Study of Barley Grain Molecular Structure for Ruminants Using DRIFT, FTIR-ATR and Synchrotron Radiation Infrared Microspectroscopy (SR-IMS): A Review

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Abstract. Barley inherent structures are highly associated with nutrient utilization and availability in both humans and animals. Barley has different degradation kinetics compared with other cereal grains. It has a relatively higher degradation rate and extent, which often cause digestive disorder in the rumen. Therefore understanding barley inherent structure at cellular and molecular levels and processing-induced structure changes is important, because we can manipulate barley inherent structures and digestive behaviors. Several molecular spectroscopy techniques can be used to detect barley inherent structures at cellular and molecular levels. This article reviews several applications of the IR molecular spectral bioanalytical techniques - DRIFT, FT/IR-ATR and SR-IMS for barley chemistry, molecular structure and molecular nutrition research.

1. Introduction
Internal molecular structures of barley affect nutrient availability and fermentation characteristics of barley. For example, Valier and Harrington barley proteins have similar chemical composition but different degradation or fermentation behaviors [1-3]. Therefore, understanding the barley inherent structure at cellular and molecular levels and the processing-induced structure changes is important, because we can manipulate barley inherent structures and digestive behaviors. However, traditional “wet” chemical analysis fails to reveal barley internal structure and to link its inherent structural information to chemical one [1,4], due to the destruction of the structure during the processing for chemical analysis. IR molecular spectroscopy techniques can be used to detect barley inherent structures at cellular and molecular levels. The objective of this article is to review the applications of several IR molecular spectral bioanalytical techniques - DRIFT, FT/IR-ATR and SR-IMS for barley chemistry, molecular structure and molecular nutrition research in my team.

2. Application A: Microprobing Molecular Spatial Distribution and Structural Architecture of Barley with SR-IMS Technique
Yu [5] reported that the advanced Synchrotron-Radiation Infrared MicroSpectroscopy (SR-IMS) is able to study cell or living cell biochemistry within cellular dimension [4]. The author used the SR-
IMS imaging to microprobe molecular spatial distribution and cell architecture of cereal grain tissue in a comprehensive way. Chemical images were collected by SR-IMS at the beamline U2B at the National Synchrotron Light Source, Brookhaven National Laboratory (NSLS-BNL, U.S. Department of Energy, New York). The molecular images were systematically carried out from the outside to the inside of the seed tissue under various chemical functional groups and their ratios. The results show that unique cell architecture and the molecular spatial distribution, intensity in the seed tissue could be generated using the SR-IMS. Figure 1 is one example. This imaging technique and methodology provide a high potential that could be used by scientists to develop a specific cereal grain variety with targeted food and feed quality; moreover, it can also be used to monitor the degree of grain maturity and grain damage, the fate of organic contaminants and the effects of chemical treatment on plant and grain seeds.
SR-IMS molecular functional group image from the pericarp (outside), to seed coat, aleurone layer and endosperm - a). Visible image; (b) 3D chemical image; (c) function group intensity ruler; and (d) Spectra corresponding to the pixel at the cross-hair in the visible image. Images were acquired with a pixel resolution of 10 × 10 μm at National Synchrotron Light Sources, Brookhaven National Laboratory (NSLS-BNL, U.S. Dept of Energy, New York) (Source: [5])

3. Application B: Molecular clustering, interrelationships and carbohydrate conformation in hull and seeds among barley cultivars

In this study [6], the authors applied molecular spectral analyses (univariate and multivariate) to Diffuse Reflectance Infrared Fourier Transform (DRIFT) spectra to study carbohydrate conformation features, molecular clustering and interrelationships in hull (Figure 2) and seed (Figure 3) among six barley cultivars, which had different degradation kinetics in rumen. The authors analyzed the spectral fingerprint regions in both hull and seeds together with Agglomerative Hierarchical Cluster (AHCA) and Principal Component (PCA) analyses. It was found that the carbohydrate molecular spectral analysis in the ca. 1185-800 cm⁻¹ region together with the AHCA and PCA were able to show that the barley seed inherent structures exhibited distinctive differences among the barley varieties. CDC Helgason had discernible differences from AC Metcalf, McLeod, CDC Cowboy and CDC Dolly in carbohydrate conformation in the seed (Figure 4). Clear molecular clusters could be distinguished and identified by using AHCA analysis and the separate ellipses could be grouped in PCA analysis. But CDC Helgason had no detectable differences from CDC Trey in carbohydrate conformation. These carbohydrate conformation/structure differences could be partially explained since the varieties were different in digestive behaviors in animals. The authors concluded that the DRIFT technique combined with AHCA and PCA molecular analyses was able to reveal carbohydrate conformation features and identify carbohydrate molecular structure differences in both hull and seeds among the barley varieties. The molecular spectroscopy technique used in this study could also be used for other plant-based feed and food structure study [6].
Figure 2. DRIFT Spectrum of barley hull in the region: 1800-800 cm$^{-1}$ (Source: [6])
Figure 3. DRIFT Spectrum of barley seed in the region: 1800-800 cm$^{-1}$ (Source: [6])
4. Application C: Using Molecular Spectroscopy with Uni- and Multi-variate Spectral Analysis Techniques to Detect Protein Molecular Structure Differences among Different Genotypes of Barley

In this study [7], the authors characterized protein molecular structure using DRIFT spectroscopy with univariate and multivariate molecular spectral analyses and identified the structure differences in both hull and seeds among six spring barley varieties. The molecular structure spectral analysis involved protein amide I and II regions ca. 1716-1485 cm⁻¹ (attributed to protein amide I C=O and C-N stretching; amide II N-H bending and C-N stretching) [9,12] together with AHCA and PCA analyses. The authors found that the techniques were able to identify the spectral differences associated with the molecular structural differences among the barley varieties. The molecular spectral analyses in the region of ca. 1715-1485 cm⁻¹ together with AHCA and PCA analyses were able to show that the molecular structures of the seeds exhibited distinctive differences among the barley varieties, while the hull did not. The authors also found that CDC Helgason is differentiated by AC Metcalfe, McLeod, and CDC Cowboy in both protein amide I and II. Figure 5 shows one example of PCA analysis [7]. The molecular spectral technique provides an approach for plant protein molecular structure and biopolymer conformation study [7,10-13].
5. Application D: Characterization of the Microchemical Structure of Seed Endosperm within a Cellular Dimension Using Synchrotron-Based Infrared Microspectroscopy

In this study [8], the authors used SR-IMS to determine the microchemical structural features in seed endosperm tissue of six developed barley varieties and to study the relationship between molecular structural characteristics and degradation kinetics and nutrient availability in six genotypes of barley. The authors found that inherent microchemical structural differences in the endosperm among the six barley varieties were detected by the SR-based analytical technique. The SR-IMS spectral profiles differed (P<0.05) among the barley samples in terms of the peak ratio, peak area and height intensities of the amide I (ca. 1650 cm\(^{-1}\)) and amide II (ca. 1550 cm\(^{-1}\)) bands, cellulosic compounds (ca. 1240 cm\(^{-1}\)) and CHO component peaks (the 1\(^{st}\) peak at the region ca. 1184-1132 cm\(^{-1}\), the 2\(^{nd}\) peak at ca. 1132-1066 cm\(^{-1}\), the 3\(^{rd}\) peak at ca. 1066-950 cm\(^{-1}\)) (Figure 6). With the SR-IMS technique, the structural characteristics of the cereal seeds were highlighted among different cultivars. The structural differences of barley seeds may be one reason for the various digestive behaviours and nutritive values for ruminants. The study [8] shows weak correlations between the functional groups’ spectral data (peak area, height intensities and ratios) and rumen biodegradation kinetics (rate and extent of nutrient degradation). Weak correlations may indicate that limited variations of these six barley varieties might be not sufficient to interpret the relationship between spectroscopic information and nutrients value of barley grain, although significant differences in the biodegradation kinetics were observed. Finally the

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Figure 5. Multivariate spectral analyses of barley structures in the whole seed: Comparison of CDC Helgason (D) and McLeod (C): PC1 and PC2 explain 98.33 and 1.33% of the variances, respectively [principal component analysis: Scatter plots of the 1\(^{st}\) principal components (PC1) vs. the 2\(^{nd}\) principal components (PC2)] (Source: [7])
authors concluded that the studies demonstrated the potential of spatially resolved synchrotron based technology (SR-IMS) to reveal the structural and chemical make-up within cellular and subcellular dimensions without destruction of the inherent structure of cereal grain tissue.

(a) Carbohydrate 1\textsuperscript{st} peak height (Region: ca. 1184-1132 cm\textsuperscript{-1})

(b) Carbohydrate 1\textsuperscript{st} peak area (Region: ca. 1184-1132 cm\textsuperscript{-1})
Figure 6. Typical synchrotron-based FTIR spectrum of endosperm tissue within a cellular dimension: Carbohydrate 1st component peak area and height: ca. 1150 cm\(^{-1}\) (source, [8])

6. Conclusion:
In conclusion, the IR molecular spectral bioanalytical techniques- DRIFT, FT/IR-ATR and SR-IMS could be used for barley cell organization architecture, barley feed chemistry, molecular structure and molecular nutrition research. These techniques can be used to detect various processing-induced or related molecular structure changes and can be used to check processing efficiency.

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