

Structural Conformation and Microlocalization of Digestion-Resistant Biopolymers Compounds in Feeds at Cellular Dimensions

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Introduction

Lignin and cellulosic compounds in feeds are biopolymers and usually play negative roles in determining feed quality, particularly in monogastric animals. Lignin is a highly condensed phenylpropanoid matrix that is relatively resistant to anaerobic fermentation by ruminal microorganisms and to intestinal digestion by enzymes. Lignin is also thought to physically encrust cell wall polysaccharides, inhibiting their fermentation, and is usually listed

as a digestion-resistant compound in feeds. The cellulosic compounds in feeds are mainly associated with phenolic-carbohydrate complexes in the feed cell wall (for example, mono-, dimer-, or trimer- ferulic acid-polysaccharides complex) [1], hemicellulose encrustation and cellulose crystallinity and are also typically listed as digestion-resistant compound. Their complex structures of cellulosic compounds reduce the susceptibility to digestive enzymes and microbial degradation [2,3].

Method

Synchrotron-based Fourier Transform Infrared Microscopy (SR-FTIRM) is a non-destructive and non-invasive technique that takes advantage of bright synchrotron light and is capable of exploring the molecular structural make-up and biopolymer conformation within inherent structures of biomaterials [4,5]. However, SR-FTIRM is rarely used for plant-based feed research [5]. The objective of this study was to localize digestion-resistant compounds in feeds within cellular and subcellular dimensions and detect differences in their structural conformation and make-up.

Results

To microlocalize the aromatic lignin and cellulosic compounds across feed intrinsic structures within cellular and subcellular dimensions, we usually check their relatively unique bands in the fingerprint region. In the MIR region, the relatively unique band of aromatic lignin in feeds is at ca. 1510 cm^{-1} . This band is considered to be primarily indicative of the aromatic character of the lignin (Figure 1). An aromatic compound gives two major bands at ca. 1600 and 1500 cm^{-1} , referred to as quadrant and semicircle ring stretch, respectively [6]. These are well exemplified in the lignin spectrum that shows bands at 1600 cm^{-1} and 1510 cm^{-1} . The first of these bands is a possible

interferent with the other bands such as feed protein amide band (ca. 1650 cm^{-1} and 1550 cm^{-1}). The second of these bands, the peak at ca. 1510 cm^{-1} , shows no significant interference with any other bands and thus is an excellent diagnostic criterion for aromatics in feeds. In a complex plant system, the major absorptions from carbohydrates are in the 1200-800 cm^{-1} region of the spectrum. The relatively unique fingerprint band of cellulosic compounds in feeds is at 1246 cm^{-1} [7,8]. The cellulosic compound in feeds is mainly associated with phenolic-carbohydrate complexes, hemicellulose encrustation and cellulose crystallinity. The complex structures of cellulosic

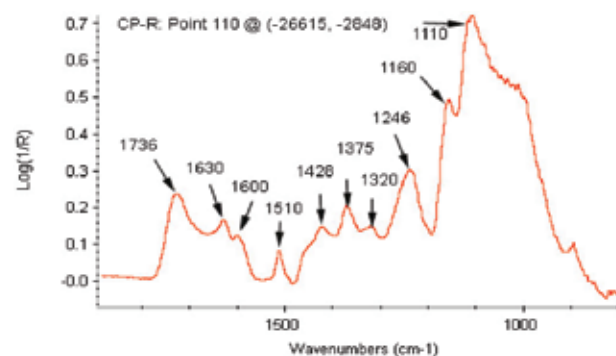
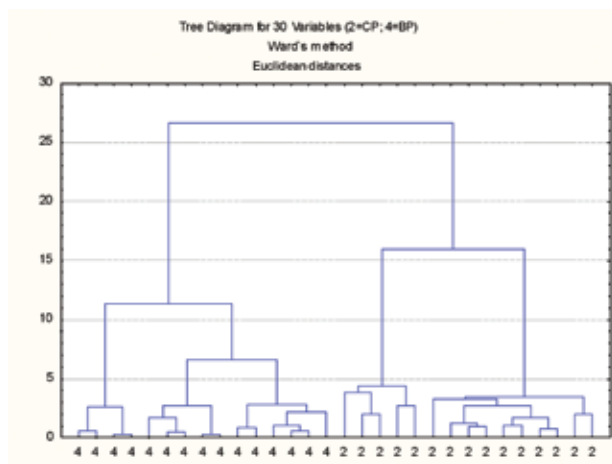


Figure 1: Ultraspacially resolved synchrotron FTIRM fingerprint bands of the undigested aromatic compound - lignin and the uneasily digested structural carbohydrates - cellulosic material in plant/seed/feed tissues at cellular and subcellular levels. Fingerprint band of the digestion-resistant aromatic compound (lignin) ca. 1510 cm^{-1} . Fingerprint band of the poorly digested structural carbohydrate (cellulosic material) at ca. 1246 cm^{-1}

compounds reduce their susceptibility to digestive enzymes. The feeds used for this pilot study were corn (cv. Pioneer) and barley (cv. Harrington) (Figure 2, 3). The results show that with SR-FTIRM, the microlocalization of the aromatic lignin and cellulosic compounds could be mapped to show the distribution and intensity across the plant seed tissues at ultra-spatial resolution.

Conclusions

With SR-FTIRM, microlocalization of two digestion-resistant biopolymers (aromatic lignin and cellulosic compounds) across different feed structural regions at cellular and subcellular levels can be obtained. Lignin and cellulosic compounds can be linked to structural information in the feeds without destruction of intrinsic structures of feeds. Use of advanced synchrotron technology can make a significant and an



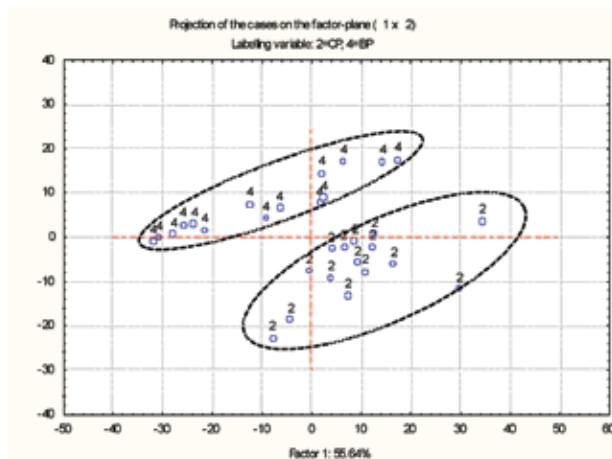
2 = Pericarp in corn (cv. Pioneer); 4 = Pericarp in Barley (cv. Harrington)

Figure 2: HCLA cluster of feed intrinsic structures showing that the clusters in the pericarp are different between the two feeds, and the clusters in the aleurone layer are different between the two feeds. [HCLA analysis: (1) Spectral region: 1800 to 800 cm⁻¹; (2) Distance method: Euclidean; (3) Cluster method: Ward's algorithm]

important contribution to feed molecular structural-chemical research.

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2 = Pericarp in corn (cv. Pioneer); 4 = Pericarp in barley (cv. Harrington)

Figure 3: PCA analysis of synchrotron-based feed molecular structure spectrum obtained from (a) aleurone layer and (b) pericarp in both corn (cv. Pioneer) and barley (cv. Harrington) at a cellular level (pixel size 10 μm×10 μm): Scatter plot of the 1st principal component (PC1) vs the 2nd (PC2) or 3rd principal component (PC3) [For the aleurone layers in both corn and barley, PC1 explains 52% of the variance while PC2 explains 20% of the variance; PC3 explains 17% of the variance; For the pericarps in both corn and barley, PC1 explains 56% of the variance while PC2 explains 19% of the variance; PC3 explains 11% of the variance].

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