NEAR INFRARED (NIR) ANALYSIS IN THE SUGARCANE FACTORY

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ABSTRACT

Near infrared (NIR) analysis has been finding widespread application in beet sugar manufacture, in sugarcane breeding programs, and in analysis of sugar products in Europe, the USA and Australia. Experience with the use of a scanning NIR spectrophotometer in a sugarcane factory laboratory is reported. Previous work has described use of filter instruments; these scanning instruments offer wavelength selectivity, wavelength combinations and accuracy far greater than those previously available. Juice samples (grower samples) were analyzed at different periods during the crop, and in different geographical locations. Results from this very rapid analysis compared favorably with the factory’s pol, Brix and purity analysis. Analysis of bagasse for moisture and sugar is also reported. Applications to analysis of raw sugars for pol, sucrose, invert, ash, color, dextran and starch are also presented and discussed.

INTRODUCTION

Near infrared (NIR) analysis is used for quality control and product evaluation in the grain, cereal, forage and fiber industries. It is finding increasing application in quality control of chemicals, pharmaceuticals and food products, including sugar containing foods and dry mixture (Osborne). The possibilities of near infrared analysis have been studied in several areas of the sugar industry; there are current applications in several areas in both the cane and beet industries. Early studies were made on whole sugarcane, shredded, by Sverzut, who found satisfactory correlation of NIR analyses with standard laboratory analyses for pol, fiber, sugar and moisture. Wood and coworkers in South Africa found NIR analysis for nitrogen in sugarcane leaves to be rapid and accurate (Wood). In studies on analysis of Brix, pol and sucrose in sugarcane juice, however, they concluded that, although NIR was rapid and required no sample clarification, it was not accurate enough for payment purposes (Meyer). Samples were filtered but not otherwise treated. The same group tried direct NIR
analysis of shredded cane for sucrose, dry matter and fiber, but found results not as satisfactory as those for juice analysis. All samples were run in reflection mode, using multiple filter instruments.

A study at the Australian Bureau of Sugar Experiment Stations used a 19-filter instrument, in reflectance mode, on sugarcane juice and whole, fibrated cane (Berding2). The purpose was to develop rapid analyses for Brix, pol, purity, recoverable sugar fiber, moisture and conductivity in cane breeding selection studies. Problems with sample homogeneity in fibrated cane, and temperature of flow-through cell for juice, were encountered, but data were sufficiently promising to expand the study and acquire scanning NIR equipment.

Another Australian study (Ames1) on raw sugar used a 20-filter instrument to measure pol, moisture, ash, reducing sugars in raw sugars. Calibrations were developed in an analytical laboratory and used on-site at a factory. Results were satisfactory for process control, but the unresolved question of bias led the authors to conclude that the analyses could not yet be used for payment. Similar conclusion were drawn in a subsequent paper from the same group (Nguyen6) on further raw sugar studies using a slow scanning instrument or a filter instrument. Studies on molasses and fermentation broths for sugar showed promise. The authors pointed out that error in calibration of raw sugars from a single mill was less than error in an overall laboratory calibration.

A successful application of the NIR technique to analyses for moisture, starch and invert sugar in powdered and agglomerated sugars was reported by Stevens8. The slight increase in error was more than compensated by the time and labor savings over the tedious traditional methods.

It is interesting to observe that in whole shredded sugarcane studies, only the work of Sverzut9 found satisfactory data. Sverzut’s study was performed at the Southern Regional Research Center (site of the senior author’s laboratory) using a scanning instrument that is a forerunner of the scanning instrument used in the study reported herein. Standard laboratory tests on samples in the Sverzut’s study were performed in the senior author’s laboratory.

Studies on application of near infra-red to sugar beet and beet sugar products have, in general, yielded more positive conclusions. Vaccari and Mantovani and co-workers have, in work related in a series of papers (Vaccari et al,10,11,12) established NIR analysis for on-line process control on raw, thin and thick juices in the beet factory. Vaccari et al,12 found, encouragingly, that calibrations set up for one factory could be used for others. A filter instrument (20 filters) in reflectance mode was used. Although Vaccari et al,12 used a smaller number of samples than other workers to set up calibration curves, the standard deviations were comparable to those of laboratory measurements.
In France, Burzawa et al. using a 9-filter near infra-red instrument in reflectance mode, have developed a satisfactory on-line control for Brix, pol and purity in beet factories (Burzawa).3

MATERIALS AND METHODS

Bonds within the sucrose molecule were examined using a saturated sucrose solution and found to display unique absorptions at 2,088 nm and 2,272 nm, with HOH group (water) absorbing at 1,435 nm and 1,940 nm.

An NIRSystems Model 6500 scanning spectrophotometer (NIRSystems, Inc., Silver Spring, MD) was used for the NIR work. This instrument scans from 400 to 2,500 nm, using a silicon detector for wavelengths between 400 and 1,000 nm and a lead sulfide detector between 1,100 and 2,500 nm. All samples were scanned over both spectral ranges. The samples were scanned at an average of 32 scans per minute, and the average analysis time was 30 seconds. NIRSystems “NSAS” NIR software was used for all calibration and prediction work. Calibrations were developed using “conventional” NIR technology procedures, which include selection of optimum wavelengths for log 1/R and first and second derivatives thereof.

Ninety-eight samples of unfiltered sugarcane juice were collected from the Sugar Cane Growers Cooperative of Florida Factory in Belle Glade, Florida. All samples were used to develop calibration equations for Brix, purity values and polarization. An independent set of juice samples were obtained from the USDA Sugarcane Juice Laboratory, Ardoyne Farms, Louisiana, and used to test the validity of the developed equations. This paper will review the concept for developing universal calibrations to predict Brix, pol, and purity in unfiltered cane juice derived from the two different geographical locations in Florida and Louisiana. In addition, the sample presentation method, including calibration development, validation, and statistical interpretation will also be discussed. No sample treatment, filtration, or chemical clarification was required. Unfiltered cane juice, from the normal laboratory growers samples, was pipetted (micropipet with rubber bulb) into a 1 mm quartz cuvette cell and the NIR spectrum recorded from 1,100 to 2,500 nm using NIRSystems Model 6,500 Spectrophotometer. All standard laboratory analyses (for pol, Brix and purity) were run by factory or USDA personnel in their normal manner. Pols were run at the Belle Glade Factory using a Membrex filtration and no chemical treatment on the juice samples. Pols were run at the USDA lab using a calcium hydroxide-aluminum chloride chemical clarification of cane juice.
The absorbance (log 1/T) spectra for three varying concentrations and a saturated sucrose spectrum are shown in Figure 1.

![Figure 1. Cane juice and saturated sucrose.](image)

The absorption bands in the NIR region are quite broad and overlapping, making it difficult to determine the optimum spectral bands for weak absorbing analytes, such as sucrose. By converting the spectra to the second derivative, shown in Figure 2, the overlapping weaker absorbers are separated and the peak intensities of these absorbers are enhanced, providing spectral bands which may be assigned to each absorbing analyte. Note that the peak maxima are converted to minima.

To isolate and identify the sucrose bands, a saturated sucrose solution was scanned and overlaid with cane juice samples of varying concentrations. Three distinct absorption bands were displayed in the combination region of the NIR spectrum, 2090, 2272, and 2380; with 2272 displaying the strongest absorption band, where the long chain C-H atoms absorb. Figure 3 isolates the 2272 band. This band was selected to monitor the sucrose content in the unfiltered cane juice.
FIGURE 2. Cane juice and saturated sucrose.

FIGURE 3. Cane juice and saturated sucrose.
SATISTICAL RESULTS

Polarization

Linear least squares regressions were performed using the second derivative spectra and the reported wet chemical pol values (sucrose) for each sample. The regression results are shown in Table 1. The y-intercept K(0) and slope K(1) are shown along with Multiple R(MR), otherwise known as the correlation coefficient; this parameter explains the amount of variation in a data set. Example: A good correlation may equal -1 or +1 multiplied by 100 = 100% variation. Using second derivative spectra, MR may be negative and provides the same fit of the data.

TABLE 1. Calibration of pol.

<table>
<thead>
<tr>
<th>File name:</th>
<th>FLORIDA Regression Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instrument:</td>
<td>6500 Standard error .185</td>
</tr>
<tr>
<td>Number of spectra:</td>
<td>98 Multiple R = -.9909</td>
</tr>
<tr>
<td>Constituent:</td>
<td>2, POL</td>
</tr>
<tr>
<td>Math:</td>
<td>2nd derivative</td>
</tr>
<tr>
<td>Segment:</td>
<td>20 K(0) = 11.465</td>
</tr>
<tr>
<td>Gap:</td>
<td>0 K(1) = -259.333 2274</td>
</tr>
<tr>
<td></td>
<td>Simple .991</td>
</tr>
</tbody>
</table>

The standard error of the calibration (SEC) is the primary parameter for evaluating the accuracy of the NIR method.

This parameter describes the total residual (Lab-NIR) error in the measurement, and is comparable to the standard deviation of the reference method (standard wet lab results). Emphasis is first placed on evaluating the SEC and secondly, on the multiple R, which is a range dependent variable and does not indicate total performance of a given calibration.

Comparison between the laboratory results vs. NIR calculated percents showed five samples with large residuals greater than two standard deviations of difference; these samples were not deleted but kept in the calibration. These differences may often be attributed to errors in the wet chemical method or in sample presentation to the instrument. Overall calibration results were acceptable and compared favorably with lab results.

A plot of the NIR vs. the laboratory using the theoretical 45° angle line are shown in Figure 4.
Similar studies were performed using refractometric Brix values. Table 2 displays the regression results. A linear summation of two wavelengths, 2276 and 1428, were selected: 2276 corresponding to C-H atoms (sucrose) and 1428 HOH groups for water and used to monitor solids (Brix). A standard error of .212 and a multiple correlation coefficient of .99 was achieved.

**TABLE 2. Calibration of Brix.**

<table>
<thead>
<tr>
<th>File name:</th>
<th>FLORIDA</th>
<th>Instrument: 6500</th>
<th>Standard error</th>
<th>0.212</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of spectra: 97</td>
<td>Regression Results</td>
<td>Multiple R = 0.963</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constituent: 1, BRIX</td>
<td>Math: 2nd derivative</td>
<td>Constants</td>
<td>Wavelength</td>
<td>Simple</td>
</tr>
<tr>
<td>Segment: 20</td>
<td></td>
<td>K(0)</td>
<td>15.684</td>
<td></td>
</tr>
<tr>
<td>Gap: 0</td>
<td>K(1)</td>
<td>-250.232</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>K(2)</td>
<td>-8.994</td>
<td>2276</td>
<td>-0.98</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1400</td>
<td>0.34</td>
</tr>
</tbody>
</table>
A tabular comparison between the lab and NIR data showed seven samples ranging from 18.66 to 20.72 with large residuals. These samples were not deleted. A 45° plot of the NIR calculated versus the lab reported is displayed in Figure 5. All samples were very close to the 45° line, indicating a good fit of the data set.

![Calibration for Brix in unfiltered cane juice](image)

**FIGURE 5. Calibration for Brix in unfiltered cane juice.**

**Purity**

In addition to pol and Brix, a calibration was developed to determine the feasibility of using NIR to predict purity in unfiltered cane. A ratio of two wavelengths were used to develop the linear least squares results; 2304/1782, both peaks absorbing the C-H region to the NIR spectrum. The standard error displayed results of .786 and a multiple R of -.9199. Results are displayed in Table 3. Overall statistical results were acceptable.

Errors are of course larger than those in the pol and Brix analyses because the error in purity is the sum of these in pol and Brix.
TABLE 3. Calibration of purity.

<table>
<thead>
<tr>
<th>File name:</th>
<th>FLORIDA</th>
<th>Regression Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instrument:</td>
<td>6500</td>
<td>Standard error</td>
</tr>
<tr>
<td>Number of spectra:</td>
<td>98</td>
<td>Multiple R = -.9199</td>
</tr>
<tr>
<td>Constituent:</td>
<td>PURITY</td>
<td></td>
</tr>
<tr>
<td>Math:</td>
<td>2nd derivative</td>
<td>Constants</td>
</tr>
<tr>
<td>Segment:</td>
<td>20</td>
<td>K(0) = 8.856</td>
</tr>
<tr>
<td>Gap:</td>
<td>0</td>
<td>K(1) = -47.175</td>
</tr>
</tbody>
</table>

VALIDATION (PREDICTION)

To evaluate the overall performance of each calibration equation, an independent set of lab analyzed samples were used as a validation set; these samples were the set of Louisiana cane juice samples. Table 4 displays a summary of the results.

TABLE 4. Statistical summary of calibration and prediction.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Calibration</th>
<th>Validation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polariometry</td>
<td>185</td>
<td>.99</td>
</tr>
<tr>
<td>Brix</td>
<td>212</td>
<td>.99</td>
</tr>
<tr>
<td>Bias¹</td>
<td>304</td>
<td>991¹</td>
</tr>
<tr>
<td>SEP²</td>
<td>.337</td>
<td>.243⁵</td>
</tr>
<tr>
<td>Simple Correlation⁵</td>
<td>.97</td>
<td>.975</td>
</tr>
</tbody>
</table>

¹ Bias: Average difference between lab and NIR; ² Standard error of performance; ³ Multiple correlation coefficient; ⁴ Standard error of calibration; ⁵ Simple correlation.

The standard error of prediction (SEP) is the primary parameter evaluated. This parameter describes the overall performance of the developed calibration, which describes the standard deviation of the residuals (differences between the primary wet chemical values, minus the NIR predicted).

Polarization

A comparison between the original calibration results, the SEC and MR were compared to the results derived from the SEP and simple C, as shown in Table 4. An increase from .185 SEC to .337 was observed with the validation set. The correlations were comparable. Such an increase is expected since differences in geographical locations of samples, lab personnel, and error from the lab methods will increase the prediction error. A slight bias of .304 was displayed. It should be noted that the lab data were not refined; all data were included, even possible outliers. The different methods for laboratory pol readings in Louisiana and Florida no doubt account for the bias.
Brix

Similar studies were performed for Brix values, comparable values for the SEC and SEP were displayed, with the SEC original value of .212 and a SEP of .243; the correlations were comparable. Deviations were observed with the bias value (Average difference between the lab-NIR). This deviation was 1.00 and once corrected, yielded acceptable results. The purity of the calibration set (Florida samples) is higher than purity of the validation (Louisiana) set; this difference may have caused the bias. Different filtration treatments in Brix analysis in Louisiana and Florida may also have contributed.

SUMMARY

In summary, acceptable and reproducible results were achieved for polarization and Brix with favorable calibration results for purity. By testing each calibration with an independent set of samples derived from a different geographical location, the performance for each constituent as pol and Brix were determined, Pol displayed comparable results, whereas Brix showed a bias. The difference in chemical reference methods in the two locations contributed to bias. Such deviations pointed to changes in crop location and lab, etc.; however, with minor adjustments to the y-intercept, these deviations were eliminated. NIR has demonstrated that pol and Brix may accurately be monitored in unfiltered cane juice. Calibration results for purity were favorable. The analysis requires no sample preparation and is a non-destructive technique which can be placed into a process or lab environment. The elimination of harsh chemicals, such as lead subacetate, is achievable with this technique. This technique is adaptable to flow through cells for routine analysis.

REFERENCES

ANALYSES DANS LE PROCHE INFRA ROUGE (NIR)
EN SUCRERIE DE CANNES

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RESUME

L’analyse dans le proche infra rouge (NIR) est utilisée en bettérave pour la selection des variétés de cannes et dans d’autres domaines sucriers en Europe, aux Etats Unis et en Australie. L’emploi du NIR dans le laboratoire d’une sucrerie de canne à sucre est décrit. Des travaux déjà publiés ont décrit l’utilisation d’instruments à filtres, ces instruments à balayage automatique offrent la selection de longeur d’onde, de combinaisons de longeur d’onde et une précision bien plus grande que précédemment. Des échantillons de jus, (échantillonnage pour les planteurs) furent analysés pendant la campagne et les résultats de cette analyse beaucoup plus rapide ont été très proche des résultats (pol, Brix, pureté) du laboratoire de la sucrerie. On a aussi analysé la bagasse et le sucre. La calibration de l’instrument, la préparation d’échantillon et la fiabilité de la méthode sont discutés. Finalement, on présente des applications en sucrerie, par exemple l’analyse du pol, des réducteurs, des cendres, de la couleur, du dextranase et de l’ amido dans le sucre.
El análisis cerca a infrarrojo (NIR) ha estado encontrando amplias aplicaciones en la fabricación de azúcar de remolacha, en programas de propagación de la caña de azúcar, y en el análisis de productos azucarados en Europa, Estados Unidos y Australia. Experiencias con el uso de un espectfotometro examinador cerca a infrarrojo (NIR) en una fábrica de azúcar son reportadas. Trabajos previos han descrito el uso de instrumentos a base de filtración; estos instrumentos examinadores ofrecen selectividad de hondas, combinaciones de hondas y mayor exactitud que los previamente disponibles. Muestras de jugos de caña fueron analizadas en diferentes ocasiones durante la zafra. Resultados de estos análisis comparan favorablemente con los análisis de polarización, Brix y pureza de la fábrica. Análisis de humedad y sacarosa en bagazo también son reportados. La calibración del instrumento, preparación de la muestra, confiabilidad y reproducibilidad del análisis son discutidos. También son presentadas y discutidas aplicaciones en análisis de azúcar para pol, sacarosa, invertidos, cenizas, color, dextrana y almidón.