

REFEREED PAPER

CAN NEAR INFRARED SPECTROSCOPY (NIRS) BE USED TO PREDICT LACTIC ACID, ACETIC ACID AND ETHANOL IN SUGARCANE MIXED JUICE?

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Abstract

The organic acids, acetic acid and lactic acid, and ethanol have been used as indicators of deteriorated cane. The conventional analytical methods to determine these analytes require sophisticated instrumentation, skilled analysts and are time consuming and costly. The development of NIRS calibrations to measure these analytes in a fraction of the time, using relatively unskilled labour at much reduced cost, is attractive. The paper presents the comparative analytical data for conventional methods of analysis and that obtained by NIRS for the three analytes in mixed juice.

Keywords: organic acids, acetic acid, lactic acid, NIRS, mixed juice

Introduction

The ability of near infrared spectroscopy (NIRS) to give rapid estimations of multiple components in liquids, suspensions and solids with minimal sample preparation by relatively unskilled staff prompted the SMRI to investigate this technique to determine the levels of acetic acid, lactic acid and ethanol in mixed juice. The harmful effects of deteriorated cane on sugar production have been thoroughly documented; hence the importance of early detection of deteriorated cane cannot be over-emphasized. The presence of the organic acids, acetic and lactic acid, and ethanol has been used as an indicator of deteriorated cane. Previous studies of these three analytes pointed out that:

- Lactic acid is not a natural constituent of sugarcane and is not found in juice extracted from freshly harvested cane. McMaster and Ravnö (1977) highlighted that the bulk of the lactic acid found in mixed juice was from incoming cane. It is also evident that substantial amounts of lactic acid can be formed by the bacterial fermentation of sucrose during diffusion.
- Assessments of acidic corrosion of vapour lines and mild steel diffusers warranted routine reliable acetic acid analyses (Purchase *et al.*, 1987; Schäffler, 1988; Cox *et al.*, 1993).
- Post harvest deterioration of burnt cane results in the formation of ethanol, and it was established by Lionnet and Pillay (1987) that this could be a useful indicator.

Mixed juice extracts are not always analysed immediately and thus need to be labelled, preserved by freezing, stored and, in most cases, transported to the SMRI laboratories before analysis by chromatography. The results are thus not available immediately. The ability of NIRS to give rapid estimations of the deterioration products *viz.* acetic acid, lactic acid and ethanol concentrations in mixed juice, with minimal sample preparation by relatively unskilled staff, would allow for rapid identification of deteriorated cane having been processed. Quick analyses of mixed juice could prepare the factory for possible effects on downstream processing. These results could also be correlated with cane consignments from large growers, especially when a spike in deterioration products is observed.

The main objective of this study was to measure the organic acids, acetic acid and lactic acid, and ethanol concentrations in mixed juice and to develop NIR calibrations. These equations together with the comprehensive range of NIRS calibrations available for the analysis of mixed juice and molasses (Simpson and Oxley, 2008) and intermediate products (Simpson and Naidoo, 2010) are part of an ongoing strategy to provide quick, reliable data, useful for process optimization and trouble shooting, and is going to be invaluable to process personnel.

Experimental

Samples

Cane Testing Services (CTS) mixed juice samples representing the weekly crush at each mill were frozen at approximately -20 °C, packed in insulated containers and sent to the SMRI where they were thawed, composited and analysed by the SMRI for factory control and cane payment purposes. A sub-sample of all South African sugar mills' mixed juice, for weeks 7, 18, 21, 22 and 23, of the 2010/11 season was analysed for this investigation. Unfortunately, due to gas chromatography problems, no late season samples were analysed. All results are expressed as mg/kg Brix.

Gas chromatography (GC)

Lactic acid and ethanol were measured on a Perkin Elmer gas chromatograph using a flame ionisation detector, a 2 m long (0.53 mm id) packed glass column (Chromosorb 101 mesh 80/100) at 135 °C for lactic acid and 150 °C for ethanol and a glass liner (with glass wool) in the injector port. Nitrogen was used as the carrier gas at a flow rate of 30 ml/min. An electronic integrator was used to measure the area under the peak.

Lactic acid

A 1000 ppm lactic acid solution was prepared by accurately weighing 0.1067 g of lithium lactate made up in distilled water as described in the SMRI test method TM305 (Anon., 2006). Appropriate dilutions were made to give the required standards, i.e. 100 and 200 ppm. One cubic centimeter of a derivatising solution (Ceric Sulphate 5.5-6.6% w/v $\text{Ce}(\text{SO}_4)_2 \cdot 4\text{H}_2\text{O}$) was added to a 0.5 cm³ portion of mixed juice and 0.5 cm³ distilled water. The solution was heated for 10 minutes at 37 °C in a circulating bath and 3 cm³ of head space gas injected. As this was preliminary work, the mixed juice will in future be diluted within the range of the 100 and 200 ppm standards.

Ethanol

A set of ethanol standards, *viz.* 100, 200 and 500 ppm were injected to determine the relative response factors for ethanol. An injection volume of 1 μL was used for all standards and samples as described in the SMRI test method TM304 (Anon., 2006).

Ion chromatography (IC)

Acetic acid

Acetic acid was measured on a Metrohm 761 ion chromatograph consisting of a pump, injector, suppressor (distilled water and 10 mmol lithium chloride (LiCl)) and conductivity detector, controlled by the Metrohm IC-Net 1.1 software was used for the analyses. Separation of acetic acid was achieved using a Supelcogel C-610H (7.8 x 300 mm) at 75 °C. A 2.00 g portion of the mixed juice samples was prepared by dilution with distilled water to 25 cm^3 . The sample solution, together with the acetic acid standard solutions of 100 and 200 ppm were chromatographed using 0.5 mmol/L perchloric acid eluant, which was pumped at a flow rate of 0.5 ml/min. All solvents and diluted samples were filtered through 0.45 μm membranes.

NIRS

The NIRS system consists of a Bruker Multi-purpose Analyser (MPA) fitted with a Metrohm 838 autosampler. Although no temperature control unit was attached to the MPA, the NIRS laboratory was maintained at 20 °C by air-conditioning at all times. All spectra were obtained in absorbance mode in the scanning range 800 to 2500 nm using a Helma flow-through sample cell with a path length of 1 mm. When not in use, the cell is filled with 3% formaldehyde to ensure that the system remains bacteria free. The NIRS software used for spectral processing and calibration creation was OPUS Version 6. This included Opus Lab, which provided a simple interface with mouse-click operations for controlling automated NIRS analysis.

Ethanol, lactic acid and acetic acid by NIRS

An unfiltered portion of the weekly composite samples of mixed juice ($\pm 200 \text{ cm}^3$) was submitted to the NIRS laboratory and analysed. Each sample was scanned in triplicate for acetic acid, ethanol and lactic acid to obtain a NIRS spectrum using Opus Lab. The predicted results from the three spectra were averaged to give the final predicted NIRS results.

Calibrations

The acetic acid, lactic acid and ethanol calibrations were added to calibrations previously generated for mixed juice (Simpson and Oxley, 2008), using the Bruker *OPUS QUANT* Software. This software uses multivariate data analysis to combine a large amount of spectral information with the corresponding reference values. Partial least squares (PLS) regressions were used to draw up the calibrations. A calibration model was built using 50% calibration samples and 50% of the test samples were used to validate the model. The method of cross validation was used to develop the equations. Spectra will be added to the calibrations during the course of the 2011/12 season to make the models more robust.

Results and Discussion

Ethanol

A regression analysis of the mixed juice ethanol values recorded during the 2010/11 season shows that excellent agreement exists ($R^2=0.97$; $n=45$) between the GC and the NIRS results for the mixed juice samples analyzed. Figure 1 illustrates the calibration equation for the sample range between 2000 and 7000 ppm on Brix. An evaluation of the method precision, depicted in Table 1, showed that the GC ethanol and NIRS analyses are essentially equally precise, with method repeatabilities of 263 ppm and 259 ppm, respectively, and an overall reproducibility of 380 ppm for both methods. Clearly the NIRS method developed for the quantification of ethanol is precise and accurate.

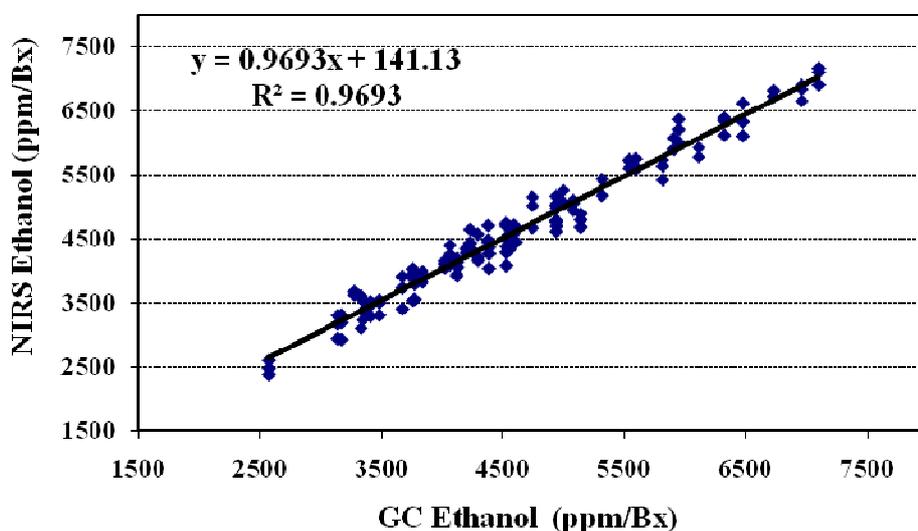


Figure 1. Conventional laboratory gas chromatography (GC) method and near infrared spectroscopy (NIRS) correlation for ethanol in mixed juice.

Acetic acid

The acetic acid results for the 30 mixed juice samples are summarised in Table 1. The calibration equation illustrated in Figure 2 shows an excellent slope and correlation coefficient ($R^2=0.98$; $n=30$). The accuracy of the NIRS predictions will improve as more results are added to the calibration database. For process control, the NIRS estimate should give a reliable trend rather than absolute accuracy. It is obvious from Figure 2 that NIRS can adequately follow the IC trends.

Table 1. Summary of near infrared spectroscopy (NIRS) prediction results for ethanol, acetic acid and lactic acid in sugarcane mixed juice.

Product		Ethanol	Acetic acid	Lactic acid
Sample count		45	30	30
Min (mg/kg)/Brix		2575	254	125
Max (mg/kg)/Brix		7099	1435	992
Range (mg/kg)/Brix		4524	1181	867
Slope		0.969	0.979	0.972
R ²		0.969	0.979	0.982
SEP (%)		108	21	21
Repeatability 95% confidence limits (mg/kg)/Brix	Lab GC/IC	263	75	68
	NIRS	259	75	67
Reproducibility 95% confidence limits (mg/kg)/Brix		380	80	77

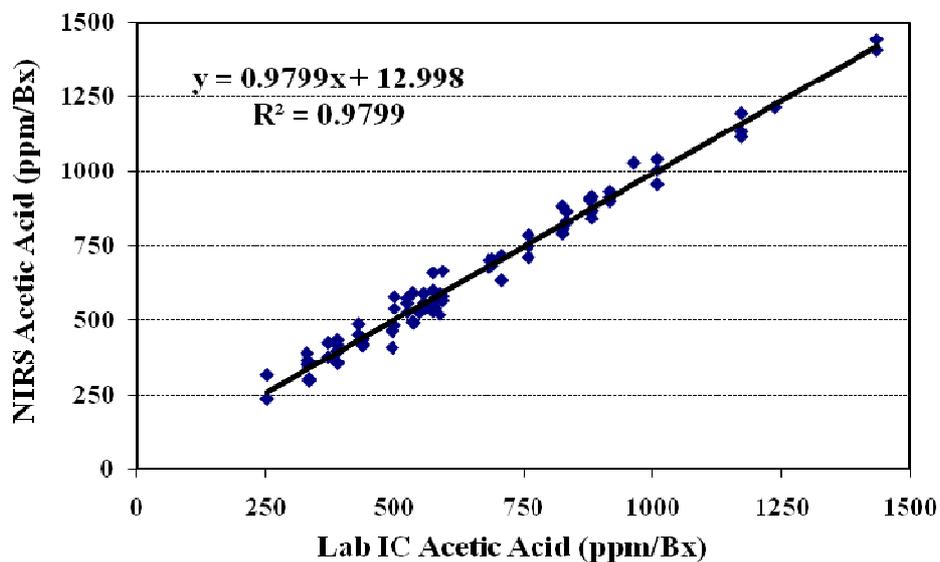


Figure 2. Conventional laboratory ion chromatography (IC) method and near infrared spectroscopy (NIRS) correlation for acetic acid in mixed juice.

Lactic acid

The regression analysis of the mixed juice lactic acid concentrations recorded during the 2010/11 season also shows that excellent agreement exists ($R^2=0.97$; $n=30$) between the GC and the NIRS results for the mixed juice samples analysed. This calibration equation is illustrated in Figure 3. An evaluation of the method precision showed that the GC lactic acid and NIRS analyses are equally precise, with method repeatabilities of 68 ppm and 67 ppm respectively, and an overall reproducibility of 77 ppm for both methods.

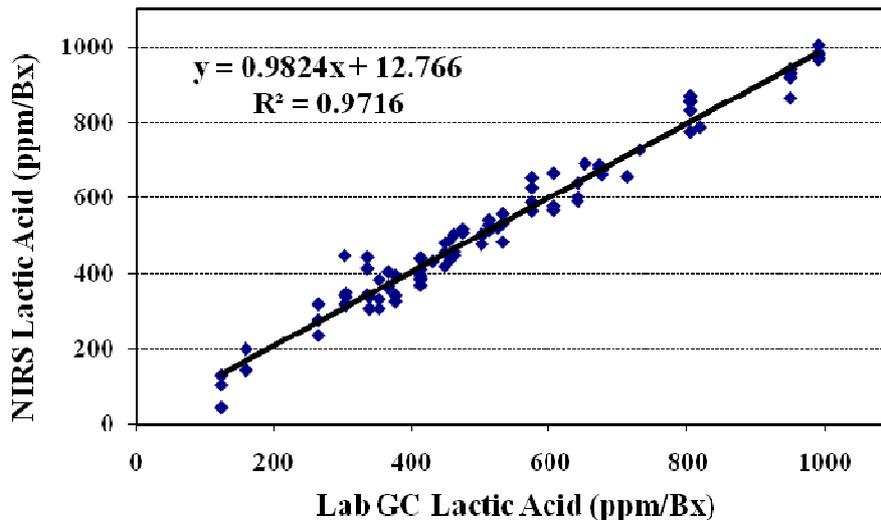


Figure 3. Conventional laboratory method and near infrared (NIRS) correlation for lactic acid in mixed juice.

The overall statistical results of the NIRS predictive capability for ethanol, acetic and lactic acids are shown in Table 1. In general, the agreement between the conventional laboratory methods and the NIRS is very good and in all cases was found to be adequate for cane input/factory control purposes. The accuracy of the NIRS predictions will improve as more results are added to the calibration database.

Future work

Additional samples will be taken to widen the range of analyte values and to improve the robustness and accuracy of the NIRS calibration equations for ethanol, acetic and lactic acids in mixed juice.

Conclusions

The results obtained in this investigation indicate that NIRS is an effective and precise method to quantify ethanol, acetic acid and lactic acid in sugar cane mixed juice. The NIRS method of analysis holds considerable promise as a more economical and rapid alternative to the more sophisticated chromatographic analyses. The method could be the basis of the

development of a tool to measure these analytes as potential indicators of deteriorated cane at line or at a consignment level.

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