

Application Note F03

Analysis of Unground Mustard Seed Using the SpectraStar 2400 NIR Spectrometer

Introduction

Near-infrared (NIR) technology has been used in the food, feed, and agriculture industries for over 50 years as a way to analyze for properties such as moisture, protein, fat, fiber, ash, amino acids, and more. NIR testing is fast (analysis in seconds), accurate, safe, usually nondestructive and requires minimal sample preparation with no reagents. NIR is extremely flexible and can be configured for the analysis of solids, liquids, oils, slurries, and suspensions. Accuracy is often equivalent to the wet chemical methods that it replaces. Its precision is almost always better.

Developed as a technique for predicting the chemical composition of a variety of unknown samples, near infrared (NIR) uses diffusely reflected light in the 800 to 2500 nanometer (nm) range to make a determination. Specifically, NIR light affects the molecular C-H, N-H, and O-H bonds. These bonds are directly related to the sample constituents of interest, such as fat, protein, moisture, fiber, starch, sugar, and amino acids, to name a few. Response to these bonds can be found throughout the NIR spectrum, but the primary combination bands for all of these properties, found above 1900 nm, are the most sensitive and generally provide the most accurate calibrations.

When NIR light hits a sample, part of the light is absorbed and part is diffusely reflected. The amount of absorbed light, at a particular wavelength, is directly proportional to the concentration of the constituent of interest. In other words, the more NIR light being absorbed at a particular wavelength, the greater the constituent (moisture, fat, protein, etc.) level in the sample.

A series of standard samples of known concentration, analyzed using a high accuracy reference method is scanned to measure the absorbance values at wavelengths throughout the NIR region. A calibration is then developed by using one of various mathematical models to correlate the reference lab values to the amount of absorbed NIR energy. The calibration can then be used to predict the constituent concentration of unknown samples.

In this report, the analysis of unground mustard seed samples is described.

Experimental

Instrumentation

All measurements were performed using a SpectraStar 2400 NIR spectrometer, equipped with a rotating drawer. The SpectraStar 2400 is a scanning monochromator-based NIR system that scans the optimum wavelength range of 1200-2400nm in 1nm steps. The SpectraStar 2400 utilizes an extended range InGaAs detector for enhanced stability and improved signal to noise ratio.

All calibration development and data management was performed using the CalStar software. CalStar is a Windows™ based software program that combines an intuitive, easy to use data management scheme along with the flexibility of using multiple calibration types, such as multiple linear regression (MLR) and partial least squares (PLS) to manage NIR data and develop calibrations.

All samples were analyzed by using the rotating cup drawer of the SpectraStar 2400. The rotating cup drawer allows for the analysis of partially ground or unground material. Rotating the sample allows for averaging over a larger surface area and helps to reduce the effects caused by sample inhomogeneity, particle size variation, and particle orientation.

Sample Preparation

Samples were mixed and loaded, without grinding, into the rotating sample cup used with the SpectraStar 2400.

Calibration Samples

Approximately 300 mustard seed samples were used in the development of moisture, protein, and oil calibrations. The calibration set includes mustard seed from various growing regions over several crop years. The following table shows the moisture, protein, and oil ranges of the calibration samples, along with the wet chemistry methods used to analyze them. As NIR is a secondary technique, it is calibrated against a primary method. Performance of the NIR will never be better than the repeatability of the wet chemistry method, which can be determined by calculating the pooled standard deviation of a set of blind duplicates. As some wet chemistry methods are better than others are, care should be taken when choosing a primary method or comparing NIR performance.

<u>Property</u>	<u>Range</u>	<u>Wet Chemistry Method</u>
Moisture	4.0 – 8.9%	Moisture Balance
Oil	24 – 52%	Acid Hydrolysis
Protein	21 – 41%	Kjeldahl

Results and Discussion

Calibration Development

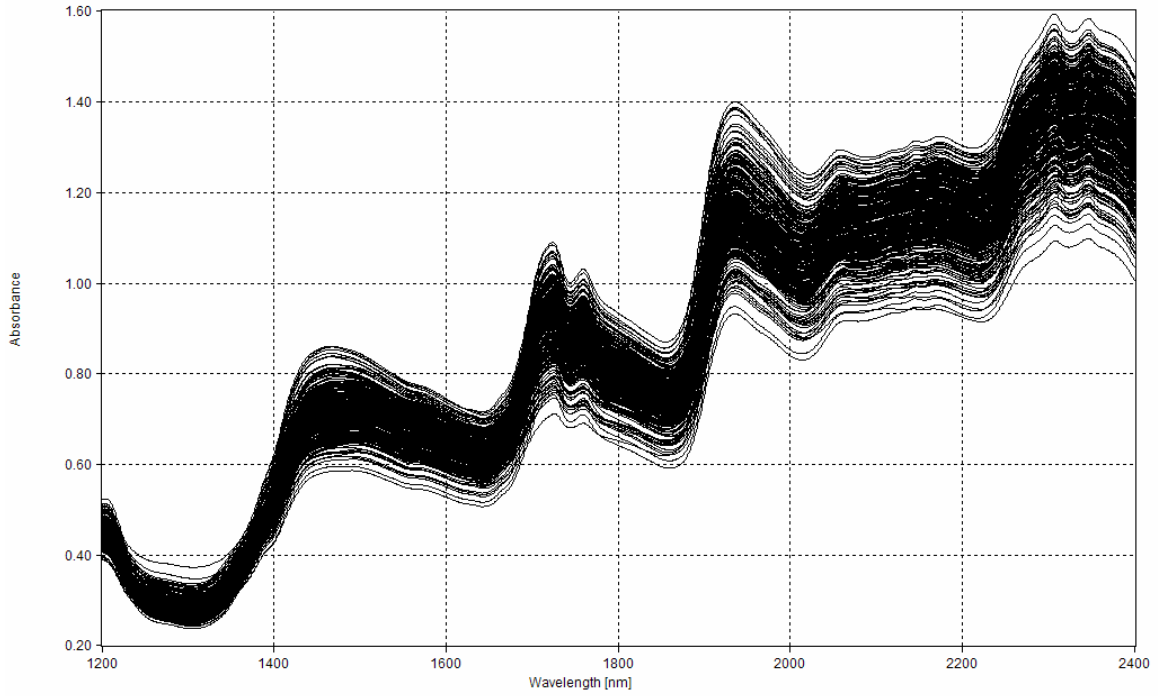
Figure 1 shows the calibration set of spectra measured using the SpectraStar 2400. Figure 2 shows the calibration set of spectra with an absorbance, standard normal variate (SNV) and 1st derivative transformation applied. Math transformations, such as SNV and first derivative, can be helpful to remove uniform baseline offset caused by varying particle size or changes in sample temperature. All calibrations developed used a combination of absorbance, SNV, and a first derivative transformation.

Partial Least Squares (PLS) calibrations were developed using the mustard seed samples for moisture, protein, and oil. The following table shows the multiple correlation coefficient and standard error of cross validation of the calibration. The multiple correlation coefficient is the agreement between the wet chemistry result and the NIR result. Perfect correlation is equal to 1. The standard error of cross validation is the performance that can be expected when using the calibration for routine analysis.

<u>Property Prediction</u>	<u>Multiple Correlation Coefficient</u>	<u>Standard Error of</u>
Moisture	0.984	0.23
Oil	0.996	0.49
Protein	0.992	0.53

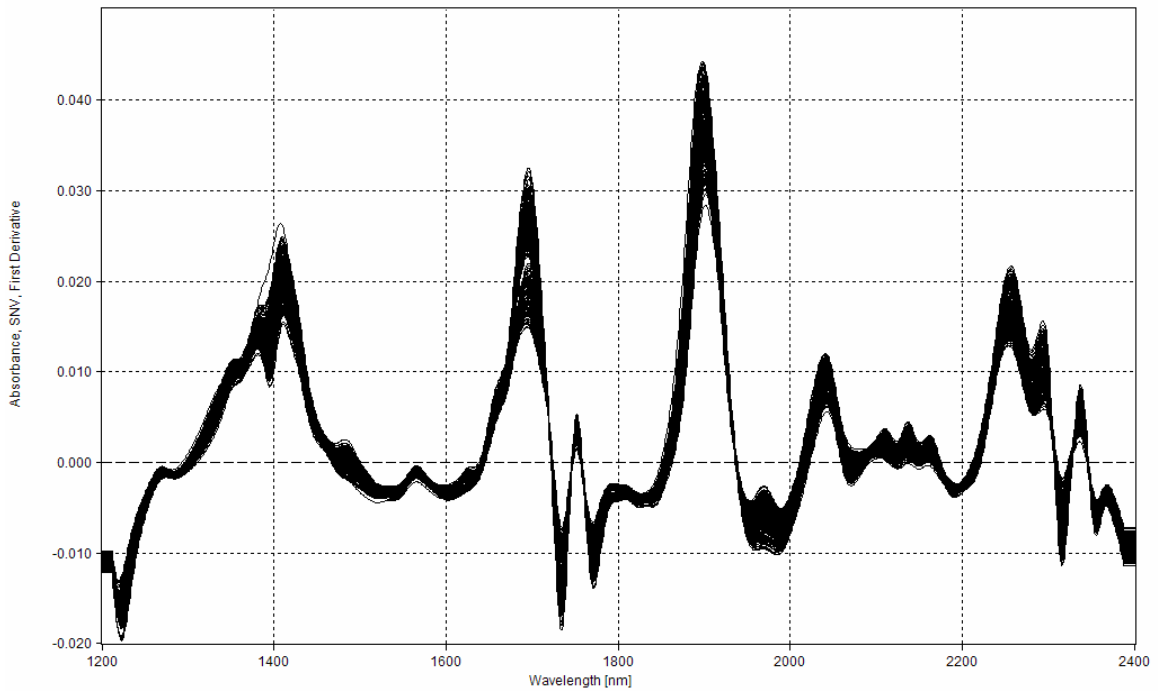
Figure 3 shows the predicted (NIR) vs. Actual (lab) plots of the protein and oil calibrations.

Figure 1



Absorbance vs. Wavelength Plot of Mustard Seed Calibration Spectra

Figure 2



Absorbance, SNV, 1st Derivative Plot of Mustard Seed Calibration Spectra

Conclusion

NIR is an important quality tool used in the pet food industry. Analysis of incoming raw materials, in-process intermediates, and finished products can help to ensure product quality and provide quick financial payback. The SpectraStar 2400 will accurately analyze mustard seed for moisture, protein, and oil. The SpectraStar's optimum wavelength range of 1200-2400nm covers the primary combination bands of C-H, N-H, and O-H bonds, which are used to analyze moisture, protein, fat, and ash. Specifically, the primary combination bands found above 1900nm are the most sensitive and generally develop the most accurate calibrations.

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