Application Note: F02

Analysis of Chocolate 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 chocolate is described.

Experimental

Instrumentation

All measurements were performed using a SpectraStar 2400 NIR spectrometer, equipped with a static 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 open cup w/ microscope slide for the SpectraStar 2400. The cup is loaded by pouring liquid chocolate into the well of the cup and covering with a microscope slide. Consistency in sample handling is crucial to accurate NIR measurements.

Sample Preparation

All samples were analyzed at approximately 40-50^0 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 developed contain both milk and dark chocolate from a wide variety of chocolate types. Approximately 560 chocolate samples were used in the development of fat calibrations. Approximately 100 samples were used in the development of the sugar calibration. The following table shows the fat and sugar 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.

Property	Range	Wet Chemistry Method
Fat	21 - 42%	Soxhlet Extraction
Sugar	42 - 58%	HPLC

Typical error of the Soxhlet method is approximately 0.20-0.30%, depending on the operator, equipment, etc. and the error of the HPLC is approximately 0.7-0.85%.

Results and Discussion

Calibration Development

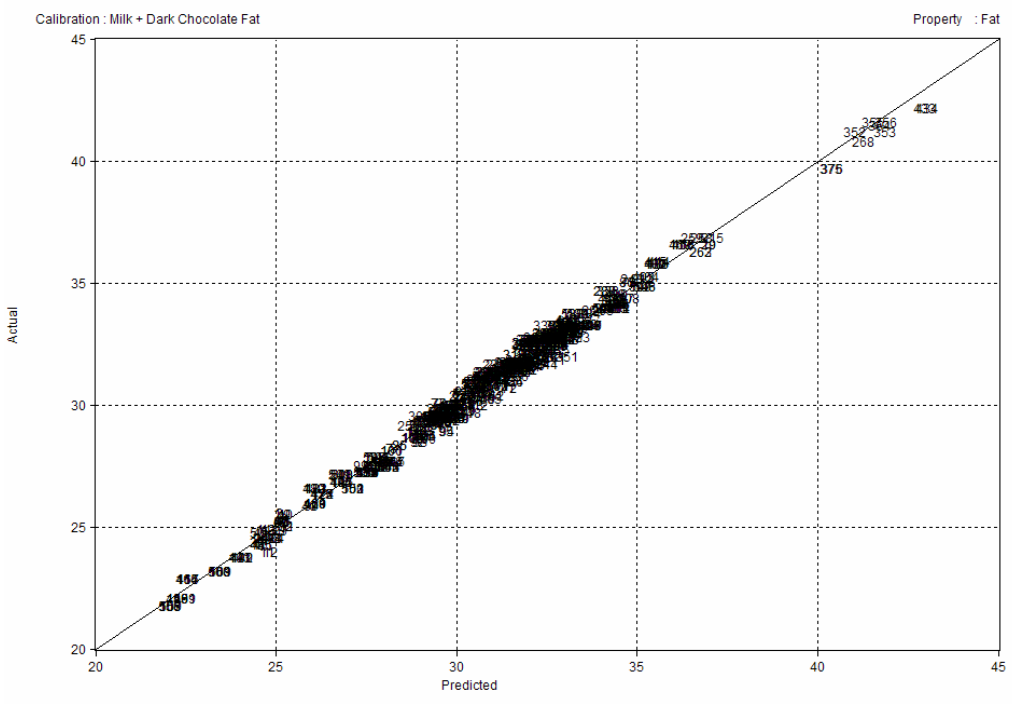
Calibrations were developed for fat, and sugar using the chocolate samples. The following table shows the multiple correlation coefficient and standard error of cross validation for the calibrations. The multiple correlation coefficient is the agreement between the wet chemistry result and the NIR result. 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.

Property	Multiple Correlation Coefficient	Standard Error of Prediction
Fat	0.995	0.343
Sugar	0.981	0.75

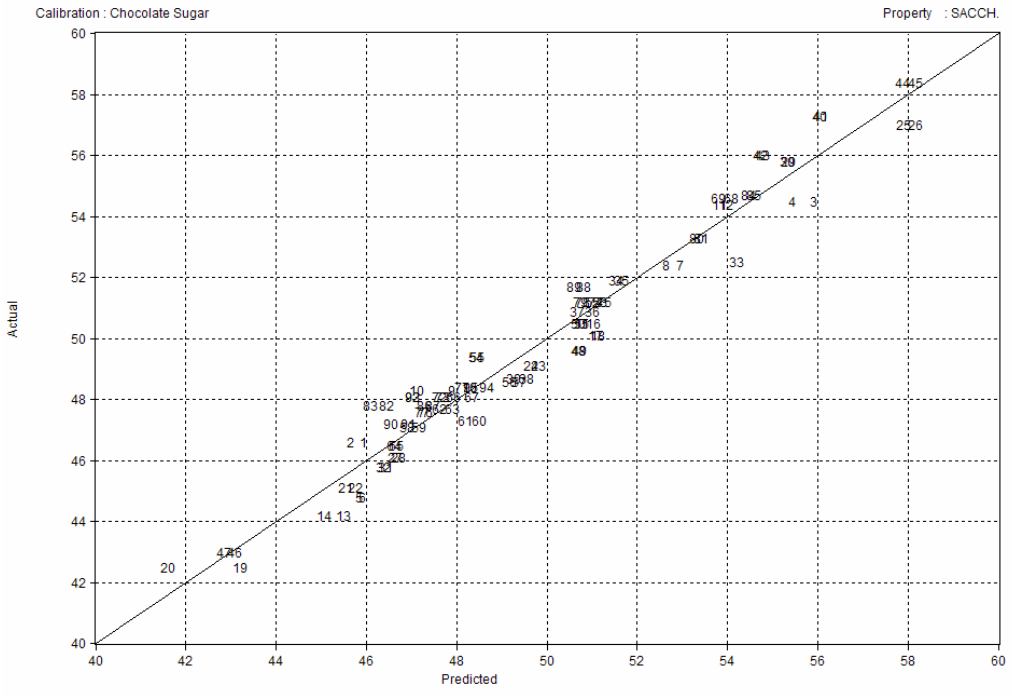
The results above are calculated using global calibrations for a wide variety of milk and dark chocolate. Performance may improve by developing a calibration for specific product types. The way in which NIR calibrations are developed will usually depend on the accuracy needed for the application.

Figure 1 shows the predicted (NIR) vs. Actual (lab) plots of the fat and sugar calibrations.

Figure 1



Predicted vs. Actual Plot of Chocolate Fat Calibration



Predicted vs. Actual Plot of Chocolate Sugar 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 chocolate for fat, and sugar. 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, and fat. Specifically, the primary combination bands found above 1900nm are the most sensitive and generally develop the most accurate calibrations.

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