Application of Near-Infrared Spectroscopy (NIRS) for the Detection of Sucrose and Betaine in Streams Surrounding the Molasses Desugaring by Chromatographic Process (MDC)

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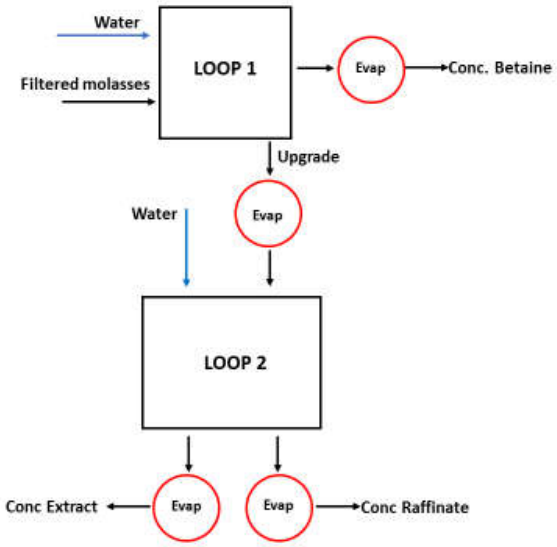
Since the mid-1980s, industrial chromatographic separators have been utilized in U.S. beet sugar factories for molasses desugarization. This technology has proven to be an advantageous extension of the factory to increase sugar yield as well as aid in the recovery of other valuable components such as betaine. A simplified layout of a typical coupled loop separator is shown below in Figure 1. It consists of two units, called loops. Softened and filtered molasses, along with water, are fed into loop1. Two fractions are then produced; the material moving ahead in the profile or the upgrade fraction (sucrose, salts, color and some organics) and the slower moving compounds or the betaine fraction (betaine and amino acids). The upgrade fraction from loop 1 serves as the feed to loop 2. In loop 2, the salts, ionized organic compounds and color (raffinate fraction) are separated from the sucrose (extract fraction). The separator is a very dynamic and complex system and to properly "tune" or optimize its operation, rapid analytical methodology is needed to monitor sucrose, betaine and refractometric dissolved solids (RDS) levels around the various separator streams.

Figure 1. Coupled Loop Molasses Separator



The analytical group at Amalgamated Research, LLC. has developed methodology, based on Near-infrared spectroscopy (NIRS), to accurately predict sucrose, betaine and RDS values across a wide range of concentrations, allowing for effective monitoring of the molasses desugaring separator. NIR spectroscopic techniques offer several advantages to traditional analytical methods. The NIR technique is a rapid method, producing data in 30-60 seconds, rather than an hour, requires zero sample preparation and uses no chemicals or consumables. The predictive performance of this technique will be discussed.

Introduction:

In 1800, Fredrick William Herschel was credited for discovering the region of the near-infrared spectrum, in his work "Experiments on the Refrangibility of the Invisible Rays of the Sun." This discovery was largely ignored for 150 years and was referred to as a "sleeper among spectroscopic techniques." The first reported application, utilizing NIR technology, was in the 1950s, mainly for measurement of moisture in hazardous materials. However, it wasn't until the 1970s that NIR techniques started to rapidly progress. The application of NIR to the food and agricultural industry began with Karl Norris, an engineer at the US Department of Agriculture, who truly recognized the potential of this technique. Norris was able to expand NIR technology into industrial practice by developing instrumentation with improved optics and electronics, coupled with computer algorithms capable of processing complex spectroscopic data. From the 1970s onward, NIRS has gained wide acceptance in many and varied industries. Typical applications include use in the pharmaceutical industry for raw material testing, product quality control and process monitoring, food (dairy, meat, grain, etc.), agrochemical quality control, petrochemical, cosmetics, medical diagnostics (ie. blood sugar and pulse oximetry), etc.

The American Society of Testing and Materials (ASTM) defines the NIR region of the electromagnetic spectrum as the wavelength range from 780 to 2,526 nm. When light energy interacts with a material, energy is absorbed at resonant frequencies associated with the atomic and molecular interactions of the material. The most prominent absorption bands occurring in the NIR region are related to overtones and combinations of fundamental vibrations of -CH, -NH, -OH and -SH functional groups.

There are three parts of an NIR spectral region. When incident NIR radiation strikes a sample, the following three absorbance phenomena take place: Transflectance, Transmission or Reflectance.

Transflectance: The 720 to 1100 nm region is most suited to transflectance through thick samples such as seeds, slurries, liquids and pastes. The absorption bands are due to 3rd overtones of the fundamental stretch bonds in the Mid-infrared (MIR) region.

Transmission: The 1200 to 1850 nm region is used for transmission through liquids and films and samples with high water content. The absorption bands are due to the 1st and 2nd overtones of the fundamental stretch bonds in the MIR region.

Reflectance: The 1850 to 2500 nm region is used for making diffuse reflectance measurements of ground or solid materials. The absorption bands are due to combination bands, such as the -CH stretch and bend combination bands.

Near-infrared spectroscopy (NIRS) is a rapid, nondestructive analytical technique that provides chemical and physical information on virtually any sample matrix. NIRS in combination with chemometrics, opens many options for development of non-invasive, rapid measurement techniques used for both qualitative and quantitative purposes.

Materials and Methods:

Sucrose, Betaine and RDS samples

The samples for this study were collected at The Amalgamated Sugar Company, LLC. Twin Falls and Nampa factories. The eleven separator streams had average sucrose, betaine and RDS concentrations ranging from 0.39 to 60.2, %m/m, 0.4 to 50.0, %m/m and 4.29 to 81.34 %m/m, respectively. Each model contained approximately 130-400 individual data points. Samples were kept refrigerated until analysis commenced. Aliquots of each sample were analyzed via the primary reference methods, gas chromatography (GC), high performance liquid chromatography (HPLC) or refractometry. The remaining sample portions were submitted for NIR scanning. All spectroscopic scans were performed at room temperature (~23 °C). Table 1 lists the separator sample streams selected for this study.

Table 1.

Model Designation	Separator Stream	Factory Location
T1	Virgin Molasses	Twin Falls
T2	Separator Feed Molasses	Twin Falls
T3	Concentrated Betaine	Twin Falls
T4	Dilute Upgrade Molasses	Twin Falls
T5	Dilute Betaine	Twin Falls
T6	Amino Acid Cut	Twin Falls
T7	Concentrated Upgrade Molasses	Twin Falls
T8	Concentrated Extract	Twin Falls
T9	Concentrated Raffinate	Twin Falls
T10	Trains A and B_Dilute Extract	Twin Falls
T11	Trains A and B_Dilute Raffinate	Twin Falls
N1	Virgin Molasses	Nampa
N2	Concentrated Betaine	Nampa
N3	Betaine Molasses	Nampa
N4	BUS_Concentrated Betaine Feed	Nampa
N5	BUS_Upgrade Betaine Dilute	Nampa
N6	BUS_Upgrade Betaine Concentrated	Nampa
N7	BUS_Reject	Nampa
N8	Loop 1 Trains A&B_Separator Feed Molasses	Nampa
N9	Loop 1 Trains A&B_Upgrade Molasses	Nampa
N10	Loop 1 Trains A&B_Betaine Cut	Nampa
N11	Loop 1 Trains A&B_Amino Acid Cut	Nampa

*BUS = Betaine Upgrade Separator

Instrumentation

An Agilent Gas Chromatograph (GC), Waters high performance liquid chromatography (HPLC) system, Rudolph Research Analytical J157 Refractometer and FOSS NIRS DS2500 NIR spectrometer were used to make the measurements for this study. The details of each instrument confuguration are listed below.

Gas Chromatography (GC)

Sucrose levels were quantified using the primary method, gas chromatography. An Agilent 6890N GC system, interfaced with a personal computer, was used to make these measurements.

GC system	
GC	Agilent 6890N (Operating software: OpenLAB CDS_EZChrom Edition)
Detector	Flame Ionization
Autosampler	7683 Series
Column	HP-5, 5% Phenyl 95% dimethylpolysiloxane, 30 m, 0.32mm, 0.25µm, 7 inch cage

Method parameters	
Sucrose std.	Sanding sugar (20 - 140 mg) Converted to its trimethylsilyl ether
Carrier gas	Helium_27 mL/min
Hydrogen to Det	25-30 mL/min
Air to Det	350-375 mL/min
Oven temp.	235℃
Inj/Det Temp.	250℃

High Performance Liquid Chromatography (HPLC)

Betaine levels were quantified using the primary method, high performance liquid chromatography. A Waters High Performance Liquid Chromatography (HPLC) system, interfaced with a personal computer, was used to make these measurements.

HPLC system	
HPLC	Waters (Operating software: Breeze 2)
Detector	Refractive index (RI 2414)
Pump	ISOpump 1515
Autosampler	Autosampler 2707
Column	Aminex HPX-87N (Na+ form), 300 X 7.8 mm, 9 µm particle size, 8% cross linkage, pH range 5-9

Method parameters	
Betaine std.	Betaine BioUltra (≥99.0% (NT))_Supplier: Sigma-Aldrich
Eluent	0.015 M Na_2SO_4
Flow rate	0.7 mL/minute
Column temp.	85℃
Detector Temp.	35℃

Refractometer

RDS levels were measured using the primary method, refractometry. A Rudolph Research Analytical J157 refractometer was used to make these measurements.

Refractometer:	
Refractometer	Rudolph J157
Measurement range	Brix 0 - 100
Accuracy	±0.01 Brix
Optical wavelength	589.3 nm (NaD line)

Near-infrared Spectrometer (NIRS)

NIR spectra were recorded with a FOSS NIRS DS2500 spectrometer, interfaced with a personal computer.

NIRS system:	
NIR Spectrometer	FOSS NIRS DS2500 (Operating software: ISIscan Nova)
Detector(s)	Silicon (400-1,100 nm) and Lead Sulfide (1,100-2,500 nm)
Sample cell	Slurry cup coupled with a gold reflector (Optical pathlength = 0.2 mm)

Method parameters:	
No. scans	32
Resolution (nm)	0.50
Spectrum range (nm)	400 - 2,500
Measurement mode	Transflectance
Final format	Log(1/R)
Optical bandwidth (nm)	8.75
Absorbance range	Up to 2 AU

Spectral data pre-treatments and regression method

The Near-Infrared (NIR) spectral pre-treatments and predictive model development were performed with WinISI (Version 4.7.0.14943). NIR spectra are affected by both physical (matrix inconsistencies) and chemical changes. Physical effects, such as sample particle size variation, can cause the baseline and maximum absorbance to vary. Spectral pre-treatments correct for these physical changes. Several pre-treatments were selected to minimize the standard error of prediction (SEP). The following spectral pre-treatments were applied: Standard Normal Variate (SNV), De-trending (DT), First Derivative and Smoothing.

Quantification models for sucrose, betaine and RDS were developed using Modified Partial Least Squares (MPLS) regression. MPLS is an improvement over the traditional partial least squares (PLS) regression. MPLS is an excellent regression technique for agricultural samples, that have a complex sample matrix, as it copes more effectively with non-analyte interference in multicomponent determinations and provides an estimate of the interference spectrum. The interference spectrum can be used to quantitatively correct the measured spectrum before determination of analyte concentrations; hence the accuracy of the NIRS prediction will be improved.

Results and Discussion;

The Near-Infrared (NIR) spectroscopic techniques allowed for the development of very good calibration models for the prediction of sucrose, betaine and RDS in eleven individual factory streams. Cross-validation (leave-one-out) was applied to all models. Cross-validation is a technique used in modeling to estimate the test error. A number of sample observations, referred to as validation sets, are partitioned from the training data set. After fitting the model on the training data set, its performance is measured against each validation set and averaged.

Two sets of samples were prepared for calibration and prediction. The calibration sets for each model consisted of 130-450 samples analyzed over a year. The validation sets consisted of 30 samples, unique from those used to create the predictive models. The quality of the prediction models was decided by a high correlation coefficient (RSQ), a low error of calibration (SEC), a low bias and a low error of prediction (SEP).

SEC, Bias and SEP were calculated by the following formulae:

$$\text{SEC} = \sqrt{\frac{\sum_{J=1}^{m}(yj - y'j)^2}{m-1-q}} \qquad \text{Bias} = \frac{\sum_{J=1}^{n}(yj - y'j)}{n} \qquad \text{SEP} = \sqrt{\frac{\sum_{J=1}^{n}(yj - y'j)^2}{n}}$$

with yj the concentration given by the primary method, y'j the concentration predicted by the NIRS for the sample j, m the number of samples in the calibration set, n the number of samples in the validation set and q the number of terms in the regression.

Table 2 lists the coefficient of correlation (RSQ), standard error of calibration (SEC) and bias for calibration models in the low to high concentration range (Calibration Set). As Table 2 shows, the predicted sucrose, betaine and RDS concentration correlate (RSQ) very strongly with those levels measured by the primary method (GC, HPLC or Refractometry).

Table 2.

Model	Constituent	Calibration Set				Validation Set	
		Mean (%m/m)	RSQ	SEC	Bias	Mean (%m/m)	SEP
T5	Sucrose	0.45	0.903	0.12	0	0.57	0.09
T4	Sucrose	21.33	0.988	0.23	0.008	22.18	0.19
T8	Sucrose	60.20	0.967	0.45	-0.007	61.02	0.38
N7	Betaine	0.65	0.999	0.02	0	0.57	0.02
N5	Betaine	17.43	0.997	0.24	-0.002	18.11	0.23
N6	Betaine	49.60	0.988	0.48	-0.002	50.26	0.34
T11	RDS	4.29	0.990	0.11	-0.001	4.07	0.09
T7	RDS	28.02	0.982	0.11	0	29.22	0.08
T1	RDS	81.34	0.992	0.30	0.001	81.75	0.25

To assess the accuracy of the NIR technique, samples independent of those used to build the models, were collected for each of the factory streams. Each, of the 30 validation samples collected, were analyzed on either the GC, HPLC or Refractometer to establish a true value. The samples were then scanned on the NIR spectrometer. The developed NIR predictive models were utilized to arrive at an estimated sucrose, betaine or RDS concentration. If a NIR prediction model is not robust, the SEP value increases with regard to the SEC value. This is mainly due to two possible causes: (1) a bias exists between the actual and predicted values of the validation sample set; (2) an additional dispersion of the values of the validation sample set after the bias has been removed. The standard error of prediction (SEP) for the validation sample set is very low and similar to the standard error of calibration (SEC). Clearly the sucrose, betaine and RDS prediction models are fit for use.

Advancements in NIRS make it a preferred measurement technique to efficiently monitor industrial process variation. The acceptance of this technique has been growing mainly because of its quick sample turnaround time, compared to conventional chemical techniques. In the sugar industry, it is likely that in the coming years, NIR spectroscopy will be a universally accepted and a preferred substitute over currently adopted methodology.

ADVANTAGES OF NIR SPECTROSCOPY

- It requires minimal or no sample preparation. There is no need for reagents or sample handling tools. This decreases analytical costs and sample handling time.
- Sample Turnaround Time (TAT) is rapid, almost instantaneous.
- The measurement technique can be designed to measure single or multi-constituents.
- NIRS is a measurement technique geared towards process control. The instrument is very robust, as it has no moving parts.
- NIRS measurements generally have better precision than primary analytical techniques because there is no need for sample preparation. The NIRS measurement accuracy is similar to accepted primary techniques.
- NIR spectrometers are very simple to operate and maintain once the analytical methodology has been developed.

LIMITATIONS OF NIR SPECTROSCOPY

- NIRS is not a primary analytical method. There are no available models that take into account the interaction between NIR energy and matter. Calibration is purely empirical.
- Developing accurate and robust prediction models require a significant investment in human resources. Model development requires using a large number of samples, which encompass all expected variations in the physical (matrix) and chemical properties of the sample.
- This technique is not a trace-method. It can only be applied to major sample constituents. A “Rule of Thumb” is that NIRS can measure organic constituents above 0.1%.

Summary and Conclusions:

The purpose of this work was to research the application of NIR spectroscopy for sucrose, betaine and RDS detection in molasses separator streams. Despite the complex sample matrices, seen in separator samples, the results observed show that each of the primary methods for quantifying sucrose (GC and/or HPLC), betaine (HPLC) and RDS (Refractometry), can be substituted by NIR spectroscopy for the rapid and non-destructive assessment of these constituents, in streams surrounding the coupled loop molasses desugarization separator.

This methodology has not yet been rigorously assessed in a factory setting, but when compared to current separator process control, based on RDS, apparent purity and conductivity measurements, the application of NIRS offers improvement. Due to the rapidity of the technique and the specificity of sucrose and betaine detection, this measurement technique should allow operators to make informed decisions regarding separator adjustments. This real time separator monitoring and tuning should result in higher initial sucrose and betaine yields, resulting in lower factory energy requirements and lower chemical and water usage.

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