecular rearrangement that occur following technological processes, too.

The conformational changes of whey proteins upon adsorption at the soy oil/water interface under high-pressure homogenization have been investigated (Lee et al. 2007). Following adsorption of whey proteins at the oil/water interface, irreversible changes in β-sheets and α-helix structures have been observed. This study demonstrated that specific conformational changes of whey proteins—unfolding, increase in α-helix structure and aggregation—are necessary to stabilize emulsions.

FT-IR spectroscopy has also been used to examine the conformation of proteins in spray-dried milk protein concentrate powders and to monitor the spectral changes responsible for nitrogen solubility of these powders (Kher et al. 2007).

Effect of brine salting on Atlantic salmon fillets with regards to quality of raw material and salt content has recently been investigated by FT-IR microspectroscopy (Bocker et al. 2008).

Cold unfolding of myoglobin has been elucidated by FT-IR spectroscopy and compared with hydrostatic pressure and heat unfolding (Meersman et al. 2002). The cold and pressure-induced unfolding yield a partially unfolded state, characterized by a persistent amount of secondary structure with a stable core of G and H helices. In contrast, the heat unfolding results in the formation of the infrared bands typical of intermolecular antiparallel β-sheet aggregation.

Similarly, molecular changes in macromolecular components of hazelnut tissues induced by radiation have also been investigated (Dogan et al. 2007). Besides changes in the lipid to protein ratio, high-dose irradiation has been found to cause alterations in the structure of hazelnut proteins. In particular, occurrence of cross-linking and aggregation of protein molecule has been evidenced, thus indicating that FT-IR spectroscopy can be successfully used to monitor food irradiation.

An interesting application concerns the study of structural modifications underlying development of technological defects, notably hard-to-cook phenomenon in common beans, by DRIFTS analysis of protein fractions (Maurer et al. 2004).

Authentication of foods by FT-IR

There is increasing interest within the food industry in rapid techniques for addressing problems of food authentication. To this respect, FT-IR spectroscopy appears to be a valuable candidate (Reid et al. 2006). Indeed, the mid-IR region (400–5,000 cm^{-1}) has proved to be very sensitive to the chemical composition of food samples.

A limited selection of examples of successful applications includes authentication of meats (Al-Jowder et al. 1997; Wu et al. 2007) and characterization of Camembert cheese (Martin-del-Campo et al. 2007).

In the former application, it has been possible to distinguish minced chicken, pork and turkey meats, as well as to differentiate between fresh and frozen-thawed samples by FT-IR analysis of characteristic protein absorption bands at about 1,650 cm^{-1} (amide I band) and at 1,550 cm^{-1} (amide II band). Also, semi-quantitative estimation of meat mixture composition (levels of turkey and pork mixed with chicken meat) has been found to be

feasible (Al-Jowder et al. 1997). Later, conformational changes correlated to denaturation of the major meat proteins in beef loin, such as myosin, actin and collagen have been clarified by FT-IR microspectroscopy (Kirschner et al. 2004). In particular, increase in β-sheet and decrease in α-helical structures could be revealed, the latter appearing to be much more pronounced for the myofibers than for the connective tissue.

Promising studies also regard evaluation of the potential of FT-IR spectroscopy to follow ripening phenomena of soft cheeses like Camembert produced at pilot scale (Martin-del-Campo et al. 2007).

FT-IR in bioavailability studies

There is a general consensus that knowledge of the structure of a food protein is essential to predict its digestive behavior, nutritive quality, utilization and amino acid availability in man and animals. However, the relationship between structure and bioavailability of proteins from food environment has been only partially clarified so far (Norris and Barness 1976; Dziuba et al. 2005; Carbonaro 2006b). There is growing evidence that protein availability and digestive behavior are closely related to food chemical composition because modifications in the protein structure, consequent to protein–protein interaction or interaction with other matrix components, are likely to occur during technological processes and gastrointestinal digestion.

Recent research by synchrotron radiation-based Fourier transform-infrared microspectroscopy has provided detailed ultrastructural-chemical information for plant feed tissues such as barley, oat and wheat (Yu 2004; Yu et al. 2004). With this technique, chemical feature of feed protein secondary structure has been revealed within amide I at an ultraspatial resolution. Percentages of α-helixes and β-sheets has been calculated for barley, oat and wheat proteins, whose relative ratio can be related to protein value of feeds.

Because essential amino acids and bioactive fragments are encrypted in the animal or plant protein sequence, the distribution and structure of bioactive fragments may favor or inhibit their release by proteinases, as it has been suggested by the result of recent findings (Dziuba et al. 2005; Carbonaro 2006a, b).

Significant changes in protein secondary structure have recently been shown by FT-IR analysis to take place upon heating of plant foods with a high protein content, such as legumes. Spectra measured in the range of the amide I band for raw and heat-processed common bean (*Phaseolus vulgaris* L.) flours are shown in Fig. 4: raw, dry thermally treated, and autoclaved samples. In each panel, the different components and resulting best-fit profiles are

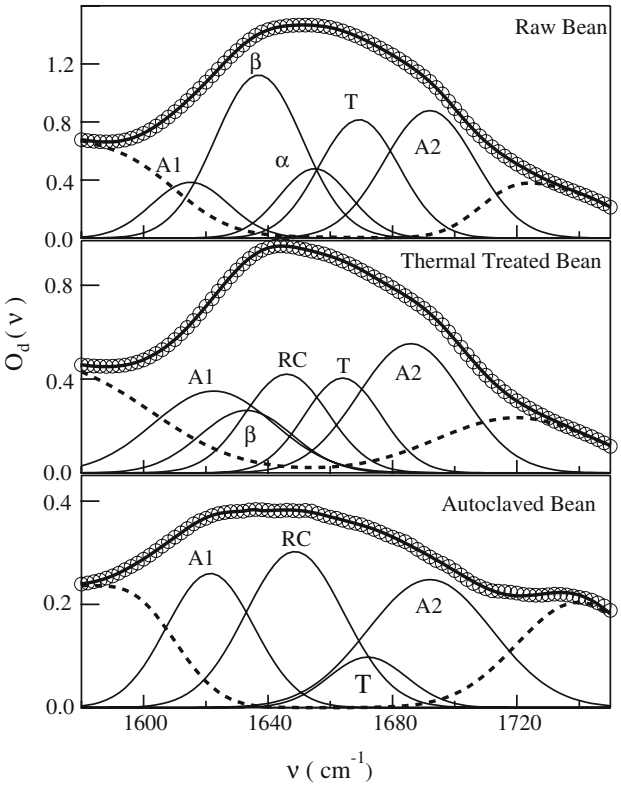

Fig. 4 FT-IR spectra of raw, thermal treated and autoclaved common bean (1% protein in KBr pellet). Gaussian components are α α-helix; β β-sheet; *T* turns, *RC* random coil, *A1*, *A2* aggregate components. From Carbonaro et al. 2008

shown separately. It is evident that for the raw common bean sample, the amide I band most significant contribution is due to the β-sheet structure. In the dry thermally treated sample, a complete disappearance of the α-helix component can be observed, together with a strong decrease in the β-sheet contribution, that was also shifted to lower frequencies, as a result of protein unfolding and denaturation. On the other hand, a good description of the amide I band of the dry thermally treated sample requires an important contribution peaked around 1,646 cm^{-1}, ascribed to RC conformation. Similarly, in the autoclaved common bean sample, the dominant contributions to the amide I band are due to RC and aggregate (A1 + A2) bands.

It is worth pointing out that such modifications involve built-up of a high amount of stable aggregates, up to 40% in autoclaved samples (Carbonaro and Nucara 2007; Carbonaro et al. 2008). The latter mechanism is likely to account for a detrimental effect on digestibility of storage proteins after thermal treatment of plant food and, possibly, of the formation of novel complexes whose bioactivity deserves further consideration (Carbonaro et al. 2005; Van Boxtel et al. 2006).

Conclusions

FT-IR has the peculiarity of providing high-quality spectra with very small amount of protein, in different environments irrespective of the molecular mass. It has recently been demonstrated to be a valuable tool for gaining insight into structural aspects of food proteins of relevance for nutritional potential and technological applications. In particular, power in pointing out mechanism of protein aggregation and stability, as well as relationship to bioavailability and bioactivity of specific components, makes this technique of increasing importance in the emerging field of food proteomics.

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References

Akkas SB, Severcan M, Yilmaz O, Severcan F (2007) Effect of lipoic acid supplementation on rat brain tissue: an FTIR spectroscopic and neural network study. Food Chem 105:1281–1288. doi:10.1016/j.foodchem.2007.03.015

Al-Jowder O, Kemsley EK, Wilson R (1997) Mid-infrared spectroscopy and authenticity problems in selected meats: a feasibility study. Food Chem 59:195–201. doi:10.1016/S0308-8146(96)00289-0

Arrondo JLR, Goni FM (1999) Structure and dynamics of membrane proteins as studied by infrared spectroscopy. Prog Biophys Mol Biol 72:367–405. doi:10.1016/S0079-6107(99)00007-3

Arrondo JLR, Young NM, Mantsch HH (1988) The solution structure of concanavalin A probed by FT-IR spectroscopy. Biochim Biophys Acta 952:261–268

Arrondo JLR, Muga A, Castresana J, Goñi FM (1993) Quantitative studies of the structure of proteins in solution by Fourier-transform infrared- spectroscopy. Prog Biophys Mol Biol 59:23–56. doi:10.1016/0079-6107(93)90006-6

Bao XL, Lv Y, Yang BC, Ren CG, Guo ST (2008) A study of the soluble complexes formed during calcium binding by soybean protein hydrolysates. J Food Sci 73:C117–C121. doi:10.1111/j.1750-3841.2008.00673.x

Barth A (2007) Infrared spectroscopy of proteins. Biochim Biophys Acta 1767:1073–1101. doi:10.1016/j.bbabio.2007.06.004

Bhattacharjee C, Saha S, Biswas A, Kundu M, Ghosh L, Das KP (2005) Structural changes of β-lactoglobulin during thermal unfolding and refolding—an FT-IR and circular dichroism study. Protein J 24:27–35. doi:10.1007/s10930-004-0603-z

Bocker U, Kohler A, Aursand IG, Ofstad R (2008) Effects of brine salting with regard to raw material variation of Atlantic salmon (Salmo salar) muscle investigated by Fourier transform infrared. J Agric Food Chem 56:5129–5137. doi:10.1021/jf703678z

Burnett GR, Rigby NM, Mills ENC, Belton PS, Fido RJ, Tatham AS (2002) Characterization of the emulsification properties of 2S albumins from sunflower seed. J Colloid Interface Sci 247:177–185. doi:10.1006/jcis.2001.8093

Byler DM, Susi H (1986) Examination of the secondary structure of proteins by deconvolved FTIR spectra. Biopolymers 25:469–487. doi:10.1002/bip.360250307

Cai S, Singh BR (1999) Identification of β-turn and random coil amide III infrared bands for secondary structure estimation of proteins. Biophys Chem 80:7–20. doi:10.1016/S0301-4622(99)00060-5

Carbonaro M (2006a) Application of two-dimensional electrophoresis for monitoring gastrointestinal digestion of milk. Amino Acids 31:485–488. doi:10.1007/s00726-006-0367-1

Carbonaro M (2006b) 7S globulins from *Phaseolus vulgaris* L.: impact of structural aspects on the nutritional quality. Biosci Biotechnol Biochem 70:2620–2626. doi:10.1271/bbb.60203

Carbonaro M, Nucara A (2007) Application of FT-IR spectroscopy in the assessment of changes in the secondary structure of food proteins taking legumes as a model. Amino Acids 33:45–46

Carbonaro M, Grant G, Cappelloni M (2005) Heat-induced denaturation impairs digestibility of legume (*Phaseolus vulgaris* L. and *Vicia faba* L.) 7S and 11S globulins in the small intestine of rat. J Sci Food Agric 85:65–72. doi:10.1002/jsfa.1940

Carbonaro M, Maselli P, Dore P, Nucara A (2008) Application of Fourier transform infrared spectroscopy to legume seed flour analysis. Food Chem 108:361–368. doi:10.1016/j.foodchem.2007.10.045

Chehin R, Iloro I, Marcos MJ, Villar E, Shnyrov VL, Arrondo JLR (1999) Thermal and pH-induced conformational changes of a β-sheet protein monitored by infrared spectroscopy. Biochemistry 38:1525–1530. doi:10.1021/bi981567j

Coleman PB (1993) Practical sampling techniques for infrared analysis. CRC Press, Boca Raton

Dev SB, Keller JT, Rha CK (1988) Secondary structure of 11 S globulin in aqueous solution investigated by FT-IR derivative spectroscopy. Biochim Biophys Acta 957:272–280

Dogan A, Siyakus G, Severcan F (2007) FTIR spectroscopic characterization of irradiated hazelnut (*Corylus avellana* L.). Food Chem 100:1106–1114. doi:10.1016/j.foodchem.2005.11.017

Dollinger G, Eisenstein L, Shuo-Liang L, Nakanishi K, Termini J (1986) Fourier transform infrared difference spectroscopy of bacteriorhodopsin and its photoproducts regenerated with deuterated tyrosine. Biochemistry 25:6524–6533. doi:10.1021/bi00369a028

Dousseau F, Pezolet M (1990) Determination of the secondary structure content of protein in aqueous solutions from their amide-I and amide-II bands: comparison between classical and partial least-square methods. Biochemistry 29:8771–8779. doi:10.1021/bi00489a038

Dousseau F, Therrien M, Pezolet M (1989) On the spectral subtraction of water from FT-IR spectra of aqueous solution of proteins. Appl Spectrosc 43:538–542. doi:10.1366/0003702894202814

Dziuba J, Niklewicz M, Iwaniak A, Darewicz M, Minkiewicz P (2005) Structural properties of proteolytic-accessible bioactive fragments of selected animal proteins. Polymers 50:424–428

Ellepola SW, Siu MC, Ma CY (2005) Conformational study of globulin from rice (*Oryza sativa*) seeds by Fourier-transform infrared spectroscopy. Int J Biol Macromol 37:12–20. doi:10.1016/j.ijbiomac.2005.07.008

Fabian H, Mantsch HH, Schultz CP (1999) Two dimensional IR correlation spectroscopy: sequential events in the unfolding process in the lambda-CroV55C repressor protein. Proc Natl Acad Sci USA 96:13153–13158. doi:10.1073/pnas.96.23.13153

Fang Y, Dalgleish DG (1997) Conformation of β-lactoglobulin studied by FTIR: effect of pH, temperature, and adsorption to the oil–water interface. J Colloid Interface Sci 96:292–298. doi:10.1006/jcis.1997.5191

Fang Y, Dalgleish DG (1998) The conformation of α-lactalbumin as a function of pH, heat treatment and adsorption at hydrophobic surfaces studied by FTIR. Food Hydrocoll 12:121–126. doi:10.1016/S0268-005X(98)00003-4

Fu F-N, DeOliveira DB, William R, Trumble R, Hemanta K, Sarkar HK, Singh BR (1994) Secondary structure estimation of proteins

using the amide III region of Fourier transform infrared spectroscopy: application to analyze calcium-binding-induced structural changes in calsequestrin. Appl Spectrosc 48:1432–1441. doi:10.1366/0003702944028065

Gianibelli MC, Larroque OR, MacRitchie F, Wringley CW (2001) Biochemical, genetic, and molecular characterization of wheat glutenin and its component subunits. Cereal Chem 78:635–646. doi:10.1094/CCHEM.2001.78.6.635

Goormaghtigh E, Cabiaux V, Ruysschaert J-M (1994) Determination of soluble and membrane protein structure by Fourier transform infrared spectroscopy III. Secondary structures. Subcell Biochem 23:405–450

Haris PI, Severcan F (1999) FTIR spectroscopic characterization of protein structure in aqueous and non-aqueous media. J Mol Catal, B Enzym 7:207–221. doi:10.1016/S1381-1177(99)00030-2

Harrick NJ (ed) (1967) Internal reflection spectroscopy. Wiley, New York

Hashimoto A, Kameoka T (2008) Application of infrared spectroscopy to biochemical, food, and agricultural processes. Appl Spectrosc Rev 43:416–451. doi:10.1080/05704920802108131

Ishiguro T, Ono T, Wada T, Tsukamoto C, Kono Y (2006) Changes in soybean phytate content as a result of field growing conditions and influence on tofu texture. Biosci Biotechnol Biochem 70:874–880. doi:10.1271/bbb.70.874

Jackson M, Mantsch HH (1995) The use and misuse of FTIR spectroscopy in the determination of protein-structure. Crit Rev Biochem Mol Biol 30:95–120. doi:10.3109/10409239509085140

Kauppinen JK, Moffatt DJ, Mantsch HH, Cameron DG (1981) Fourier self deconvolution: a method for resolving intrinsically overlapped bands. Appl Spectrosc 35:271–276. doi:10.1366/0003702814732634

Kher A, Udabage P, McKinnon I, McNaughton D, Augustin MA (2007) FTIR investigation of spray-dried milk protein concentrate powders. Vib Spectrosc 44:375–381. doi:10.1016/j.vibspec.2007.03.006

Kirschner C, Ofstad R, Skarpeid HJ, Host V, Kohler A (2004) Monitoring of denaturation processes in aged beef loin by Fourier transform infrared microspectroscopy. J Agric Food Chem 52:3920–3929. doi:10.1021/jf0306136

Kong J, Yu S (2007) Fourier transform infrared spectroscopic analysis of protein secondary structure. Acta Biochim Biophys Sin (Shanghai) 39:549–559. doi:10.1111/j.1745-7270.2007.00320.x

Kubelka P (1948) New contributions to the optics of intensely light-scattering materials. Part I J Optic Soc Am 38:448–457. doi:10.1364/JOSA.38.000448

Kubelka P (1954) New contributions to the optics of intensely light-scattering materials. Part II J Optic Soc Am 44:330–335. doi:10.1364/JOSA.44.000330

Kumosinski TF, Unruh JJ (1996) Quantitation of the global secondary structure of globular proteins by FTIR spectroscopy: comparison with X-ray crystallographic structure. Talanta 43:199–219. doi:10.1016/0039-9140(95)01726-7

Lee DC, Haris PI, Chapman D, Mitchell RC (1990) Determination of protein secondary structure using factor analysis of infrared spectra. Biochemistry 29:9185–9193. doi:10.1021/bi00491a012

Lee S-H, Lefèvre T, Subirade M, Paquin P (2007) Changes and roles of secondary structures of whey protein for the formation of protein membrane at soy oil/water interface under high-pressure homogenization. J Agric Food Chem 55:10924–10931. doi:10.1021/jf0726076

Lefevre T, Subirade M (2000) Molecular differences in the formation and structure of fine-stranded and particulate β-lactoglobulin. Biopolymers 54:578–586. doi:10.1002/1097-0282(200012)54:7<578::AID-BIP100>3.0.CO;2-2

Li-Chan ECY (2007) Vibrational spectroscopy applied to the study of milk proteins. Dairy Sci Technol 87:443–458. doi:10.1051/lait:2007023

Lorenz-Fonfria VA, Padros E (2008) Method for the estimation of the mean Lorentzian bandwidth in spectra composed of an unknown number of highly overlapped bands. Appl Spectrosc 62:689–700. doi:10.1366/000370208784658129

Ma CY, Rout MK, Mock WY (2001) Study of oat globulin conformation by Fourier transform infrared spectroscopy. J Agric Food Chem 49:3328–3334. doi:10.1021/jf010053f

Mangavel C, Barbot J, Popineau Y, Gueguen J (2001) Evolution of wheat gliadins conformation during film formation: a Fourier transform infrared study. J Agric Food Chem 49:867–872. doi:10.1021/jf0009899

Mantsch HH, Chapman D (eds) (1996) Infrared spectroscopy of biomolecules. Wiley-Liss, New York

Martin-del-Campo ST, Picque D, Cosio-Ramirez R, Corrieu G (2007) Middle infrared spectroscopy characterization of ripening stages of Camembert-type cheeses. Int Dairy J 17:835–845

Maurer GA, Ozen BF, Maurer LJ, Nielsen SS (2004) Analysis of hard-to-cook red and black common beans using Fourier transform infrared spectroscopy. J Agric Food Chem 52:1470–1477. doi:10.1021/jf035083s

Meersman F, Smeller L, Heremans K (2002) Comparative Fourier transform infrared spectroscopy study of cold-, pressure-, and heat-induced unfolding and aggregation of myoglobin. Biophys J 82:2635–2644. doi:10.1016/S0006-3495(02)75605-1

Meng G-T, Ma C-Y (2001) Fourier-transform infrared spectroscopy study of globulin from *Phaseolus angularis* (red bean). Int J Biol Macromol 29:287–294. doi:10.1016/S0141-8130(01)00178-7

Mills ENC, Marigheto NA, Wellner N, Fairhurst SA, Jenkins JA, Mann R, Belton PS (2003) Thermally induced structural changes in glycinin, the 11S globulin of soya bean (*Glycine max*)—an in situ spectroscopic study. Biochim Biophys Acta 1648:105–111

Murayama K, Tomida M (2004) Heat-induced secondary structure and conformation change of bovine serum albumin investigated by Fourier transform infrared spectroscopy. Biochemistry 43:11526–11532

Naumann D (2001) FT-infrared and FT-Raman spectroscopy in biomedical research. In: Gremlich H-U, Yan B (eds) Infrared and Raman spectroscopy of biological materials. Marcel Dekker Inc, New York, pp 323–378

Norris KH, Barness RF (1976) Infrared reflectance analysis of nutritive value of feedstuffs. In: Fonnesbeck PV, Harris LE, Kearl LC (eds) Feed composition, animal nutrient requirements and computerization of diets. Proceedings of the 1st international symposium. Utah State University, Logan, UT, pp 237–241

Pelton JT, McLean LR (2000) Spectroscopic methods for analysis of protein secondary structure. Anal Biochem 277:167–176. doi:10.1006/abio.1999.4320

Pietrzak LN, Miller SS (2005) Microchemical structure of soybean seeds revealed in situ by ultraspatially resolved synchrotron Fourier transformed infrared microspectroscopy. J Agric Food Chem 53:9304–9311. doi:10.1021/jf050608x

Popineau Y, Bonenfant S, Cornec M, Pezolet M (1994) A study by infrared-spectroscopy of the conformation of gluten proteins differing in their gliadin and glutenin composition. J Cereal Sci 20:15–22. doi:10.1006/jcrs.1994.1040

Reeves JBIII (1994) Near- versus mid-infrared diffuse reflectance spectroscopy for the quantitative determination of the composition of forages and byproducts. J Near Infrared Spectrosc 2:49–57

Reeves JBIII, Zapf CM (1998) Mid-infrared diffuse reflectance spectroscopy for discriminant analysis of food ingredients. J Agric Food Chem 46:3614–3622. doi:10.1021/jf9801926

Reid LM, O'Donnell CP, Downey G (2006) Recent technological advances for the determination of food authenticity. Trends Food Sci Technol 17:344–353. doi:10.1016/j.tifs.2006.01.006

Roeges NPG (1994) A guide to the complete interpretation of infrared spectra of organic structures. New York, Wiley

Sawyer L, Holt C (1993) The secondary structure of milk proteins and their biological function. J Dairy Sci 76:3062–3078

Secundo F, Guerrieri N (2005) ATR-FT/IR study on the interactions between gliadins and dextrin and their effects on protein secondary structure. J Agric Food Chem 53:1757–1764. doi:10.1021/jf049061x

Severcan M, Haris PI, Severcan F (2004) Using artificially generated spectral data to improve protein secondary structure prediction from Fourier transform infrared spectra of proteins. Anal Biochem 332:238–244. doi:10.1016/j.ab.2004.06.030

Shewry PR, Halford NG (2002) Cereals seed storage proteins: structure, properties and role in grain utilization. J Exp Bot 53:947–958. doi:10.1093/jexbot/53.370.947

Torii H, Tasumi M (1992) Model calculations on the amide-I infrared bands of globular proteins. J Chem Phys 96:3379–3387. doi:10.1063/1.461939

Toyran N, Turan B, Severcan F (2007) Selenium alters the lipid content and protein profile of rat heart: an FTIR microspectroscopic study. Arch Biochem Biophys 458:184–193. doi:10.1016/j.abb.2006.12.012

Troullier A, Reinstädler D, Dupont Y, Naumann D, Forge V (2000) Transient non-native secondary structures during the refolding of α-lactalbumin detected by infrared spectroscopy. Nat Struct Biol 7:78–86. doi:10.1038/71286

Van Boxtel EL, Van Beers MCM, Koppelman SJ, Van den Broek LAM, Gruppen H (2006) Allergen Ara h 1 occurs in peanuts as a large oligomer rather than as a trimer. J Agric Food Chem 54:7180–7186. doi:10.1021/jf061433+

Venyaminov SY, Kalnin NN (1990) Quantitative IR spectrophotometry of peptide compounds in water (H_2O) solution. Spectral parameters of amino acid residue absorption bands. Biopolymers 30:1243–1257. doi:10.1002/bip.360301309

Wellner N, Belton PS, Tatham AS (1996) Fourier transform IR spectroscopic study of hydration-induced structure in the solid state of ω-gliadins. Biochem J 319:741–747

Wu Z, Bertram HC, Bocker U, Ofstad R, Kohler A (2007) Myowater dynamics and protein secondary structural changes as affected by heating rate in three pork qualities: a combined FT-IR microspectroscopic and 1H NMR relaxometry study. J Agric Food Chem 55:3990–3997. doi:10.1021/jf070019m

Yu P (2004) Application of advanced synchroton radiation-based Fourier transform infrared (SR-FTIR) microspectroscopy to animal nutrition and feed science: a novel approach. Br J Nutr 92:869–885. doi:10.1079/BJN20041298

Yu P, McKinnon JJ, Christensen CR, Christensen DA (2004) Using synchroton transmission FTIR microspectroscopy as a rapid, direct and non-destructive analytical technique to reveal molecular microstructural-chemical features within tissue in grain barley. J Agric Food Chem 52:1484–1494. doi:10.1021/jf035065a