



Application Note: F04

Analysis of Cocoa Butter 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 cocoa butter is described.

Experimental

Instrumentation

All measurements were performed using a SpectraStar 2400 NIR spectrometer, equipped with a standard 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 Unity transfectance cup for the SpectraStar 2400. The pathlength of the transfectance cup is 0.30 mm. The cup is loaded by using a transfer pipette to place a few drops of cocoa butter on the reflecting surface before applying the cover. Consistency in sample handling is crucial to accurate NIR measurements.

Sample Preparation

All samples were analyzed at approximately 40-50⁰ C. Samples that had cooled to room temperature were kept in an oven to maintain temperature. Samples were stirred and mixed before loading into the Unity open cup.

Calibration Samples

The calibrations that were developed contained cocoa butter samples from around the world. Approximately 400 cocoa butter samples were used in the development of the free fatty acids calibration. Approximately 200 samples were used in the development of the iodine value calibration. The following table shows the free fatty acids and iodine value ranges of the calibration samples, along with the wet chemistry methods used to analyze them. As a secondary technique, NIR instruments are 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, care should be taken when choosing a primary method or comparing NIR performance.

<u>Property</u>	<u>Range</u>	<u>Wet Chemistry Method</u>
Free Fatty Acids	0.5 – 2.3%	Titration
Iodine Value	33 - 42%	Titration

Results and Discussion

Calibration Development

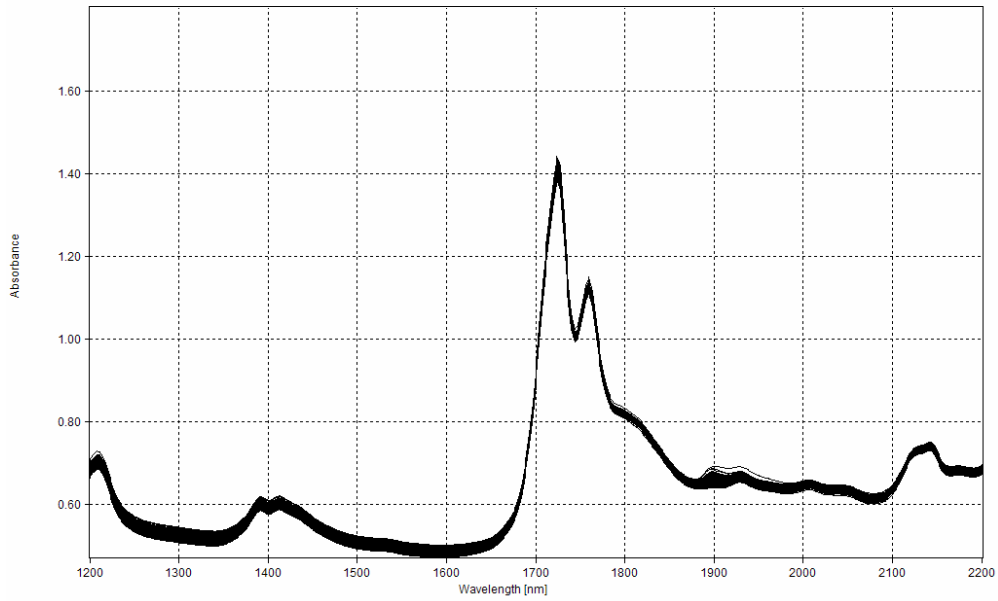
Figure 1 shows the calibration set of samples used in the development of free fatty acids and iodine value samples. Figure 2 shows the calibration set of spectra with an absorbance, standard normal variate (SNV), and first derivative transformation. Math treatments, such as the SNV, are helpful to eliminate baseline offset in the cocoa butter calibration spectra caused by scattering effects of particulate matter in the sample.

Calibrations were developed for free fatty acids and iodine value using the cocoa butter samples. The following table shows the multiple correlation coefficient and standard error of cross validation for the calibrations. 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</u>	<u>Multiple Correlation Coefficient</u>	<u>Standard Error of Prediction</u>
Free Fatty Acids	0.993	0.046
Iodine Value	0.996	0.20

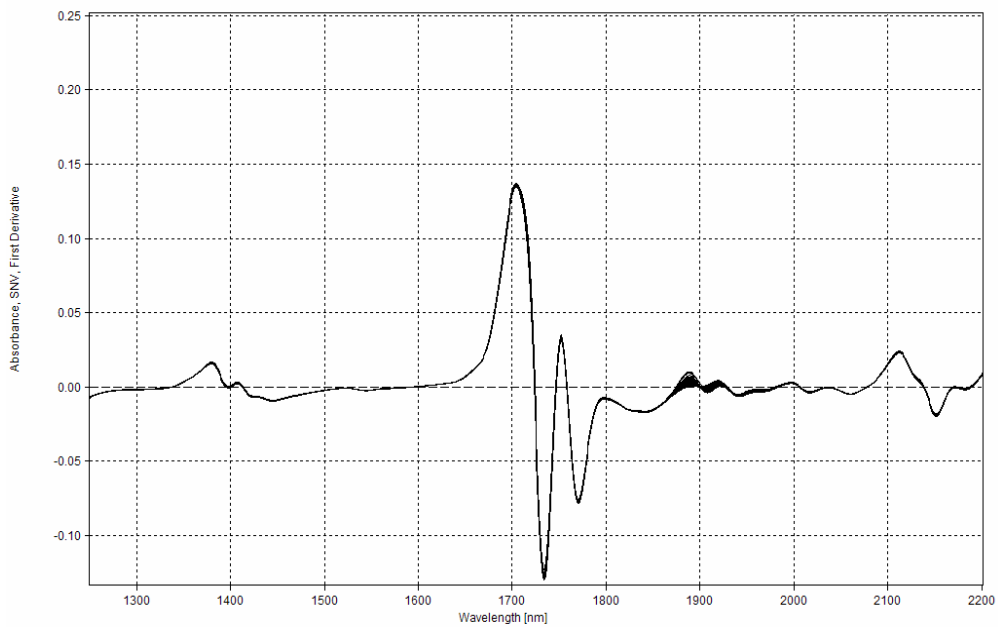
Figure 3 shows the predicted (NIR) vs. Actual (lab) plots of the free fatty acids and iodine value calibrations.

Figure 1



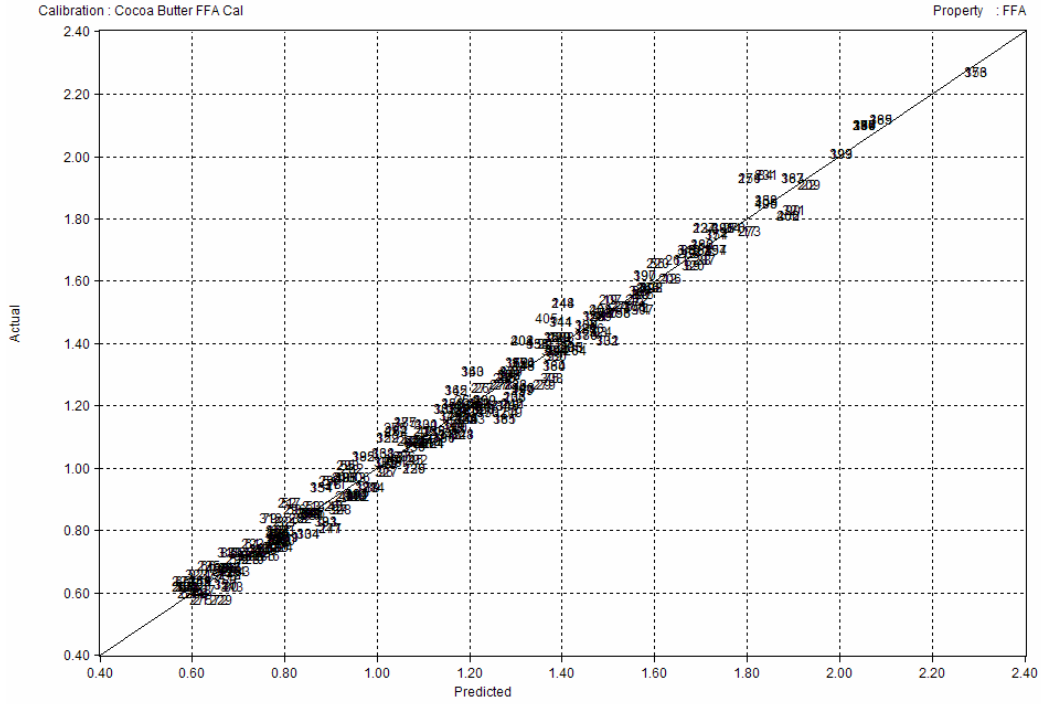
Absorbance vs. Wavelength Plot of Cocoa Butter Calibration Spectra

Figure 2

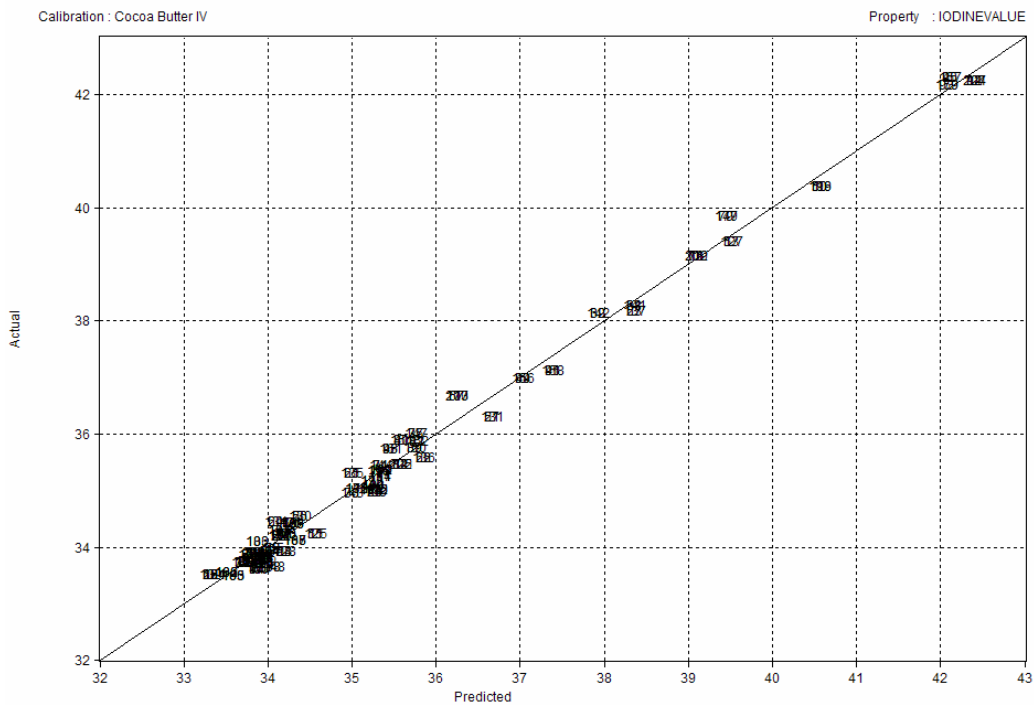


Absorbance, SNV, 1st Derivative Plot of Cocoa Butter Calibration Spectra

Figure 3



Predicted vs. Actual Plot of Cocoa Butter FFA Calibration



Predicted vs. Actual Plot of Cocoa Butter IV Calibration

Conclusion

NIR is an important quality tool used in the 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 cocoa butter for free fatty acids and iodine value. The SpectraStar's optimum wavelength range of 1200-2400nm covers the primary combination bands for C-H, N-H, and O-H bonds. These bonds are critical to accurately analyzing constituents such as moisture, protein, fat, and sugar. Specifically, the primary combination bands found above 1900nm are the most sensitive and generally develop the most accurate calibrations.

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