

Correlation spectroscopy: near infrared, mid-infrared and Raman spectral regions as complementary techniques for the study of wheat properties

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Introduction

Wheat is grown worldwide and year round and has been for 2000 years. Wheat grown and exported in the USA is divided into seven classes, durum, hard red spring (HRS), hard red winter (HRW), soft red winter, white, unclassified and mixed.¹ During the time wheat has been a cultivated crop, its genetics have been modified by cross-breeding and back-crossing. As such, the question of classification has become more difficult in recent years. In addition, the classification system is not uniform worldwide. Near infrared (NIR) spectroscopy first successes were in the determination of moisture and protein in wheat.^{2,3} Wheat is composed of starch, protein, moisture and a small portion of lipid and bran, all of which have been determined by NIR spectroscopy in some commodity.⁴ It seems logical to develop a classification system based solely on the spectra of wheat. Therefore, a real understanding of the wheat spectrum and what spectral characteristics can be used to describe functional properties is needed.

The spectra of wheat in any region of the electromagnetic spectrum is very consistent. Only the use of statistical/chemometric methods has been successful in NIR spectroscopy in determining composition and discriminating by hardness. This is primarily due to the high signal-to-noise ratio of the NIR spectroscopic instrumentation. The mid-infrared, nuclear magnetic resonance (NMR) and Raman spectrum all have greater information content with respect to the interpretation of specific functionality within the wheat kernel and wheat meal or flour, but have found limited analytical utility. Two-dimensional correlation spectroscopy across the NIR and mid-infrared regions has been used to explain the NIR spectra and provide additional confidence in the analytical models developed with empirical data.^{5,6} Recent studies have shown that the major C–H stretching vibrations and some of the aromatic C–H and ring stretching vibrations and the minor vibrations in the “fingerprint” region also are correlated. Degradation studies with carbohydrates and pure cultures of microbial organisms have shown that specific C–O stretches can be distinguished with the two-dimensional techniques.^{7,8} There has been ample evidence from the work of Bietz that the proteins of wheat can be used for variety identification.⁹

We have made use of NIR spectroscopy, mid-infrared, NMR and Raman spectroscopy in an attempt to understand what differentiates wheat. In this study, we examined the spectral properties

that differentiate “hard and soft”, “winter” and “spring” and differences in information between mid-infrared and Raman.

Experimental

Samples

Two sets of wheat samples were obtained from the USDA ARS Grain Marketing Research laboratory in Manhattan, KS, and supporting Wheat Quality Laboratories. One set of 15 and another of 25 samples comprising HRW, HRS, White and Soft wheats. The samples were ground through a UDY cyclone mill with the kernels introduced via a vibrating tray to allow for a uniform grind.

Instrumentation

NIR

An NIRSystems Inc. (Silver Spring, MD) model 6500 interfaced to a PC running Infracore International's ISI-3 software package was used for the NIR spectra. A total of 64 scans was accumulated for each of the five samples and three samples of each were run and their scans averaged (3×64). Fourier self-deconvolution (FSDC) was accomplished on reference NIR spectra with the arithmetic routines in GRAMS, ver. 3.0 and with software written for the National NIR Spectroscopy Research Project¹³. The baseline was flattened by rational interpolation and line width reduced by approximately 25% for all spectra.

Mid-infrared

The mid-infrared spectra were obtained on a Nicolet Analytical Instruments (Madison, WI) model 800, 740 and/or a model 750 interfaced to a Nicolet 620 computer workstation for data acquisition. Data were transferred to a PC-compatible disk file on a diskette. The sample's spectra, a total of 128 scans, were taken using a Globar source, a KBr beamsplitter and an MCT-B detector at 4 cm^{-1} resolution. Diffuse reflectance spectra were obtained with a Spectra-Tech-Nicolet advanced diffuse reflectance accessory.

Raman

Raman spectra were obtained on a Nicolet Analytical Instruments 910 Raman spectrometer interfaced to a Nicolet 620 workstation, a Nicolet 950 Raman spectrometer or a Bruker IFS 66 spectrometer with an FRA 106 Raman accessory. For both instruments, laser light at $1.1 \mu\text{m}$ was used and 1024 scans collected. An MCT-A detector was used for the Nicolet 910 and Bruker IFS66. A liquid nitrogen cooled germanium detector was used for the Nicolet 750.

Software/mathematical algorithms

The ISI file was converted to NIRSystems NSAS format with an ISI conversion routine. The NSAS NIR file and the Nicolet and Bruker mid-infrared and Raman files were both put into Galactic Industries (Salem, NH) GRAMS, ver. 3.0 software package. GRAMS can accept files from more than 100 different instruments and is the only package where these files can be combined to run the correlations. A special routine was written by Galactic Industries to conduct the correlations as previously described.⁵ The new GRAMS 3D package was used to produce some of the maps and slices.

Results and discussion

The first attempt to model wheat by its component spectra was by Hruscka and Norris in 1982.¹⁰ These authors found the “Least Squares” method of curve fitting to be inadequate to the task of adequately describing wheat. Wheat is a matrix of starch, protein, some lipid and bran in a somewhat hydrated state. The matrix contains these components in complimentary linear combinations, but with non-linear interactions which perturb linear models beyond their normal usefulness, such as differences in internal moisture which affects particle size. Figure 1 is the mid-infrared spectrum of a hard red spring wheat sample plotted with a soft wheat sample spectrum. The baseline shift between the two spectra is the result of the difference in hardness. Although the difference in hardness is quite large the spectra are very similar. The major differences can be seen in the 1100–1500 cm^{-1} where the amide bands absorb. The major absorbances of wheat in the NIR (Figure 2) are the 2180 nm band attributed to $-\text{CONH}$ amide III (carbonyl) and N–H combination stretch, the 1780 and 2090 nm bands for C–H first overtone and O–H combination in the starch. The O–H stretch combination band for moisture absorbs at 1960 nm, lower for moisture associated with the protein matrix (1949 nm).¹¹ The major absorbances in the mid-infrared and Raman follow the same pattern as the NIR, but since we are looking at the fundamental vibrations the spectrum is much richer. In general, the same bands occur in both mid-infrared and Raman spectroscopy, but they are usually complementary in strength, i.e. if strong in the mid-infrared they are weak in the Raman and *vice versa*. The proteins are characterized by the Amide A & B around 3300 and 3100 cm^{-1} , respectively and by a series of bands known as Amide I, 1600–1680 cm^{-1} [C=O stretch, N–H deformation (def.) and C–N stretch]; Amide II, 1480–1575 cm^{-1} (C–N stretch and N–H def. usually not seen in Raman); and Amide III 1230–1300 cm^{-1} (C–N stretch and N–H def.). There are Amide IV–VII bands but they are in the fingerprint region and would be obscured by other absorbances.¹² The major C–H stretches are the asymmetric around 2900–2930 cm^{-1} and the symmetric at 2825–2860 cm^{-1} . These are about the same strength for both mid-infrared and Raman. The carbohydrates exhibit many medium to strong bands in the mid-infrared around 1160–1000 cm^{-1} involving C–O stretch of COH and COC groups.^{13–15} Carbohydrates exhibit medium to weak C–H stretch in the 2900 cm^{-1} region and multiple medium bands in the 1460–1200 cm^{-1} region involving CH_2 def. and CH and CH_2 wag

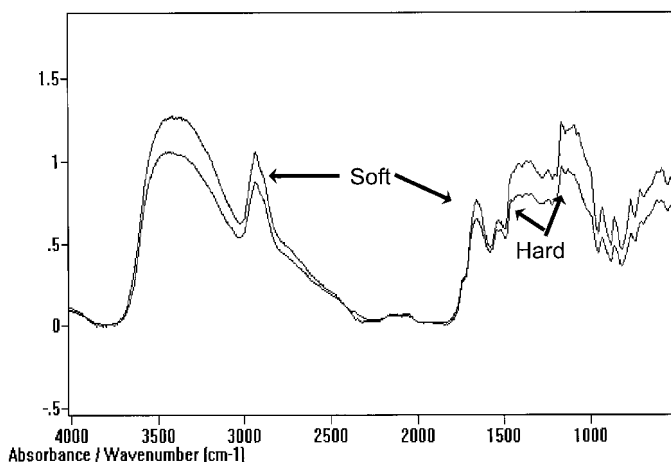


Figure 1. A plot of the mid-infrared spectra of a hard and a soft wheat.

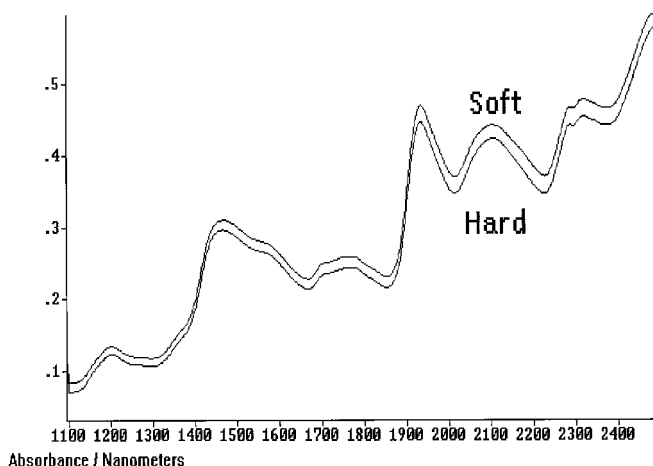


Figure 2. A plot of the near infrared spectra of a hard and a soft wheat.

and OH in-plane def. There are multiple (2–4) medium to weak bands in the $960\text{--}730\text{ cm}^{-1}$ region to distinguish types of carbohydrates. The pyranose ring absorbs at the lower frequencies and the five member furanose rings around $900\text{--}800\text{ cm}^{-1}$. These are associated with in- and out-of phase ring stretch. Starch, the alpha-D-glucose polymer, has five bands in this region compared to only two for cellulose.¹⁶ The O–H stretch in water is very strong in the mid-infrared and very weak in

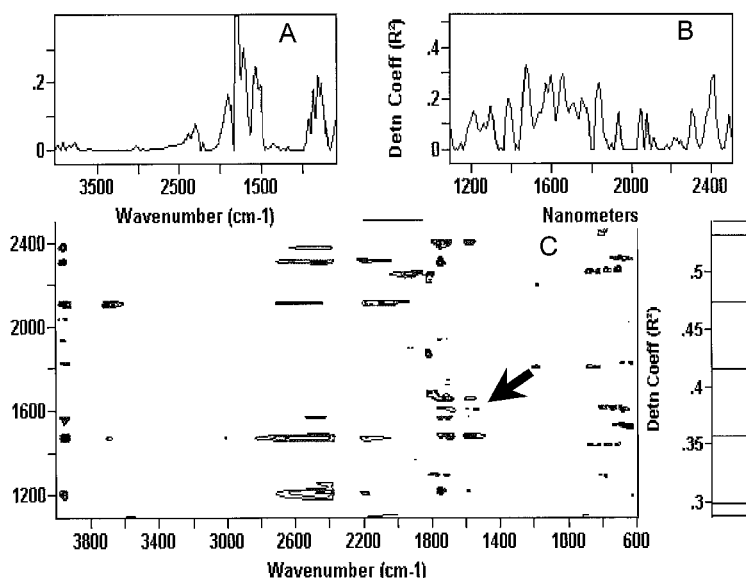


Figure 3. A 2-D contour map of the mid-infrared and near infrared spectra of a file of hard red spring and hard red winter wheat. A: the mid-infrared correlation “slice” at 1672 nm; B: the near infrared correlation “slice” at 1515 cm^{-1} and C: the contour map (arrow indicates the coordinates of the two “slices”).

the Raman at $3650\text{--}3000\text{ cm}^{-1}$.¹² This accounts in part for the renewed interest in Raman for high moisture applications.

The two-dimensional vibration spectroscopy technique allows us to see the correlations of any two of the spectral regions. The spectra of a set of samples (taken as identically as possible on each instrument) is taken in each region and arranged in a file so that the sample is paired for the two regions being correlated. A point-for-point simple least squares regression is performed and the resulting contour map plotted. These maps reveal some expected as well as some unexpected results. Figure 3 is a contour map of the mid-infrared (X-axis) vs. NIR (Y-axis) of hard red winter and hard red spring wheats. The area shown is centered on the aromatic ring puckering stretch at 1515 cm^{-1} . The NIR is at 1670 nm (first overtone of aromatic C–H stretch). Contours are present for each of the strongest NIR bands found by Williams and Norris for protein.¹⁷

Conclusions

The results of this study show that two-dimensional techniques can be used to find subtle differences and confirm other qualitative differences in wheat. Further, it may be possible to devise spectral based classification schemes based on these differences. Finally, these techniques allow us to focus attention on areas of research where improvements in crop quality and handling can provide for better end use of a most valuable agricultural commodity.

Disclaimer

Reference to specific products is made for identification purposes only and does not imply endorsement by the United States government.

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