MONITORING CHANGES IN MOLECULAR STRUCTURES OF PROTEINS AND CARBOHYDRATES DURING FEED PROCESSING USING DSC AND DRIFT: AN OVERVIEW

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In the feed industry, many forms of technological processing are applied to manipulate the site and extent of digestion in ruminants. Technological processing applied in feed industries are usually involve heat, pressure and shear forces. Such processing manipulates the site of digestion in ruminants by reducing the size of feed particles,(Goelema et al., 1996) protecting the proteins from being degraded in the rumen,(Prestløkken, 1999; Ljøkjel et al., 2003c) and making the starch granules more accessible for microbial digestion (Arieli et al., 1995; Ljøkjel et al., 2003c). Different methods have been developed to determine the extent of protein denaturation and starch gelatinization occurs due to application of feed processing. Thermal analysis such as differential scanning calorimetry (DSC) has proved to be a suitable tool to study phase transitions, such as the denaturation of proteins (Wright and Boulter, 1980) and gelatinization of starch. The technique provides the temperature of denaturation and gelatiniztion (Tp and Tg) and the enthalpy change associated with the transition (δH). The δH value represents a combination of exothermic reactions such as those associated with disruption of hydrophobic interactions and aggregation of the molecule, and of the endothermic contributions of disruption of nitrogen bonds and unfolding of the polypeptide chains (Wright and Boulter, 1980). The DSC has been successfully applied to determine to what extent proteins have been denatuarted and starch has been gelatinized when a hydro-thermal processing applied to feedstuffs. Goelema (1999) found that enthalpy change associated with the protein denaturation was highly and negatively correlated to digested undegraded protein in dairy cows in several legume seeds including faba beans, peas, lupins and soybeans. Azarfar et al. (2012; unpublished data) found that enthalpy change associated with starch gelatinization was negatively correlated to maximum fractional rate of gas production *in vitro* in barley, maize, peas and lupins.

Beside thermal analysis, vibrational spectroscopic methods such as diffused reflectance infrared Fourier transformed spectroscopy were successfully applied to reveal molecular structural features related to proteins, carbohydrates and lipids (Yu, 2005c; Yu et al., 2011; Azarfar et al., 2013) and that the changes occur to these macromolecules when technological processes applied to feedstuffs. The technique is based on the fact that exposure to IR radiation cause vibration and rotation of molecules between different quantized discrete energy levels E0, E1 and 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, 2000b; 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). 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 nondestructive, 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). IR 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, 2000b; 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).

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, 2000a; Miller, 2002). 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, 1996; 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 (Wetzel and LeVine, 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 feed evaluation (Yu, 2005b, 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 nonstructural carbohydrates (Yu, 2005a). Starch exhibits absorption

bands at ca. 1025 cm-1(Wetzel et al., 1998a; Wetzel and LeVine, 2001). For structural CHO, cellulose is characterized at 1170–1150, 1050, 1030 cm-1 and hemicellulose is centered at 1732 and 1240 (Wetzel and LeVine, 2001; Stuart, 2004; Yu, 2004, 2005a).

The characteristic lignin absorbance is represented at ca. 1590 and 1510 cm-1, in which the aromatic character can be detected (Yu, 2005a). The amide I band and lignin band were found overlapped in the pericarp of corn (Yu, 2005a). 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). 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. 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 (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, 2005b).

In conclusion, thermal analyses as well as IR spectroscopic method can be successfully used to reveal molecular structures of feed's macromolecules, and changes occur in these macromolecules when feeds subjected to technological processes.

References

Arieli, A., I. Bruckental, O. Kedar, and D. Sklan. 1995. Insacco disappearance of starch nitrogen and fat in processed grains. Anim. Feed Sci. Technol. 51: 287-295.

Azarfar, A., A. Jonker, and P. Yu. 2013. Protein structures among bio-ethanol co-products and its relationships with ruminal and intestinal availability of protein in dairy cattle. Int J Mol Sci 14: 16802-16816.

Barth, A. 2007. Infrared spectroscopy of proteins. Biochim. Biophys. Acta. 1767: 1073-1101.

Budevska, B. O. 2002. Vibrational spectroscopy imaging of agricultural products. In: J. M. Chalmers and P. R. Griffiths (eds.) Handbook of Vibrational Spectroscopy. p 3720-3732. J. Wiley, Hoboken, NJ.

Dumas, P., G. D. Sockalingum, and J. Sule-Suso. 2007. Adding synchrotron radiation to infrared microspectroscopy: what's new in biomedical applications? Trends Biotechnol. 25: 40-44.

Goelema, J. O. 1999. Processing of legume seeds: effects on digestive behaviour in dairy cows. PhD Thesis, Wagningen University, Wageningen.

Goelema, J. O., G. Hof, A. F. B. van der Poel, and S. Tamminga. 1996. Effect of particle size, cold pelleting, steam pelleting and expander treatment on the rumen degradability of a compound feed for ruminants. J. Dairy Sci. 79 (Suppl. 1): 142.

Griffiths, P. R., and J. A. De Haseth. 1986. Fourier transform infrared spectrometry. Wiley, New York.

Jackson, M., and H. H. Mantsch. 1996. Biomedical Infrared Spectroscopy. In: H. H. Mantsch and D. Chapman (eds.) Infrared Spectroscopy of Biomolecules. p 311-340. Wiley-Liss, New York.

Jackson, M., and H. H. Mantsch. 2000a. Ex Vivo Tissue Analysis by Infrared Spectroscopy. In: R. A. Meyers (ed.) Encyclopedia of Analytical Chemistry No. 1. p 131-156. Jhon widely & Sons, New York.

Jackson, M., and H. H. Mantsch. 2000b. Infrared spectroscopy ex vivo tissue analysis. In: R. A. Myers (ed.) Encyclopedia of Analytical Chemis. Wiley & Sons Ltd, Chichester, England.

Ljøkjel, K., A. Skrede, and O. M. Harstad. 2003c. Effects of pelleting and expanding on vegetable feeds on in situ protein and starch digestion in dairy cows. J. Anim. Feed Sci. 12: 435-449.

Messerschmidt, R. G., and M. A. Harthcock. 1988. Infrared microspectroscopy: theory and applications. H. Dekker, New York, NY.

Miller, L. M. 2002. Infrared microspectroscopy and imaging. National Synchrotron Light Source, Brookhaven National Laboratory, Internal publication.

Prestløkken, E. 1999. *In situ* ruminal degradation and intestinal digestibility of dry matter an protein in expanded feedstuffs. Anim. Feed Sci. Technol. 77: 1-23.

Stuart, B. 2004. Infrared spectroscopy: fundamentals and applications. J. Wiley, Chichester, West Sussex, England.

Wetzel, D. L., A. J. Eilert, L. N. Pietrzak, S. S. Miller, and J. A. Sweat. 1998a. Ultraspatially-resolved synchrotron infrared microspectroscopy of plant tissue in situ. Cell. Mol. Biol. (Noisy-Le-Grand). 44: 145-168.

Wetzel, D. L., and S. M. LeVine. 1993. In situ FT-IR microspectroscopy and mapping of normal brain tissue. Spectroscopy 8: 40-45.

Wetzel, D. L., and S. M. LeVine. 2001. Biological applications of infrared microspectroscopy. In: H. U. Gremlich and B. Yan (eds.) Infrared and Raman Spectroscopy of Biological Materials. p 101-142. Marcel Dekker Inc., NY.

Wetzel, D. L., J. A. Reffner, G. L. Carr, and L. Cho. 1998b. Synchrotron powered FT-IR microspectroscopy permits small spot ATR sampling of fiber finish and other materials. In: J. A. d. Haseth (ed.) Fourier Transform Spectroscopy. p 657-660. American Institute of Physics, Woodbury, NY.

Wright, D. J., and D. Boulter. 1980. Differential scanning calorimetry study of meals and constituents of some food grain legumes. J. Sci. Food Agric. 31 1231 - 1241.

Yu, P. 2004. Application of advanced synchrotron-based Fourier transform infrared microspectroscopy (SR-FTIR) to animal nutrition and feed science: a novel approach. Br. J. Nutr. 92: 869-885.

Yu, P. 2005a. Applications of hierarchical cluster analysis (CLA) and principal component analysis (PCA) in feed structure and feed molecular chemistry reserach, using synchrotron-based feed Fourier transform infrared (FTIR) microspectroscopy. J. Agric. Food Chem. 53: 7115-7127.

Yu, P. 2005b. Multicomponent peak modeling of protein secondary structures: comparison of gaussian with lorentzian analytical methods for plant feed and seed molecular biology and chemistry research. Appl. Spectrosc. 59: 1372-1380.

Yu, P. 2005c. Protein secondary structures (α-Heilx and β-Sheet) at a cellular level and protein fractions in relation to rumen degradation behaviours of protein: a new approach Br. J. Nutr. 94: 655-665.

Yu, P. 2006a. An emerging method for rapid characterization of feed structures and feed component matrix at a cellular level and relation to feed quality and nutritive value. Arch. Anim. Nutr. 60 229-244.

Yu, P. 2006b. Synchrotron IR microspectroscopy for protein structure analysis: Potential and questions. Spectroscopy 20: 229-251.

Yu, P. 2008. Modeling protein structures in feed and seed tissues using novel synchrotron-based analytical technique. Anim. Feed Sci. Technol 140: 199-206.

Yu, P., D. Damiran, A. Azarfar, and Z. Niu. 2011. Detecting Molecular Features of Spectra Mainly Associated with Structural and Non-Structural Carbohydrates in Co-Products from BioEthanol Production Using DRIFT with Uni- and Multivariate Molecular Spectral Analyses. International Journal of Molecular Sciences 12: 1921-1935.