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Understanding the differences in molecular conformation of carbohydrate and protein in endosperm tissues of grains with different biodegradation kinetics using advanced synchrotron technology

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Abstract

Conventional “wet” chemical analyses rely heavily on the use of harsh chemicals and derivatization, thereby altering native seed structures leaving them unable to detect any original inherent structures within an intact tissue sample. A synchrotron is a giant particle accelerator that turns electrons into light (million times brighter than sunlight) which can be used to study the structure of materials at the molecular level. Synchrotron radiation-based Fourier transform IR microspectroscopy (SR-FTIRM) has been developed as a rapid, direct, non-destructive and bioanalytical technique. This technique, taking advantage of the brightness of synchrotron light and a small effective source size, is capable of exploring the molecular chemistry within the microstructures of a biological tissue without the destruction of inherent structures at ultraspatial resolutions within cellular dimensions. This is in contrast to traditional ‘wet’ chemical methods, which, during processing for analysis, often result in the destruction of the intrinsic structures of feeds. To date there has been very little application of this technique to the study of plant seed tissue in relation to nutrient utilization.

The objective of this study was to use novel synchrotron radiation-based technology (SR-FTIRM) to identify the differences in the molecular chemistry and conformation of carbohydrate and protein in various plant seed endosperms within intact tissues at cellular and subcellular level from grains with different biodegradation kinetics. Barley grain (cv. Harrington) with a high rate (31.3%/h) and extent (78%), corn grain (cv. Pioneer) with a low rate (9.6%/h) and extent of (57%), and wheat grain (cv. AC Barrie) with an intermediate rate (23%/h) and extent (72%) of ruminal DM degradation were selected for evaluation. SR-FTIRM evaluations were performed at the National Synchrotron Light Source at the Brookhaven National Laboratory (Brookhaven, NY).

The molecular structure spectral analysis involved the fingerprint regions of ca. 1720–1485 cm^{-1} (attributed to protein amide I C=O and C—N stretching; amide II N—H bending and C—N stretching), ca. 1650–950 cm^{-1} (non-structural CHO starch in endosperms), and ca. 1185–800 cm^{-1} (attributed to total CHO C—O stretching vibrations) together with agglomerative hierarchical cluster and principal component analyses. Analyses involving the protein amide I

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features consistently identified differences between all three grains. Other analyses involving carbohydrate features were able to differentiate between wheat and barley but failed however to differentiate between wheat and corn. These results suggest that SR-FTIRM plus the multivariate analyses can be used to identify spectral features associated with the molecular structure of endosperm from grains with different biodegradation kinetics, especially in relation to protein structure. The Novel synchrotron radiation-based bioanalytical technique provides a new approach for plant seed structural molecular studies at ultraspatial resolution and within intact tissue in relation to nutrient availability.

Keywords

Plant; Molecular chemistry; Structural make-up; Protein and carbohydrate conformation; Seed endosperms; Biodegradation kinetics

1. Introduction

A synchrotron is a giant particle accelerator that turns electrons into light [1]. Synchrotron light is extremely bright (millions of times brighter than sunlight). The beam is non-divergent, intense and extremely fine which allows us to explore molecular structures in biomaterials [2–5].

Synchrotron radiation-based Fourier transform IR microspectroscopy (SR-FTIRM) has been developed as a rapid, direct, non-destructive and bioanalytical technique. This technique, taking advantage of synchrotron light brightness and a small effective source size, is capable of exploring the molecular chemistry [2,3,5–7] and conformation of biological components (biopolymer) [8] within the microstructures of a biological tissue. This is achieved without destroying inherent structures and can be done at ultra spatial resolutions that are within subcellular dimensions [5,9–13]. This is in contrast to traditional ‘wet’ chemical methods, which, during processing for analysis, often result in the destruction of the intrinsic structures of plant seeds. To date there has been very little application of this technique to the study of seed inherent structures and biopolymer conformation in relation to nutrient utilization.

Common cereals, corn (cv. Pioneer 39P78), barley (cv. Harrington) and wheat (cv. AC Barrie) differ in rumen degradation kinetics and fermentation characteristics which results in differences in terms of nutrient availability. Selection of barley grain for brewing has resulted in the highest rate (31.3%/h) and extent (78%) of rumen DM degradation [14]. However, in ruminants these fast degradation characteristics can lead to digestive upset including acidosis and bloat. Corn exhibits the lowest rate (9.6%/h) and extent (57%) of rumen DM degradation [15] which reduces the risk of digestive upset. Wheat is intermediate with a rumen DM degradation rate of 23%/h and extent of 72% [16]. These biological differences may be related to differences in molecular structure and differences in biological components (or biopolymers) conformation in the endosperms of the different grains. Conventional “wet” chemical analyses rely heavily on the use of harsh chemicals and derivatization which can destroy the native physiochemical and molecular structures [17].

The Synchrotron-based technique (SR-FTIRM) was applied to detect the difference in starch granules between malting-type and feed-type barleys [18].

The objective of this study was to use the non-destructive and non-invasive synchrotron-based Technology (SR-FTIRM) as a novel approach to identify the differences of molecular structure and biological components conformation (protein and carbohydrate) in the endosperm of grains with differing biodegradation kinetics. It was expected that differences in the structural conformations of the endosperm would provide insight on the differences in grain biodegradation behavior. This study also demonstrated that the novel synchrotron radiation-based analytical technology will provides a new approach and new opportunity for plant seed and food internal structure research in relation to nutrient availability.

2. Materials and methods

2.1. Barley, corn and wheat sample preparation

Barley seed samples (cv. Harrington) were obtained from Brian Rossnagel, Crop Development Center, The University of Saskatchewan (SK, Canada). Corn samples (cv. Pinoneer 39P78) were obtained from Henry Penner (Pox 1028, Morden, MB, R6M 1A9), arranged by Prairie Feed Resource Centre (Canada) (Director, Vern Racz). Wheat samples (cv. AC Barrie) were provided by Pierre Hucl, Crop Development Center, The University of Saskatchewan (SK, Canada). The unstained cross-sections of seed tissues were mounted onto IR microscope slides and BaF₂ windows for SR-FTIR. The detailed procedure was reported in Yu et al. [6,18].

2.2. Synchrotron radiation-based FTIR microspectroscopy

These experiments were performed at the U2B and U10B beamlines of the National Synchrotron Light Source at Brookhaven National Laboratory (NSLS-BNL, US Department of Energy, NY). The beamlines were equipped with FTIR spectrometers with KBr beamsplitters and liquid nitrogen-cooled MCT detectors coupled with Continuum infrared (IR) microscopes with a Schwarzschild 32× objective and a 10× condenser. Synchrotron radiation from the VUV storage ring at the beamlines (800 MeV) entered the interferometer via a port designed for infrared emission. The IR spectra were collected from 15 samples of each grain in the mid-IR range 4000–800 cm⁻¹ at a resolution of 4 cm⁻¹ with 64 co-added scans and an aperture setting of 10 μm × 10 μm. Stage control, spectrum data collection and processing used using OMNIC 6.0 (Spectra Tech, Madison, WI). Scanned visible images were obtained with a charge-coupled device camera linked to the infrared images.

2.3. Synchrotron spectral data collection

The synchrotron IR spectra data of the tissues were collected, corrected for the background spectrum, displayed and analyzed using OMNIC 6.0 (Spectra Tech, Madison, WI). The data were displayed as either a series of spectroscopic images collected at individual wavelengths, or as a collection of IR spectra obtained at each pixel position in the image. Spectral features associated with chemical functional groups were identified according to published reports [9,19,20]. Regions of specific interest in the present study include the amide I (ca. 1650 cm⁻¹) and amide II (ca. 1550 cm⁻¹) bands in the mid-infrared regions of

ca. 1720–1485 cm^{-1} , and multiple peaks associated with the total carbohydrate region of ca. 1185–800 cm^{-1} and the single peak at ca. 1025 cm^{-1} in the non-structural carbohydrate region ca. of 1065–950 cm^{-1} .

2.4. Univariate and multivariate spectral data analyses

Two approaches to analyze spectral data collected under SR-FTIRM usually include univariate and multivariate methods. The univariate methods of analysis consist of various spot sampling and mapping displays of spectral data [3,21]. The multivariate methods of data analysis create spectral corrections by utilizing the entire spectral information. The multivariate analyses included cluster analysis (CLA), using Ward's algorithm method without prior parameterization, and principal component analysis (PCA), which were performed using Statistica software 6.0 (StatSoft Inc., Tulsa, OK, USA).

2.5. Statistical analysis

Statistical analyses were performed using Proc Mixed of SAS (2003) with a CRD model. The Tukey test was used to determine the differences among the treatments. Significance was declared at $P < 0.05$.

3. Results and discussion

3.1. Advanced synchrotron-based FTIR microspectroscopy

The molecular spectroscopic techniques exist including Near IR (NIR), Raman and global sourced FTIR spectroscopy. The NIR technique is a computerized optical technique, in which absorption of near IR radiation is measured on a set of ground samples and a mathematical relationship is established between the optical data and chemical composition obtained by other methods [22,23]. The fatal disadvantages of this NIR technique are dependent on an appropriate calibration to the direct measurement of the variable for which the NIR method predicts a value, the lack of adequate theory to support the measurement [22]; and the requirement to use ground samples, which means that the sample's inherent structures are destroyed and the spatial origin and distribution of the components of interest is lost. The other two IR spectroscopic techniques (Raman and FTIR spectroscopy) are not common in food and feed industry. FTIR has limited flexibility for analysis solids. Raman instruments suitable for routine use are only recently becoming available and their practical utility require study. Again the fatal disadvantage of the two is the need for the sample to be ground. Therefore the NIR, Raman and FTIR spectroscopy could not link the inherent structural information to nutrient utilization and could not reveal the chemical features at cellular and sub-cellular levels. Using a standard global (conventional thermal IR) sourced FTIR microspectroscopy cannot reveal chemical feature of micro-biomaterials, which is $<35\text{--}50\text{ }\mu\text{m}$ (depending on the type of infrared microspectrometer). The normal plant feed cell size is around $5\text{--}30\text{ }\mu\text{m}$. With a global source, a very poor signal to noise ratio is obtained within plant cellular dimensions [2,3,9,24]. The synchrotron-based analytical technique (SR-FTIRM) allows a very small area to be explored, provides higher accuracy and precision, allows faster data collection, reaches diffraction limit as a few μm and provides very good signal to noise ratio at ultra-spatial resolutions [2,3,11,24,25]. It can reveal feed structural–chemical features within cellular dimensions. The experiment also

shows that the synchrotron IR source is unable to damage any biological tissue. The detailed comparisons between the conventional globar and synchrotron sourced FTIR microspectroscopy and applications of synchrotron-based FTIR microspectroscopy have been reported in details by Holman et al. [25], Miller [2,3], Yu [4] and Miller and Dumas [5].

The SR-FTIRM can be used to identify molecular functional groups at ultra-spatial resolution [8,20]. Each biological component in biological samples has unique molecular chemical–structural features. This results in relatively unique infrared absorption and thus unique infrared spectrum [9,26]. The infrared spectrum involving the fundamental vibration from ca. 4000–800 cm^{-1} has been a useful tool for describing the molecular structure of biological compounds. Fig. 1a and b is a FTIR spectrum example from wheat endosperm tissue (pixel size 10 $\mu\text{m} \times 10 \mu\text{m}$) with various spectral features identified. From the spectrum, the presence or absence of various organic functional groups is readily observed [9,20]. SR-FTIRM has been used to increase the fundamental understanding of the inherent structures of plant/feed/food tissues at ultra-spatial resolutions [9,27].

3.2. Endosperm differences in protein molecular structural make-up/conformation among wheat, corn and barley

3.2.1. Identify the differences in the absorbed intensity of protein amides I and II and their ratios in the endosperm tissues among wheat, corn and barley—

The plant protein spectrum has two primary features, the amide I (ca. 1650 cm^{-1}) and amide II (ca. 1550 cm^{-1}) bands in the mid-infrared regions of ca. 1720–1485 cm^{-1} (Fig. 1c and d), which arise from specific stretching and bending vibrations of the protein backbone. The amide I band arises predominantly from the C=O stretching vibration (80%) of the amide C=O group plus C—N stretching vibration [21]. The vibrational frequency of the amide I band is particularly sensitive to protein secondary structure [3,28,25,11,13,27] and can be used to predict protein secondary structures in relation to protein values [8,29,30]. The amide II (predominantly an N—H bending vibration (60%) coupled to C—N stretching (40%) is also used to assess protein conformation [21]. Table 1 shows the structural characteristics of protein amide I and II (mid-infrared absorbed intensities) and their ratios in the plant seed endosperm regions of wheat (cv. AC Barrie), corn (cv. Pioneer) and barley (cv. Harrington). Compared with Harrington barley and Pioneer corn, AC Barrie wheat was lower ($P < 0.05$) in area intensities of both protein amide I (9.47 vs. 20.16 and 21.19) and amide II (2.21 vs. 5.49 and 6.81), but higher ($P < 0.05$) in the amide I to II intensity ratio (4.63 vs. 3.79 and 3.26), indicated the differences in the protein structural make-up or protein biopolymer conformation between AC Barrie wheat and Pioneer corn and between AC Barrie wheat and Harrington barley.

3.2.2. Discriminate and classify protein internal structures in the endosperm tissues among wheat, corn and barley seeds—

Fig. 2 shows the cluster analysis of the protein molecular structures (spectral region: ca 1720–1485 cm^{-1}) in the endosperms: compared AC Barrie wheat with Pioneer corn (Fig. 2a) and AC Barrie wheat with Harrington barley (Fig. 2b). Cluster analysis is a multivariate analysis of which function performs an (agglomerative hierarchical) cluster analysis of an infrared spectra data set and

displays the results of cluster analysis as dendrograms. First, it calculates a distance matrix, which contains information on the similarity of spectra. Then, in hierarchical clustering, the algorithm searches within the distance matrix for the two most similar infrared spectra (minimal distance). These spectra are combined into a new object (called a “cluster” or called “hierarchical group”). The spectral distances between all remaining spectra and the new cluster are re-calculated (Cytospec, 2004). It is a technique which clusters infrared spectra based on similarity with other spectra. In this study, the Ward’s algorithm method was used without any prior parameterization of the spectral data in the IR region (ca. 1720–1485 cm^{-1}). The results of this method can allow us to discriminate the differences in the structural make-up between the tissues.

Compared AC Barrie wheat with Pioneer corn (Fig. 2a), three classes can be distinguished below a linkage distance less than 7, with Pioneer corn and AC Barrie wheat groups forming two separate groups. The third class consisted of three cases of wheat protein spectra and six cases of corn protein spectra. Compared AC Barrie wheat with Harrington barley (Fig. 2b), it was also found that three classes can be distinguished below a linkage distance less than 5, with Harrington barley and AC Barrie wheat groups forming two separate groups. The third class consisted of two cases of wheat protein spectra and four cases of barley protein spectra were mixed together. These cluster analysis results indicated that the structural make-up or conformation of protein in AC Barrie differs from that in Pioneer corn and Harrington barley, but not fully distinguished.

Fig. 2 shows the principal component analysis results of the seed spectra at the amide I and II regions of 1720–1485 cm^{-1} obtained from the endosperms of AC Barrie wheat, Pioneer corn and Harrington barley. Principal component analysis is a statistical data reduction method. It transforms the original set of variables to a new set of uncorrelated variables called principal components. The first few principal components will typically account for >95% variance. The purpose of principal component analysis is to derive a small number of independent linear combinations (principal components) of a set of variables that retain as much of the information in the original variables as possible. This analysis allows studying globally the relationships between P quantitative characters (e.g. chemical functional groups such as amide I and II and carbohydrates) observed on n samples (e.g. Fourier Transform infrared spectra of seed structures). The basic idea is to extract, in a multiple variable system, one, two or sometimes more principal components that carry maximum information. These components are independent (orthogonal) of each other and the first factor generally represents maximum variance. As factors are extracted, they account for less and less variability and the decision of when to stop basically depends on the point when there is only very little significant variability left, or merely random noise. Thus, the reduction of data provides a new coordinate system where axes (eigenvectors) represent the characteristic structure information of the data and the spectra may then be simply described as function of specific properties, and no longer as a function of intensities. The outcome of such an analysis can be presented either as a 2D (two principal components) or 3D (three principal components) scatter plots [31]. In this study of seed inherent structure, principal component analysis was used to identify the main sources of variation in the protein amide I and II spectra in the region 1720–1485 cm^{-1} of AC Barrie wheat, Pioneer corn and Harrington

barley and identify features that differ between the seeds in the endosperm regions in terms of protein conformation.

Compared AC Barrie wheat with Pioneer corn protein amides spectra, AC Barrie wheat and Pioneer corn can be grouped into separate ellipses (Fig. 2a). The first three principal components explain 93.9, 2.7, and 1.8% of the variation in the protein amides spectrum data set. Compared AC Barrie wheat with Harrington barley protein amides spectra, AC Barrie wheat and Harrington barley can be almost grouped in separate ellipses (Fig. 2b). The first three principal components explain 94.7, 3.3, and 1.5% of the variation. The results showed that the principal component analysis could distinguish the spectral pattern difference between AC Barrie wheat and Pioneer corn and between AC Barrie wheat and Harrington barley using the protein amide I and II spectra at the region of 1720–1485 cm^{-1} . No published results have been found for discrimination of internal protein structures in different types of seed endosperms.

3.3. Endosperm differences in carbohydrate internal structural make-up among wheat, corn and barley

3.3.1. Identify the differences in the absorbed infrared intensity of carbohydrates and their ratios in the endosperm tissues among wheat, corn and barley—The major absorption bands from carbohydrates in plants are found in the ca. 1185–800 cm^{-1} region of the mid-infrared spectrum (Fig. 1e and f) and are attributed to C—O stretching vibrations. The bands in this region are very complex. When studying plant materials, it is customarily to look for structural carbohydrates such as cellulosic material and non-structural carbohydrates such as starch [9]. A peak area at ca. 1420 cm^{-1} band can be used to look for a particular type of carbohydrate- β -glucan [9]. In the portion of the spectrum from ca. 1550–800 cm^{-1} , strong carbohydrate bands are present for both structural and non-structural carbohydrate, particularly in the region 1100–1025 cm^{-1} . A major difference between these two forms of carbohydrate is the presence of bands of moderate intensity at approximately 1420, 1370 and 1335 cm^{-1} which indicates characteristics of structural carbohydrates [9,20]. Carbohydrate band peaks between 1100 and 1025 cm^{-1} depend on whether the carbohydrate is structural or non-structural. A peak at ca. 1025 cm^{-1} indicates non-structural carbohydrate such as starch in the endosperm of cereal grain [9,20]. A peak at ca. 1240 cm^{-1} indicates a structural carbohydrate such as cellulosic material. Table 1 shows the characteristics of the carbohydrates and their ratios in the plant seed endosperm regions of AC Barrie wheat, Pioneer corn and Harrington barley. The peak area absorbed intensities at ca. 1185–800 cm^{-1} (total carbohydrate, CHO) and 1650–950 cm^{-1} (non-structural carbohydrate, NSC) for AC Barrie wheat, Harrington barley and Pioneer corn endosperm were presented. Compared with Harrington barley, AC Barrie wheat was higher ($P < 0.05$) in total CHO (143.7 vs. 45.0) and NSC (82.7 vs. 22.9) absorbed intensities and higher in NSC to total CHO ratio ($P < 0.05$, 0.58 vs. 0.51). Compared with Pioneer corn, AC Barrie wheat was lower ($P < 0.05$) in total CHO (143.7 vs. 179.4) and NSC infrared absorbed intensity (82.7 vs. 105.7) with no differences in NSC to total CHO ratio ($P > 0.05$, 0.58 vs. 0.59). Table 1 shows that the structural characteristics of the amide I to carbohydrate ratios in the plant seed endosperms of AC Barrie wheat, Harrington barley and Pioneer corn. The results showed that there were significantly different ($P < 0.05$) between AC Barrie

wheat and Harrington barley in the ratios of amide I to total CHO and amide I to NSC. However, there were no significant differences between AC Barrie wheat and Pioneer corn. These results indicated that there are structural differences in the make-up of the seeds endosperm.

3.3.2. Discriminate and classify carbohydrate internal structures in the endosperm tissues of wheat, corn and barley seeds—Fig. 3 shows the cluster analysis of the spectra data from AC Barrie wheat, Pioneer corn and Harrington barley endosperms in the region of ca. 1185–800 cm^{-1} . This region is the total carbohydrate region. If the structural make-up differs among the plant seeds, the patterns of the spectra will be different. Compared AC Barrie wheat with Pioneer corn (Fig. 3a), there are no two classes which can be distinguished between AC Barrie wheat and Pioneer corn endosperm spectra in the region of ca. 1185–800 cm^{-1} . Pioneer Corn and AC Barrie wheat groups did not form two separate groups (Fig. 3a). Compared AC Barrie wheat with Harrington barley (Fig. 3b), two classes can be distinguished below a linkage distance less than 10, with Harrington barley and AC Barrie wheat groups forming two separate groups. These cluster analysis results indicated that the spectra from AC Barrie wheat in the region of ca. 1185–800 cm^{-1} are distinguishably different from Harrington barley but not from Pioneer corn.

Fig. 3 shows the results from principal component analysis of the spectra data obtained from the AC Barrie wheat, Pioneer corn and Harrington barley endosperms in the region of ca. 1185–800 cm^{-1} . The first two principal components were plotted. Fig. 3a shows that it cannot be completely grouped in separate ellipses between AC Barrie wheat and Pioneer corn because of overlapping of each group. However, when compared with Harrington barley, the results show that principal component analysis could fully distinguish between AC Barrie wheat and Harrington barley (Fig. 3b).

Both cluster and principal component analyses results indicated that the carbohydrate spectra between AC Barrie wheat and Pioneer corn were not fully discriminated but the carbohydrate spectra between AC Barrie wheat and Harrington barley were fully discriminated. This indicates that the carbohydrate molecular structures/conformation were different among the seeds.

3.3.3. Discriminate and classify non-structural carbohydrate internal structures in the endosperm tissues of wheat, corn and barley seeds—Further study was focused on the NSC make-up and spectral pattern in the seed endosperms. Fig. 4 shows the cluster analysis of the spectra data from AC Barrie wheat, Pioneer corn and Harrington barley endosperms in the region of ca. 1065–950 cm^{-1} (NSC region). Compared AC Barrie wheat with Pioneer corn (Fig. 4a), there were no two classes which could be distinguished between AC Barrie wheat and Pioneer corn spectra in the region of ca. 1065–950 cm^{-1} . Pioneer corn and AC Barrie wheat groups did not form two separate groups. Compared AC Barrie wheat with Harrington barley (Fig. 4b), two classes can be fully distinguished below a linkage distance less than 7, with Harrington barley and AC Barrie wheat groups forming two separate groups. These cluster analysis results indicated that the spectra from AC Barrie wheat in the region of ca. 1065–950 cm^{-1} are fully distinguishably from Harrington barley but not from Pioneer corn. These results were further verified by

principal component analysis. Fig. 4 shows results from the principal component analysis of the spectra data obtained from AC Barrie wheat, Pioneer corn and Harrington barley endosperms in the region of ca. 1065–800 cm^{-1} . The first two principal components were plotted. Fig. 4a shows that it cannot be grouped in separate ellipses between AC Barrie wheat and Pioneer corn because of overlapping of each group. However, when compared with Harrington barely, the principal component analysis could fully distinguish between AC Barrie wheat and Harrington barley (Fig. 4b). Both Cluster and principal component analyses results indicated that the NSC spectra between AC Barrie wheat and Pioneer corn were not fully discriminated but the NSC spectra between AC Barrie wheat and Harrington barley were fully discriminated. This indicates that the NSC molecular structures were different between the seeds. No published results have been found for discrimination of carbohydrate internal structures in different types of seed endosperms.

The different spectral patterns, different absorbed intensities in protein amide I and II and carbohydrates and different absorbed intensity ratios of protein amide I and II and carbohydrate in the endosperms between the seeds can explain different pattern of biodegradation kinetics and fermentation characteristics. Further study is needed to understand the quantitative relationship between the spectral characteristics of internal structures (peak intensity, intensity ratios, and spectral pattern of biological components) and biodegradation kinetics and fermentation behaviors.

4. Conclusions

In conclusion, synchrotron radiation-based technology (SR-FTIRM) identified spectral differences associated with molecular structural differences within the endosperm of sectioned grains. The spectral analysis at the regions of ca. 1720–1485 cm^{-1} (attributed to protein amide I and II), ca. 1650–950 cm^{-1} (attributed to NSC), and ca. 1185–800 cm^{-1} (attributed to total CHO) together with the agglomerative hierarchical cluster and principal component analyses were able to show that the molecular structures (or structural make-up) of the endosperm tissues exhibited distinguishable differences among AC Barrie wheat, Pioneer corn and Harrington barley. Further study is needed to understand the quantitative relationship between internal structure spectral characteristics (peak intensity, intensity ratios, and spectral pattern of biological components) and biodegradation kinetics and fermentation behaviors. The novel synchrotron radiation-based bioanalytical technique provides a new approach for plant/seed/feed/food structural molecular study and biopolymer conformation study in relation to nutrient availability within intact tissue at ultra-spatial resolution.

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Fig. 1.

Synchrotron-based FTIR spectrum in plant seed endosperm tissue (wheat) (pixel size: 10 μm × 10 μm): (a) whole region: ca. 4000–800 cm⁻¹; (b) fingerprint region: ca. 1800–800 cm⁻¹; (c) protein amide I region: 1720–1575 cm⁻¹; (d) protein amide II region: 1575–1485 cm⁻¹; (e) total carbohydrate region: ca. 1185–800 cm⁻¹; (f) non-structural carbohydrate (starch) region: ca. 1065–950 cm⁻¹.

Fig. 2.

Multivariate spectral analyses of protein internal structures in the endosperms: compared AC Barrie wheat with Pioneer corn and AC Barrie wheat with Harrington barley. I: cluster analysis (1) select spectral region: amides I and II 1720–1485 cm^{-1} ; (2) distance method: Euclidean; (3) cluster method: Ward's algorithm]; II: principal component analysis: Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2).

Fig. 3.

Multivariate spectral analyses of carbohydrates internal structures in the endosperms: compared AC Barrie wheat with Pioneer corn and AC Barrie wheat with Harrington barley. I: cluster analysis (1) select spectral region: ca. 1185–800 cm^{-1} ; (2) distance method: Euclidean; (3) cluster method: Ward's algorithm]; II: principal component analysis: Scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2).

Fig. 4. Multivariate spectral analyses of the non-structural carbohydrate (NSC) structures in the endosperms: compared AC Barrie wheat with Pioneer corn and AC Barrie wheat with Harrington barley. I: cluster analysis (1) select spectral region: ca. 1065–950 cm^{-1} ; (2) distance method: Euclidean; (3) cluster method: Ward's algorithm]; II: principal component analysis: scatter plots of the 1st principal components (PC1) vs. the 2nd principal components (PC2).

Table 1

The structural characteristics of protein amide I and II, carbohydrates and their ratios in the plant seed endosperm regions, revealed using synchrotron-based Fourier transform infrared microscopy: Comparison of wheat (cv. AC Barrie) with barley (cv. Harrington) and corn (cv. Pioneer) ($n = 15$ samples per each type of seeds)

Items	Peak center (cm ⁻¹)	Region (cm ⁻¹)	Baseline (cm ⁻¹)	Molecular characteristics of seed in terms of synchrotron mid-IR absorbed peak area and their ratios (Infrared Absorbed intensity unit)		
				Wheat	Barley	Corn S.E.M. ^a
Amide I	~1650	1720–1575	1720–1485	Based on the amide I and II peak area	20.16 a	21.19 a 1.375
Amide II	~1550	1575–1485	1720–1485		5.49 a	6.81 a 0.514
Ratio of Amide I to II	~1650/~1550	–	–		3.79 b	3.26 b 0.170
Total carbohydrate (CHO)	–	1185–800	1185–800	Based on the CHO peak area	44.99 c	179.43 a 4.357
Non-Structural CHO (NSC)	~1025	1650–950	1185–800		22.93 c	105.68 a 2.647
Ratio of NSC to CHO	~1650/~1550	–	–		0.51 b	0.59 a 0.059
Ratio of Amide I to CHO	–	–	–	Based on the Amide I and CHO peaks area	0.46 a	0.12 b 0.023
Ratio of Amide I to NSC	–	–	–		0.91 a	0.21 b 0.046

^a S.E.M. = pooled standard error of means; means with the different letter in the same column are significantly different ($P < 0.05$).