Ocean Optics

Application Note

Keywords

- Agricultural samples
- Powders and grains
- Sample heterogeneity

Techniques

- NIR spectroscopy
- Diffuse reflection
- Calibration modeling

Applications

- Compositional analysis
- Moisture and fat determination
- Process control

Near-Infrared Spectroscopy Analysis of Powders and Grains

NIR spectra are composed of broad peaks resulting from molecular vibrations caused by interaction of molecules with light in the NIR wavelength region (~800-2500 nm). Information can be extracted from these broad peaks for the quantitative determination of chemical composition using chemometrics. Because NIR spectroscopy measures liquids and powders without the need for sample preparation, the technique is an important tool for the food industry, where quantitative information on parameters like fat, moisture and protein content is critical. In this application note, NIR spectroscopy is used for the quantitative characterization of powders and grains. The importance of sampling strategy and multivariate statistics are discussed with tips to optimize the measurements for the most accurate compositional information.

Background

NIR spectroscopy is used extensively for the compositional analysis of samples in the food and agricultural industries. In the agriculture and food industries, NIR spectroscopy techniques are employed in solids processing to control parameters like protein, moisture, fiber and fat.

Spectral data measured in the NIR region typically are composed of broad, often overlapping peaks related to the chemical composition of the sample. When quantitative analysis of moisture, fat or protein is the goal, there are a number of important parameters to consider. The accuracy of the compositional information extracted from NIR spectral data depends not only on a good calibration model but one created from representative spectral data. With an accurate and robust calibration model, NIR spectroscopy can be used for the rapid analysis of samples on a process line, in a lab or in the field.

Experiment Considerations

The reference spectrum is a critical part of any spectroscopy measurement. For accurate spectral data, the reference must be fully representative of the sample containing everything present in the light path. In the case of food and agricultural samples, samples are often placed in a plastic bag or other container with measurements made through the container. Many of these materials absorb light in the NIR region so they must be included in the reference measurement to account for their spectral contribution to the overall spectrum.

Figure 1: An NIR spectrometer is configured with light source and sampling fixture for diffuse reflection measurements.

To illustrate this, we used a diffuse reflection stage with NIR illumination (model name Vivo) and an NIR spectrometer covering the wavelength range from 900-2050 nm (NIRQuest256-2.1) to measure NIR spectra for soybeans (Figure 1). NIR spectra were measured for soybeans using a Spectralon® diffuse reflectance standard as the reference. The reference standard was placed directly on the reflection stage or sampled through a plastic bag.

Results

The NIR spectra for the diffuse reflection standard are shown in Figure 2. As evidenced by the significant spectral differences observed between 1680 nm and 1800 nm, the plastic sample bag absorbs NIR light in this region. If the reference spectrum acquired without the plastic sample bag in the light path was used to provide soybean composition analysis, the composition obtained using this data would be inaccurate. The absorption due to the presence of the plastic bag in the light path would not be taken into account, resulting in incorrect compositional values.

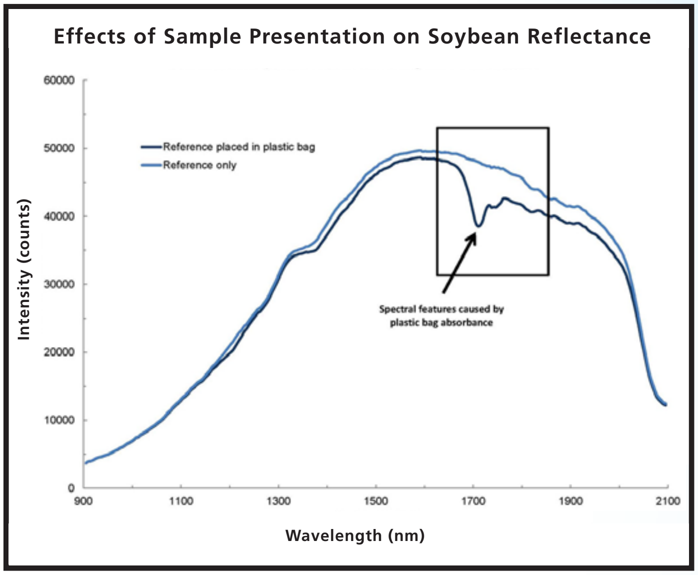

Figure 2: Accurate NIR measurements of food and other packaged samples require consideration of the effects of any packaging in the light path.

The impact of using an improper reference to measure soybean absorbance is demonstrated in Figure 3. NIR spectra were measured for soybeans using a diffuse reflection standard as the reference. Soybean absorbance was measured for soybeans placed either on the Vivo reflection stage (no plastic bag in the light path) or for soybeans contained in a plastic bag. The reference spectra used for the absorbance measurements were acquired with either the reference placed directly on the reflection stage or in a plastic sampling bag. As shown in Figure 3 for the same soybean sample, significant spectral differences are observed when plastic bag absorbance is not accounted for in the measurements.

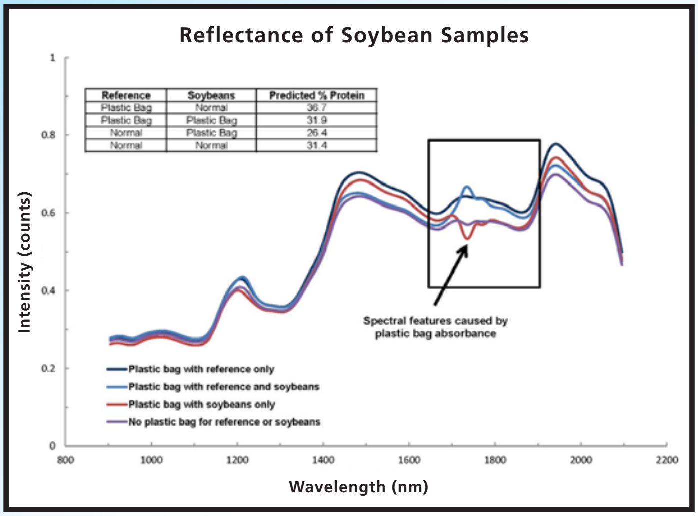

Figure 3: Variability in sampling media will affect NIR measurement results, as these soybean sample spectra demonstrate.

The impact of the spectral differences observed in Figure 3 is also demonstrated by the variability in the soybean protein composition predicted from these spectra. For the two spectra where the reference and soybean spectra were measured under different conditions, the predicted protein concentrations were very different (approximately 26% and 36%) even though the spectra were measured for the same soybean sample. With a reference that accounted for all the materials in the light path, the predicted protein concentration was different from the other spectra but very similar to one another at ~31%. The variability in these protein composition predictions for the same soybean sample demonstrates the impact a non-representative reference can have on the determination of composition from spectral data.

The spectral differences observed in Figure 3 are related to the absorption of the plastic sampling bag and have nothing to do with soybean sample composition. Quantitative analysis of the soybeans measured with a reference that is not representative of the sample leads to inaccurate compositional information if the calibration model created for the sample includes the region where the sampling bag absorbs. Inaccurate compositional information could lead to improper sorting and grading of the soybeans. It is critical to ensure that the reference and all sample measurements used for compositional analysis include all potentially interfering materials present in the light path.

Sample Heterogeneity

Most NIR samples are inherently non-uniform. Measurements must take into account both the location-dependent variability of a non-uniform sample like a batch of corn kernels and the distribution of the constituents. An example of the spectral variability obtained when multiple locations are sampled in a batch of corn kernels is shown in Figure 4. For these measurements, absorbance spectra were measured for corn kernels from 900-1900 nm.

As shown in Figure 4, the heterogenous nature of the samples causes significant spectral variability in both spectral shape and peak intensity when spectra are measured at different locations on the batch of corn. If not accounted for, this variability will lead to compositional data that is not representative and repeatable for critical parameters like moisture, fat and fiber content.

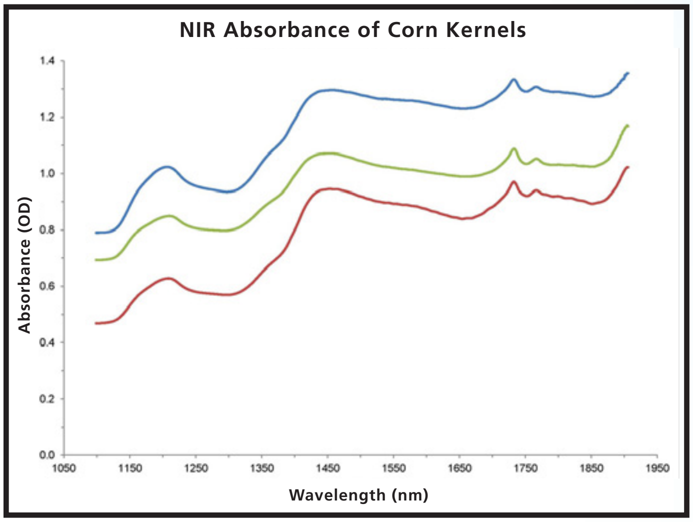

Figure 4: Proper NIR measurements account for subtle differences in samples such as corn, which have a non-uniform distribution of compounds within each kernel.

While the impact of sampling at different locations is not as significant for a homogeneous sample like sugar as it is for a non-uniformly shaped sample like corn, spectral variability is still observed and must be addressed with a measurement technique that averages out this variability. In the case of corn, sample non-uniformity is not limited to the size and shape of the corn kernels in a given batch. Each kernel has a non-uniform distribution of compounds that also affects the spectral data. For example, the fiber content is highest near the surface of the kernel with starch concentrated deepest within the kernel. Protein and fat also can be found in distinct areas of the kernel, resulting in spectral variability even if sampling is limited to a single corn kernel. This variability must be addressed to ensure representative sampling, or compositional information will be inaccurate and affect determination of sample quality for the sale or sorting of product.

The impact of this non-uniformity can be overcome by averaging multiple spectra collected at different locations in the sample. This sampling can be done by changing the sampling location or through the use of a mechanical rotating sampling accessory. The rotating cup enables easy acquisition of multiple spectra at various locations on a non-uniform sample, allowing the user to generate an average spectrum that is more representative of the non-uniform sample. When multiple average measurements are made using a rotating cup accessory, the spectra are nearly identical leading to much more accurate and representative compositional analysis.

Conclusions

After representative, repeatable spectral data has been acquired for a sample, the next step is to take the spectral data measured under optimal conditions (including a sample set with known concentration values) and create a calibration model for the quantitative analysis of samples with unknown composition. Quantitative information cannot be obtained without creating a calibration model to use in the determination of unknown sample compositions. Creation of an accurate and robust calibration model starts with representative data that accounts for sample non-uniformity and all potential interferences present in the sample path. With a good calibration model, NIR spectroscopy techniques can be applied to the measurement of critical sample parameters in unknown samples in the field, on a production line or as part of an inbound or outbound QC procedure. The ability to extract such important information from NIR spectroscopy has made it a very important technique for the food and agricultural industries. Detailed compositional information all starts with good measurement techniques.

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