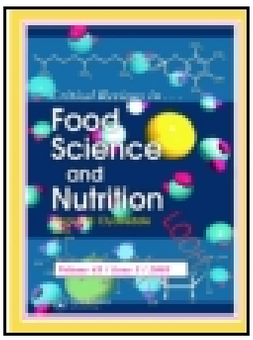

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Recent Research and Progress in Food, Feed and Nutrition with Advanced Synchrotron-Based SR-IMS and DRIFT Molecular Spectroscopy

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Running head: Recent Progress in Food and Feed Structure Research Using Molecular Spectroscopy

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ABSTRACT:

Ultraspatially resolved synchrotron radiation based infrared microspectroscopy is able to detect the structure features of a food or feed tissue at cellular and molecular levels. However, to date, this advanced synchrotron-based technique is almost unknown to food and feed scientists. The objective of this article was to introduce this novel analytical technology, ultra-spatially resolved synchrotron radiation based infrared microspectroscopy (SR-IMS) to food, feed, conventional nutrition and molecular nutrition scientists. The emphasis of this review focused on the following areas: (1) Principles of molecular spectroscopy for food and feed structure research, such as protein molecular structure, carbohydrate conformation, heating induced protein structure changes, and effect of gene-transformation on food and feed structure; (2) Molecular spectral analysis methodology; (3) Biological applications of synchrotron SR-IMS and DRIFT spectroscopy; and (4) Recent progress in food, feed and nutrition research program. The information described in this article gives better insight in food structure research progress and update.

Keywords: Molecular Structure, Molecular Spectroscopy, DRIFT, Synchrotron Radiation, Food and Feed, Molecular Nutrition

1. INTRODUCTION

Ttraditional wet chemical analysis has long been employed to study chemical composition of a food or feed. Although chemical analysis methods are in common usage, they fail to take the structural information into account. As reviewed previously, all the traditional wet analysis methodologies involve chemical reagents, chemical treatment and digestive process (Wetzel and LeVine 1993; Budevska 2002). The grinding of the sample usually makes it difficult to explore and evaluate microstructure or chemical distribution of biological samples. The locally distributed compounds are thus diluted and evenly mixed. These undesired influences from traditional chemical analysis make it difficult to restore the original status of the food or feed, thus resulting in loss of structure. These limitations cannot be overcome unless a new analytical tool is introduced. Therefore, traditional analysis is no longer sufficient for further investigation of grain in food and feed science, but needs to be complemented with other novel techniques.

II. PRINCIPLES OF MOLECULAR SPECTROSCOPY FOR STRUCTURE RESEARCH

2.1. Infrared Spectroscopy Principles

Mid-IR is commonly defined to cover the region of the electromagnetic spectrum from ca. 4000 to 400 cm^{-1} (Messerschmidt and Harthcock 1988). The wavelength (λ) of infrared (IR) region is longer than that of visible light. Atoms vibrate with certain frequencies in a molecule. Vibration of chemical bonds are not completely isolated, but may be affected by other parts of the molecule, especially the neighbouring groups. At the same time, with this interaction, the molecule itself does vibrate in different modes (Messerschmidt and Harthcock 1988). Generally,

there are two major vibrational modes: stretching and bending. For the stretching vibration, it includes symmetrical stretching vibration (ν_s) and asymmetrical stretching vibration (ν_{as}). For bending vibration, it includes (1) in plane bending vibration (δ), which can be separated to scissoring vibration (δ) and rocking vibration (ρ), and (2) out-of-plane bending vibration (γ), which can be separated to wagging vibration (ϖ) and twisting vibration (τ) (Messerschmidt and Harthcock 1988; Jackson and Mantsch 2000; Stuart 2004). All molecules can be imagined as the superposition of a simple harmonic oscillator formed by atoms. They move around at a certain frequency. The atom mass and the chemical bond between atoms are two factors which can affect this motion (Jackson and Mantsch 2000). Stronger bonds and smaller atom masses usually mean higher vibration frequency (Messerschmidt and Harthcock 1988). When the molecule encounters energy with the same vibration frequency as that of its normal modes of vibration, it can absorb the energy at this frequency. The unabsorbed energy will pass through the sample and can be measured. Different atoms vibrate at different frequencies with different modes, which results in complicated matrix structural information. Different compounds can exhibit their own characteristic IR absorption pattern (Messerschmidt and Harthcock 1988; Jackson and Mantsch 2000; Stuart 2004).

Exposure to IR radiation cause vibration and rotation of molecules between different quantized discrete energy levels E0, E1, E2 (Stuart 2004). Whenever IR radiation transmits through a sample, energy uptake by a molecule occurs and results in transition between different energy levels (Stuart 2004; Barth 2007). Primarily, various IR spectra patterns result from the corresponding vibration, a kind of internal motion of the molecule. Therefore, it is possible to identify the unknown organic compounds and determine the composition of a mixture according

to the frequencies, lineshapes, intensities and patterns of the characteristic peaks (Messerschmidt and Harthcock 1988; Jackson and Mantsch 2000; Stuart 2004). There can also be a slight shift of characteristic peaks resulting from diverse specimens, while the typical IR absorbance patterns do not change. Differences in chemical and structural composition, therefore, can be shown in the IR spectra. By analyzing with uni- and multi-variate analysis methods, informative differences can be probed. Various chemical compounds have various IR spectra. IR spectra are specific and characteristic, which therefore can be used as "fingerprints" for identifying or discriminating sample conformation (Messerschmidt and Harthcock 1988).

Infrared spectroscopy has been successfully used as an analytical technique in organic chemistry since 1940s. IR spectroscopy is widely applied in analysis of chemical composition, because it can accomplish rapid analysis with simple operation and simultaneously determine multi-nutrient composition in a non-destructive and non-pollutive manner (Barth 2007). This technique only requires a small amount of sample. Additionally, with flexible accessories, IR spectroscopy is capable of analyzing a sample in different status (gases, liquids and solids) or with different composition (organics/inorganics, macro/micro molecules) (Wetzel et al. 1998b; Stuart 2004). Samples are mounted on the path of IR radiation in an IR spectroscope. When a sample is exposed to IR radiation, if the molecules of the sample are "active" to the corresponding IR frequencies, the certain electric dipole moment in the molecule can be altered and characteristic absorption occurs. By measuring the absorption, spectroscopic information can be obtained (Stuart 2004).

2.2. Spectral Band Assignments and Characteristics

Infrared spectroscopy is designed to characterize chemical functional groups by measuring the IR absorption in the sample. The IR spectrum is displayed as a function of frequency. All types of chemical functional groups have their own unique absorption frequency associated with energy. When the energy or frequency of IR meets any vibrational frequency of molecules in the sample, absorption occurs. A detector can record the absorption to determine the chemical function groups and examine the chemical composition of the complex matrix (Budevska 2002). Characteristic absorption peaks can be used to analyze a large variety of compound classes. For example, in the ca. 4000-2500 cm^{-1} region, there are bands caused by O–H, C–H and N–H stretching. Triple-bond stretching usually exhibits absorption in the ca. 2500-2000 cm^{-1} region. In contrast C=C and C=O stretching absorptions are located at the ca. 2000–1500 cm^{-1} region (Jackson and Mantsch 2000; Stuart 2004). The wavenumber range of IR from 1800 to 800 cm^{-1} is the so-called “fingerprint region”. The fingerprint region usually accounts for almost all the characteristics of biological molecules. Bands in the fingerprint region are particularly sensitive to molecular structure. However, it should be pointed out that not every band can guarantee the representation of a certain corresponding chemical structure because the vibration of chemical bonds vary in sensitively depending on the sample status and experiment circumstance (Griffiths and De Haseth 1986; Messerschmidt and Harthcock 1988; Yu 2006b).

III. MOLECULAR SPECTRAL ANALYSES

3.1. Spectral Analysis

Infrared spectroscopy provides comprehensive information on composition and characteristics of samples. Following exposure to IR radiation, chemical functional groups exhibit characteristic absorption at certain frequencies, which enable the detection of chemical and structural differences. The typical IR absorption peaks of the relevant biopolymers have been well documented (Jackson and Mantsch 2000; Miller 2002). The typical IR spectral bands of biological compounds have been summarized in Table 1.

Protein IR absorption bands are related to the corresponding amide group. The corresponding absorbance of amide groups occurs at around 1700-1500 cm^{-1}. Two are commonly used in biological applications. One is amide I (centered at ca. 1650 cm^{-1}), resulting from 80% C=O stretching, 10% C–N stretching and 10% N–H bending (Jackson and Mantsch 1991; Stuart 2004). The amide II absorbance appears at ca. 1550 cm^{-1}, which is from 40% C-N stretching associated with 60% N-H deformation (Jackson and Mantsch 1991; Wetzel 1993; Stuart 2004). However, the amide II band is usually overlapped with other bands. Hence, it is used less in protein analysis than amide I. Generally, in complex biological specimens, protein conformation is composed of various biopolymer structures. The amide I band can also be used to analyze the secondary structure of the protein because it usually contains a variety of subcomponents referring to secondary protein structures with 2nd derivative or Fourier self deconvolution (FSD) analysis. For example, this has been used in membrane protein analysis (Stuart 2004) and food and feed evaluation (Yu 2005c, 2008).

Carbohydrates have absorption at ca. 3000-2800 cm^{-1} due to C-H stretching. Characteristic IR bands of carbohydrates also appear at ca. 1200-800 cm^{-1}, which are caused by C-O and C-C stretching vibrations and C-O-H deformation (Stuart 2004). However, these bands may be assigned to either structural or non-structural carbohydrates (Yu 2005b). Starch exhibits absorption bands at ca. 1025 cm^{-1} (Wetzel et al. 1998a; Wetzel and LeVine 2001). For structural carbohydrates, cellulose is characterized at 1170–1150, 1050, 1030 cm^{-1} and hemicellulose is centered at 1732 and 1240 cm^{-1} (Wetzel et al. 1998a; Wetzel and LeVine 2001; Stuart 2004; Yu 2004, 2005b).

The characteristic lignin absorbance is represented at ca. 1590 and 1510 cm^{-1}, in which the aromatic character can be detected (Himmelsbach et al. 1998; Yu 2005b). The amide I band and lignin band were found overlapped in the pericarp of corn (Yu 2005b). The IR spectra of lipid gives absorption bands at ca. 3000-2800 cm^{-1} due to the C-H stretching. The band at ca. 1745-1725 cm^{-1} is due to the C=O stretching. The C=C-H bending of lipid also results in a peak centered at ca. 967 cm^{-1} (Dumas et al. 2007). Carbon dioxide and H_2O also absorbs IR strongly, thus dry nitrogen is usually used to fill the sample cell (Miller and Dumas 2006).

More information referring to band assignments are detailed and summarized (Wetzel et al. 1998a; Jackson and Mantsch 2000; Wetzel and LeVine 2001; Marinkovic et al. 2002; Yu 2004; Yu et al. 2004d, 2004b; Marinkovic and Chance 2005; Miller and Dumas 2006; Dumas et al. 2007). As previously mentioned, IR spectroscopy can display the characteristic peak patterns of chemical compounds in terms of frequency, lineshape, and intensity. Commonly, data treatments including uni- and multi-variate analyses, are conducted to characterize the sample information after the spectra collection.

3.2. Univariate Spectral Analysis

For the interpretation of spectra, converting the spectra to absorbance display mode provides us the opportunity to read the absorbance value and relate it to the relative content of the biopolymers of interest. Peak intensity ratio calculation and mapping analysis of certain chemical functional groups or ratios are also common methods to deal with IR spectroscopic data (Miller 2002; Yu 2005a, 2006a). An earlier study applied this analysis method and found that yellow- and brown-seed canola showed different characteristic peak intensities and chemical functional groups ratios, which indicated microstructure differences between canola varieties (Yu et al. 2005).

3.3. Multivariate Spectral Analysis

How to deal with the large and complicated data sets obtained from IR spectra is a primary question that researchers face. In spectral analyzing applications, multivariate methods are more appropriate for complex situations, including agglomerative hierarchical cluster analysis (AHCA), principal component analysis (PCA), artificial neural networks (ANN), linear discriminant analysis (LDA), and fuzzy C-means clustering (FCM) (Dumas et al. 2007).

Hierarchical cluster analysis (AHCA) and principal component analysis (PCA) are commonly applied in spectral data analysis (Jain et al. 1999; Yu 2005b). Hierarchical cluster analysis is used to sort objectives according to a defined similarity (Kaufman and Rousseeuw 1990). AHCA

builds the cluster by combining the objectives according to the distance measurement. The objectives are treated and linked into larger groups by calculating criterion distance step by step. The previously established clusters are progressively merged into larger groups. Eventually, these algorithms form a hierarchical dendrogram to express the possible structure of the data (Romesburg 1984; Kaufman and Rousseeuw 1990; Gan et al. 2007). The dendrogram, appearing as a tree structure, can be generated to visibly present the results.

The PCA is also used to deal with spectral data. This method can transform the original data set into a new data set with smaller dimensions. The new data set is composed of uncorrelated variables, which are principle components (PCs). The first several PCs usually can explain greater than 95% of the total variance. Researchers can use the new data set, which is composed of fewer variables than the original data, to describe as much information as the original observation (Dunteman 1989; Jolliffe 2002; Davies and Fearn 2004; Miller et al. 2007).

These two statistical tools are used to reduce the number of variables and extract maximal information content from the informative spectra data. For application in food and feed science, these two analysis methods have been used to make comparisons between raw and heated flaxseed with amide I FSD spectral information (Yu 2006a), investigate differences in the endosperm molecular structures from wheat, corn and barley (Yu 2005b), and reveal the similarities of normal and transgenic alfalfa (Yu et al. 2009a). Cluster analysis for imaging chemical functional groups has proved powerful in corn structure examination (Yu 2005a).

IV. SYNCHROTRON-BASED SR-IMS AND DRIFT SPECTROSCOPY

4.1. Fourier Transform Infrared Spectroscopy

Fourier Transform Infrared Spectroscopy (FTIR) detects the signal as a function of the difference of path lengths between the two beams generated by the Michelson interferometer. The mathematical method of Fourier Transform is to convert the symmetric interferogram into functions with frequency components to form the continuous transmittance or absorbance spectra (Stuart 2004). A typical FTIR spectrometer device usually consists of thermal (globar) light source, Michelson interferometer, detector, and computer. The MCT detector is commonly used in FTIR spectroscopy (Stuart 2004). In comparison with conventional dispersive spectroscopy, FTIR spectroscopy exhibits more effective and powerful properties as it has excellent sensitivity, larger optical throughput, and good signal to noise (S/N) ratio. The entire IR region can be detected simultaneously with the FTIR technique. With the faster scan speed, FTIR spectroscopy can produce spectra in less than 2 minutes. It is thereby possible to spend a short time scanning the entire IR region (Stuart 2004). In summary, FTIR methods enable us to apply broadband IR radiation on the sample simultaneously and with multi-scans, high quality spectra can be produced in a short time.

4.2. Diffuse Reflectance Fourier Transform Infrared Spectroscopy

If IR radiation strikes the uneven surface of a solid sample, two kinds of light reflection are generated: specular reflection with the same incidence and reflectance angles, and diffuse reflection, which results in scattered reflectance. When coarse sample is exposed to IR radiation, the light may be reflected in all directions. Using Diffuse Reflectance Fourier Transform Infrared

Spectroscopy (DRIFT), the diffusely reflected energy can be collected and a spectrum produced that is related to the sample chemical composition (Griffiths and De Haseth 1986; Stuart 2004). Diffuse reflectance technique is usually utilized on samples that are powdered, or with irregular surfaces, or on non-transparent samples (Stuart 2004). Therefore, DRIFT spectroscopy is particularly suitable for the examination of food and feed samples. DRIFT spectroscopy is capable of investigating ground food and feedstuffs for spectral analysis to reveal structural and chemical differences.

4.3. Synchrotron Radiation Based FTIR Microspectroscopy (SR-IMS)

4.3.1. Synchrotron Radiation

A synchrotron is a particle accelerator using electric fields to speed up electrons and magnetic fields to adjust the direction of the travelling particles in a circular track (Dumas et al. 2007). A synchrotron is composed of six main components, including electron gun, linear accelerator, booster ring, storage ring, beamline and experimental station. Electrons, generated from the electron gun, are accelerated in the linear accelerator and booster ring, a circular accelerator. The high-energy electrons are then transmitted into the storage ring (circular vacuum environment), in which they travel at a speed up to 99% of light. A series of magnetic fields transfer the electrons into circular paths. When the track is bent, the high speed electrons emit radiation beam tangentially from the orbit. Synchrotron radiation covers the full region of the spectrum from X-ray, ultraviolet, visible light, to IR. The radiation is transported through the beamline to the experimental stations where the light is available for study (Dumas and Miller 2003; Dumas et al. 2007).

In 1947, synchrotron radiation was accidentally discovered in the USA from high-energy physics application. Today, FTIR microspectroscopy coupled with synchrotron radiation (SR-IMS) is widely used in biological applications, making it possible to get the visible image and spectral information together, to characterize the microscopic sample area at the cellular or subcellular levels (Marinkovic and Chance 2005; Messerschmidt and Harthcock 1988; Yu 2012).

4.3.2. Strong Point of Synchrotron Light

Conventional thermal (globar) light source has limitations when examination is performed within the cellular dimension (5-30 μm) due to the limitation from the wavelength of IR light and the aperture size of the instrument (Raab and Martin 2001; Marinkovic et al. 2002; Yu 2004; Miller and Dumas 2006; Miller et al. 2007). This drawback in terms of diffraction and poor S/N ratio can be overcome by the use of an advanced light source.

Synchrotron light has led to a further development of IR techniques. The synchrotron beam is preferred as the light source in terms of high brightness, continuous bands of spectrum, less signal loss and ultra-spatial resolution, which result from its highly illuminated, well collimated and non-divergent characteristics (Wetzel et al. 1998b; Dumas and Miller 2003).

The excellent brightness and light source size of synchrotron radiation makes it possible to investigate the molecular chemistry information within cellular and subcellular dimension of biological tissues. Synchrotron light source reduces the influence of diffraction and assists researchers to gain high quality spectra with ultra-spatial resolution (3 to 10 μm) on the microscopic area.

V. BIOLOGICAL APPLICATION OF SYNCHROTRON SR-IMS AND DRIFT MID-IR SPECTROSCOPY AND RECENT PROGRESS

Specimens from plant tissue are mixtures of lignin, various CHO, proteins and other biological components. To overcome the difficulties that traditional methods cannot provide with regard to spatial information of the sample, new approaches to detect structural make-up are required in complex plant based food and feed. Biological application of Mid-IR spectroscopic techniques can be traced back to the middle period of last century (Dumas et al. 2007). A number of biological investigations were conducted using IR spectroscopy. In the past decade, the use of IR spectroscopy in biology has brought applications to food and feed science as well.

5.1. Application of FTIR Techniques

There is a broad range of scientific fields that apply FTIR techniques in research for qualitative and quantitative analyses. FTIR spectroscopy is a powerful analytical tool to estimate the chemical composition in conjunction with additional structural information. From paints to pharmaceutical materials and food/feed samples, numerous organic and inorganic materials can be identified and characterized using FTIR spectroscopy (Stuart 2004; Jonker et al., 2012).

It has been recognized that compositional and structural differences are responsible for the spectroscopic differences. The peak intensity, shape and pattern differences can be detected to reveal structural and chemical characteristics of tissue histopathology. For example, spectroscopic *in situ* analysis has been documented to compare white and gray matter in the rat

brain. Peak position and intensity changes were found in diseased brain tissue of mice (Levine and Wetzel 1993; LeVine and Wetzel 1994). Spectroscopic information also revealed the existence of oxidative products in brain tissue of humans with multiple sclerosis (LeVine and Wetzel 1998). Additionally, there have been reports on disease diagnosis and microorganism characterization using FTIR spectroscopy (LeVine et al. 1999; Stuart 2004). This technique was also applied to secondary structure of protein in amyloid plaques and D_2O effects on chemical functional groups in the brain (Wetzel 1998; Wetzel et al. 1998c). Another application was the examination of pathological and normal retina (Homan et al. 2000). The nucleic acids, DNA and RNA, have also been studied using FTIR spectroscopy (Stuart 2004).

5.2. Application of FTIR Microspectroscopy

Integration of the IR microscope and spectrometer as an associated instrument allows us to focus on a particular micro area of biological tissue. FTIR microspectroscopy provides us opportunities for the investigation of localized chemical compounds of plant cells which require no or minimal modification. This technique offers a solution to the problem that chemical agents may destroy the intact structure of sample. Wetzel described that his group first applied FTIR microspectroscopy to investigate wheat kernels (Wetzel and LeVine 2001). This was an early attempt accomplished with conventional (thermal) FTIR spectrometer. FTIR Microspectroscopy has also been used to investigate other plant tissues, including wheat aleurone cells, primary root cells, rye, corn, oats, and soybean, which have been reviewed previously (Wetzel et al. 1998a). However, conventional FTIR spectroscopy is restricted to exploring microstructure, owing to the properties of conventional light source.

5.3. Application of Synchrotron-Radiation FTIR Microspectroscopy (SR-IMS)

In order to expand the ability of FTIR microspectroscopy techniques, synchrotron radiation was developed to overcome the disadvantage of conventional (thermal) light source. Synchrotron radiation has made it possible to reveal the features and distribution of localized chemical compounds. Ideal brightness and the excellent S/N ratio are of particular importance to obtain the spectroscopic information on chemical constituents without the destruction of sample.

Presently, the interdisciplinary application of synchrotron radiation has integrated various sciences. In 1993, a FTIR microspectrometer instrument was equipped with the synchrotron radiation at the National Synchrotron Light Source (NSLS) (Wetzel et al. 1998a). Thereafter many experiments have been conducted to characterize and localize the distribution of chemical compounds within cellular and subcellular dimensions. For example, SR-IMS has served as a powerful tool to investigate single mouse hybridoma B cell, human hair and skin at ultraspatial resolution (Dumas and Miller 2003; Miller and Dumas 2006; Dumas et al. 2007) and provide data in cancer diagnosis (Dumas et al. 2007) and protein folding research (Marinkovic et al. 2002). The SR-IMS has also been used to probe the biological specimen of plant tissues (Dokken et al., 2005). A diversity of plants (wheat, barley, soybeans, rye, oats and corn) has been sectioned for FTIR microspectroscopic examination and characterization. Differences in chemical and structural composition from various tissues, including kernel, root, and vascular bundle sheath, have been identified (Wetzel et al. 1998a; Pietrzak and Miller 2005; Yu et al. 2008; Liu and Yu 2010; Liu et al. 2012).

The SR-IMS technique is applied in feed science to gradually expand knowledge of crop quality and value of food. In practice, chemical functional group imaging (or mapping) can graphically reveal the chemical and structural information of specimens by examining the sample in a defined rectangle using SR-IMS techniques (Yu 2011; Yu 2012). The AHCA and PCA have also been used on SR-IMS spectral data analysis to examine and distinguish different structures. For instance, chemical functional groups, which are associated with nutrients of plant tissue from wheat, barley, corn, canola, and flaxseed have been detected (Yu 2006a; Yu et al. 2004b; 2005; 2007). Ultra-structural studies suggested that SR-IMS can successfully image the structural and chemical features and distribution of barley grain (Yu 2007c; Yu et al. 2004d). The yellow- and brown-seeded *Brassica* rape were also examined using SR-IMS technique to reveal the inherent chemical composition (Yu 2004). Barley variety for food/feed purpose (Valier) and malting purpose (Harrington) were differentiated in the make-up of endosperm tissue (Yu et al. 2004a).

Protein secondary structure is difficult to detect due to the complexity and variation of conformation involving α-helices, β-sheets and turns (Nelson and Cox 2005). In previous work, barley varieties have been characterized using SR-IMS to reveal the molecular structure of barley protein (Yu 2007a). The results indicated that large differences in protein structure among barley varieties were detected and with the application of PCA analysis, some varieties could be distinguished with the SR-IMS spectral data. The seed protein structure of wheat, feather, winterfat, oats and barley were also examined (Yu 2005c, 2006b, 2007a, 2007b; Yu et al. 2009a). High β-sheets content were detected in feather meal partially accounting for its lower

digestibility using multi-component modelling (Yu 2004; Yu et al. 2004c; Yu 2006a). Golden and brown flaxseeds were distinguished by analyzing the amide I FSD spectra and heat treatment was found to have impact on the protein secondary structure of flaxseed tissue (Yu 2006a, 2007b, 2008). A recent study to estimate the quality of alfalfa in terms of protein molecular structure using SR-IMS microspectroscopy in conjunction with Gaussian and Lorentzian methods of multi-component peak modeling was conducted (Yu et al. 2009b). An article summarized plant-based food and feed protein structure changes induced by gene-transformation, heating and bio-ethanol processing in the novel synchrotron-based molecular structure and molecular nutrition research program (Yu 2010). Very recently, Withana-Gamage et al. (2012, unpublished) applied the synchrotron SR-IMS technique in food and nutrition research in transgenic Arabidopsis seeds by chemical characterization of microstructure and assessment of protein degradability of Arabidopsis seeds. This group also use the SR-IMS to study cruciferin mutant Arabidopsis lines and genetic changes in seed storage protein expression that affects protein intrinsic structure and their bioaccessibility.

Synchrotron-based FTIR microspectroscopy is capable of exploring the chemical make-up and structural features simultaneously within intact biological tissues at ultra-spatial resolution (3 to 10 μm), therefore, this technique is extremely powerful when dealing with heterogeneous biological specimens. This technique allows us to extend information at a desired cellular or subcellular level and localize the chemical information to the structure of heterogeneous samples.

The above biological applications of FTIR spectroscopy provide evidence as to the potential and power of this technique. Moreover, fluorescence-assisted IR microspectroscopy, near-IR (NIR) spectroscopy, synchrotron X-ray, Raman and other spectroscopic techniques are also used to examine complex plant tissues (Wetzel and LeVine 2001; Dumas and Miller 2003; Stuart 2004; Miller and Dumas 2006).

VI. CONCLUSIONS

The FTIR spectroscopy is sensitive to structural and chemical differences and can therefore be used to identify and discriminate biological materials. Spectroscopic parameters such as wavenumber, intensity and band shape can be used to reflect the chemical constituents and structural information of the sample. Infrared region 1800 to 800 cm^{-1} can be regarded as the fingerprint region for identification, since molecules have their unique absorption patterns in this region. FTIR techniques have a high scan speed, shorter sampling time and good S/N ratio. Associated with PCA and AHCA analysis, it is possible to effectively deal with a large body of data and interpret maximum information from IR spectra.

Furthermore, with the use of superior synchrotron light source, it is capable to extend our knowledge at cellular or molecular scale of biological materials. Compared to the conventional thermal (globar) source, synchrotron light source is particularly advantageous because of the excellent spatial resolution, brightness, and S/N ratio (without the thermal noise). Therefore,

ultra-spatially resolved synchrotron light sourced FTIR microspectroscopy can provide more complementary information referring to the structural and chemical conformation of the barley.

In conclusion, the adoption of IR spectroscopy techniques such as DRIFT and SR-IMS techniques for investigating the structural and chemical information of food and feeds with complex biological tissues provide advantages such as rapidity, efficiency and specificity over traditional chemical analysis.

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Table 1. Brief summary of typical infrared spectral bands of biological compounds.

Item	Wavenumber (cm^{-1})	Peak assignment	Reference (s)
Amide I	1650	80% C=O stretching, 10% C–N stretching and 10% N–H bending	Jackson and Mantsch 1991; Stuart 2004
Amide II	1565	40% C-N stretching associated with 60% N-H deformation	Wetzel 1993; Stuart 2004
Lignin	1510	aromatic ring stretching;	Himmelsbach et al. 1998; Wetzel et al. 1998a; Yu 2005b
Cellulosic compound	1246	C-O stretching	Wetzel et al. 1998a; Wetzel and LeVine 2001; Stuart 2004; Yu 2004, 2005b
Carbohydrates	1200-800	C-O and C-C stretching vibrations and C-O-H deformation	Wetzel et al. 1998a; Wetzel and LeVine 2001; Stuart 2004