

## USING NEAR INFRA RED SPECTROSCOPY FOR RAPID QUANTIFICATION OF INTERMEDIATE SUGAR FACTORY PRODUCTS

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### Abstract

The Sugar Milling Research Institute analytical laboratory has been exploring the feasibility of using NIR spectroscopy as a processing tool for intermediate products, following the successful implementation of this novel application for the analysis of C-molasses for both factory control and as part of cane payment during the 2009/2010 South African crushing season. NIRS is an attractive method for process engineers, as rapid multi-component analysis of intermediate products may be possible. Sample sets of approximately 300 clear juices, syrups, A-, B- and C-masseccutes and A- and B-molasses have been analysed by standard reference methodologies for sucrose, fructose, glucose, pol and Brix, and the results used to develop NIRS calibrations for rapid multi-component analysis. The correlation coefficients achieved for most of the intermediate products for most analytes were greater than 0.9. The data prove that NIRS is capable of providing an analytical prediction tool suitable for process control. The method, compared with conventional analysis, would allow for a larger number of analyses to be conducted within a relatively short period of time, thereby enabling faster process control decision-making.

*Keywords:* NIRS, molasses, masseccutes, syrup, clear juice, calibration

### Introduction

In South Africa both mill and cane testing laboratories have, in recent years, strategically recognised the need to improve the cost effectiveness of their laboratories in order to remain globally competitive. The downsizing of their operations has put pressure on the remaining testers with an increased likelihood of analytical errors occurring. The ability of 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 with a view to implementation in the Sugar Milling Research Institute (SMRI), mill and cane testing laboratories. Calibrations for mixed juice and molasses were reported by Schaffler and De Gaye (1997). This work was conducted using a NIRS systems 5000 spectrophotometer and the recommendation was that an instrument be installed in one of the South African mills for the 1997-1998 season. Loss of wavelength accuracy and bias adjustment were necessary on lamp failure with this instrument and precluded its entry into the mill laboratories. Calibrations for mixed juice and final molasses were subsequently developed by Simpson and Oxley (2008) using a Bruker MPA spectrophotometer, and culminated in the acceptance of the NIRS technique for cane

payment for molasses. Schaffler and De Gaye (1997) noted at the time that, "if stable calibrations could be developed for these two streams, then training of NIR models for the intermediates should also be achievable." The possibility of providing the process manager with a bouquet of calibrations from front-end to back-end of the raw sugar factory was an extremely attractive idea. The more sophisticated reference techniques to determine fructose, glucose, true sucrose and dry solids are virtually impossible to perform reliably in mill laboratories. The availability of these results in a fraction of the time by NIRS would represent a significant step change in the quality and the speed of information available from the mill laboratory in order to make informed decisions concerning factory performance. The calibrations developed in the present study coupled with the calibrations available for mixed juice and molasses from the previous study by Simpson and Oxley (2008) are believed to provide the most comprehensive range of NIRS calibrations available for the analysis of intermediate products in raw sugar production.

## Experimental

### *Instrumental*

*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 Hellma 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.

*Polarimeter:* The reference (or laboratory) pol was measured on a Schmidt & Haensch Universal Polartronic at 589 nm. Samples were clarified using the standard lead clarification method.

*Refractometer:* The reference (or laboratory) Brix was measured by refractometry using the Bellingham and Stanley RFM 500 refractometer.

*Gas chromatography (GC):* Fructose, glucose and sucrose for clear juice and syrup were determined on a Varian 3900.

*High Performance Anion Exchange Chromatography (HPAEC):* Molasses and massecuite fructose, glucose and sucrose were determined on the Perkin Elmer 200 series, using a PE200 pump, PE 200 autosampler and a Dionex II pulsed amperometric detector connected to Peak Simple chromatography software for data acquisition.

### *Samples*

Weekly composite samples of clear juice, syrup, A-, B- and C-massecuite and A- and B-molasses from the previous three seasons, i.e. 2007-2008, 2008-2009 and 2009-2010 were sent to the SMRI, on a weekly basis, from five different geographically located Southern African sugar factories, viz. Malalane (ML), Felixton (FX), Noodsberg (NB), Nakambala (NK) and Umzimkulu (UK). These samples were analysed during the season.

*Clear juice and syrup sample preparation*

**Clear juice:** An unfiltered portion of the clear juice composite ( $\pm 200 \text{ cm}^3$ ) was submitted to the NIRS laboratory and analysed within two hours of initial thawing. A 150 g portion was lead clarified for pol measurement and the remaining 50 g was filtered for Brix, while a 2 g Brix filtrate portion was analysed by GC for sucrose, fructose and glucose.

**Syrup:** A 60 g portion was diluted to  $240 \text{ cm}^3$  and lead clarified for pol measurement and 50 g was filtered for Brix. A 5 g portion of syrup was diluted to 35 g and analysed by GC for sucrose, fructose and glucose. Syrup samples were diluted at 20 g to  $100 \text{ cm}^3$  for NIRS analyses. Each sample was analysed in triplicate and a NIRS spectrum for each obtained using Opus Lab. The predicted results from the three spectra were averaged to give the final predicted NIRS results.

**C-massecuite and A-, B-molasses sample preparation** Weekly composite samples of A-, B- and C-massecuite and A- and B-molasses were submitted to the SMRI. The samples were manually homogenised and sub-sampled. A single sub-sample was used for all test methods and the NIRS sample preparation. The samples were clarified and filtered for pol and brix analyses. Massecuite and molasses samples were diluted as shown in Table 1 and analysed for fructose, glucose and sucrose by HPAEC. A-massecuite was diluted at 15 g to  $100 \text{ cm}^3$  while the B-massecuite was diluted at 14.5 g to  $100 \text{ cm}^3$  and C-massecuite was diluted at 14 g to  $100 \text{ cm}^3$ . A- and B-molasses were diluted at 18 g and 17.5 g to  $100 \text{ cm}^3$ . Each prepared sample was analysed in triplicate and a NIRS spectrum was obtained for each. The predicted results from the three spectra were averaged to give the final predicted NIRS results.

A quality control procedure was set up using three molasses control samples of established composition by conventional laboratory methods and NIRS and run with each batch of samples tested. These were used to monitor the NIRS performance on a weekly basis. All laboratory results were generated using approved methods from the SASTA Laboratory manual (Anon, 2005) and SMRI test methods (SMRI TM300 and TM301). Table 1 summarises the dilutions for intermediate product analyses.

**Table 1. Summary of dilutions for intermediate product analyses**

| Product      | Pol and Brix               | Fructose, glucose and sucrose (GC) | Fructose, glucose and sucrose (HPAEC) | NIRS                         |
|--------------|----------------------------|------------------------------------|---------------------------------------|------------------------------|
| Clear juice  | $150 \text{ cm}^3$         | $2 \text{ cm}^3$ (undiluted)       | -                                     | undiluted                    |
| Syrup        | 60 g to $240 \text{ cm}^3$ | 5 g to 35 g                        | -                                     | 20 g to $100 \text{ cm}^3$   |
| A-massecuite | 50 g to $250 \text{ cm}^3$ | -                                  | 3 g to $100 \text{ cm}^3$             | 15 g to $100 \text{ cm}^3$   |
| B-massecuite | 50 g to $250 \text{ cm}^3$ | -                                  | 3 g to $100 \text{ cm}^3$             | 14.5 g to $100 \text{ cm}^3$ |
| C-massecuite | 50 g to $250 \text{ cm}^3$ | -                                  | 3 g to $100 \text{ cm}^3$             | 14 g to $100 \text{ cm}^3$   |
| A-molasses   | 50 g to $250 \text{ cm}^3$ | -                                  | 0.6 g to $100 \text{ cm}^3$           | 18 g to $100 \text{ cm}^3$   |
| B-molasses   | 50 g to $250 \text{ cm}^3$ | -                                  | 0.8 g to $100 \text{ cm}^3$           | 17 g to $100 \text{ cm}^3$   |

### Calibrations

Calibrations previously generated for C-molasses were used to develop the intermediate product calibrations 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. Where the number of samples was less than 300, the method of cross validation was used to develop the equations. Spectra were added to the initial calibrations during the course of the seasons to make the models more robust.

## Results

### Clear juice

The statistical results for over 300 clear juice samples presented in Table 2 show excellent slope, correlation coefficient (RSQ) and standard error of predictions (SEP) between laboratory and NIRS results for all analytes. This calibration is a good example of the precision of the NIRS, especially where the sample solutions have minimal impurities. Clear juice samples require no sample preparation when scanned on the NIRS hence fundamental analytical errors are eliminated.

**Table 2. Summary of NIRS predictions for clear juice.**

| Clear juice  | Slope | RSQ  | SEP (%) | 95% confidence limits (%) |             |
|--------------|-------|------|---------|---------------------------|-------------|
|              |       |      |         | Laboratory method         | NIRS method |
| Pol (°Z)     | 1.00  | 1.00 | 0.02    | ±0.33                     | ±0.33       |
| Brix (°Bx)   | 1.00  | 1.00 | 0.01    | ±0.12                     | ±0.12       |
| Fructose (%) | 1.00  | 1.00 | 0.01    | ±0.09                     | ±0.09       |
| Glucose (%)  | 0.96  | 0.96 | 0.01    | ±0.08                     | ±0.07       |
| Sucrose (%)  | 1.00  | 1.00 | 0.07    | ±0.59                     | ±0.58       |

SEP = standard error of prediction, RSQ = correlation coefficient squared

### Syrup

The syrup samples also achieved statistical results similar to that of clear juice. The exceptional agreements shown in Table 3 are largely due to the high quality of laboratory data.

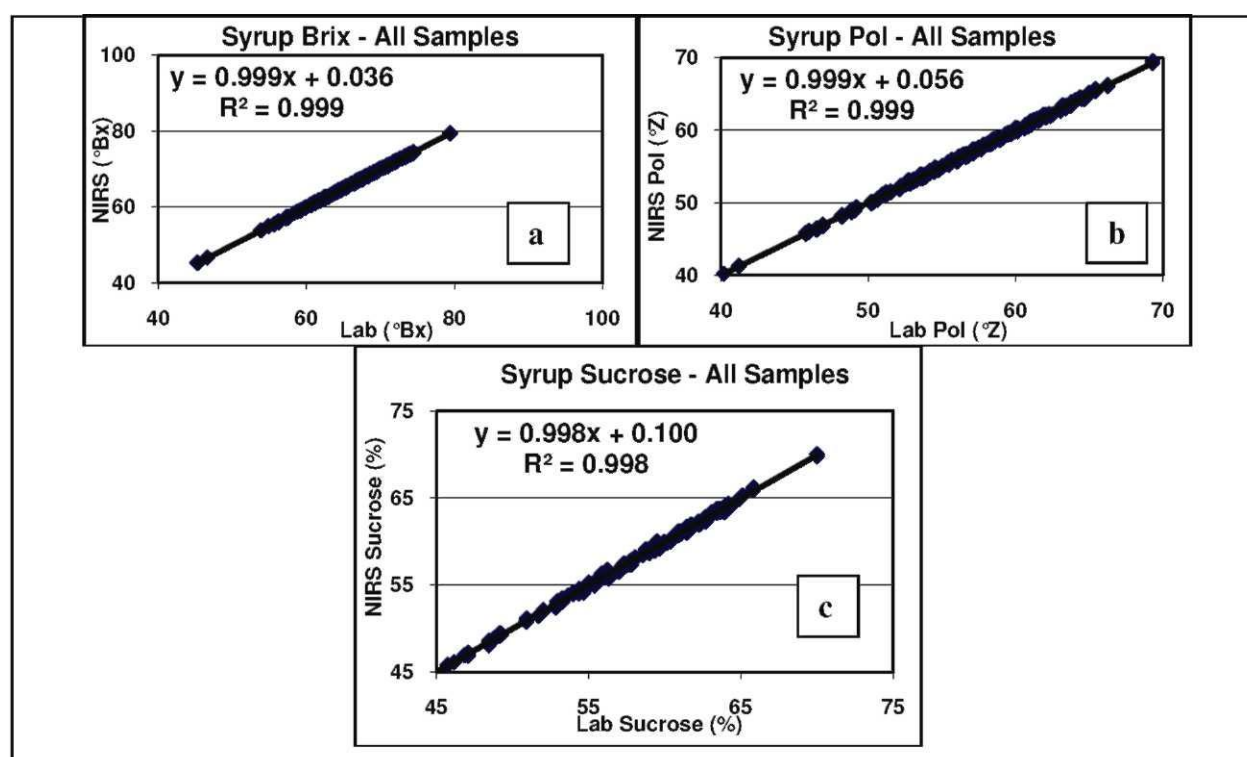
The prediction data for syrup gave very good precision results, as shown by the SEPs of better than 0.13 units. The RSQ of 1.00 achieved for pol, Brix and sucrose were excellent.

Figure 1 shows graphically the comparison between the laboratory data and NIRS predicted data for syrup pol, Brix and sucrose (SEPs of 0.09%, 0.07% and 0.13%, respectively).

**Table 3. Summary of NIRS predictions for syrup**

| Syrup        | Slope | RSQ  | SEP (%) | 95% confidence limits (%) |             |
|--------------|-------|------|---------|---------------------------|-------------|
|              |       |      |         | Laboratory method         | NIRS method |
| Pol (°Z)     | 1.00  | 1.00 | 0.09    | ±0.50                     | ±0.50       |
| Brix (°Bx)   | 1.00  | 1.00 | 0.07    | ±0.52                     | ±0.52       |
| Fructose (%) | 0.94  | 0.94 | 0.03    | ±0.02                     | ±0.02       |
| Glucose (%)  | 0.94  | 0.94 | 0.04    | ±0.03                     | ±0.03       |
| Sucrose (%)  | 1.00  | 1.00 | 0.13    | ±0.60                     | ±0.60       |

SEP = standard error of prediction, RSQ = correlation coefficient squared



**Figure 1. Conventional laboratory method and NIRS correlation for syrup (a) Brix, (b) pol and (c) sucrose.**

*A-, B- and C-massecuite*

The prediction data for A-, B- and C-massecuite presented in Table 4 shows the correlation between laboratory and NIRS results for pol, Brix, fructose, glucose and sucrose. The massecuities showed good overall predictions (SEP 0.02% to 0.68%). With the exception of A-massecuite glucose and C-massecuite fructose, the massecuities illustrated excellent slope and correlation

coefficient (RSQ) statistics for all components. RSQ results were acceptable (0.91 to 1.00). Comparing the linearity and SEP to the precision of the laboratory method is a measure of the NIRS predictive capability. In all cases it was found to be adequate for factory control purposes. Although the SEP for A- and C-masseccuite glucose and fructose is acceptable at 0.15 and 0.19 respectively, additional samples could to be taken to widen the range of analyte values and to improve the correlation between NIRS and conventional laboratory testing.

**Table 4. Summary of NIRS prediction results for A-, B- and C-masseccuites.**

| Product              | Slope       | RSQ         | SEP (%) | 95% confidence limits (%) |              |
|----------------------|-------------|-------------|---------|---------------------------|--------------|
|                      |             |             |         | Laboratory method         | NIRS method  |
| <b>A-masseccuite</b> |             |             |         |                           |              |
| <b>Pol (°Z)</b>      | 0.96        | 0.96        | 0.27    | ±0.26                     | ±0.26        |
| <b>Brix (°Bx)</b>    | 0.97        | 0.96        | 0.10    | ±0.10                     | ±0.09        |
| <b>Fructose (%)</b>  | 0.91        | 0.91        | 0.09    | ±0.06                     | ±0.06        |
| <b>Glucose (%)</b>   | <b>0.80</b> | <b>0.80</b> | 0.15    | <b>±0.11</b>              | <b>±0.10</b> |
| <b>Sucrose (%)</b>   | 0.93        | 0.93        | 0.68    | ±0.54                     | ±0.52        |
| <b>B-masseccuite</b> |             |             |         |                           |              |
| <b>Pol (°Z)</b>      | 0.99        | 1.00        | 0.13    | ±0.33                     | ±0.33        |
| <b>Brix (°Bx)</b>    | 0.99        | 0.99        | 0.08    | ±0.12                     | ±0.12        |
| <b>Fructose (%)</b>  | 0.94        | 0.94        | 0.07    | ±0.09                     | ±0.09        |
| <b>Glucose (%)</b>   | 0.95        | 0.95        | 0.08    | <b>±0.08</b>              | <b>±0.07</b> |
| <b>Sucrose (%)</b>   | 0.95        | 0.95        | 0.56    | ±0.59                     | ±0.58        |
| <b>C-masseccuite</b> |             |             |         |                           |              |
| <b>Pol (°Z)</b>      | 0.99        | 0.99        | 0.02    | ±0.28                     | ±0.28        |
| <b>Brix (°Bx)</b>    | 0.93        | 0.93        | 0.15    | ±0.10                     | ±0.10        |
| <b>Fructose (%)</b>  | <b>0.76</b> | <b>0.76</b> | 0.19    | <b>±0.09</b>              | <b>±0.08</b> |
| <b>Glucose (%)</b>   | 0.95        | 0.95        | 0.11    | ±0.12                     | ±0.12        |
| <b>Sucrose (%)</b>   | 0.97        | 0.97        | 0.29    | ±0.41                     | ±0.41        |

#### *A- and B-molasses*

Table 5 presents the predictions for A- and B-molasses (RSQ 0.93 to 1.00). The high SEP of 1.55 for B-molasses pol is attributed to analytical errors in laboratory analyses associated with sample inhomogeneity as some B-molasses samples received contained crystals. The 95% confidence limits achieved by NIRS for B-molasses pol was better (±0.94) than that of the laboratory (±0.97).

**Table 5: Summary of NIRS prediction results of A- and B- molasses**

| Product             | Slope | RSQ  | SEP (%) | 95% confidence limits (%) |              |
|---------------------|-------|------|---------|---------------------------|--------------|
|                     |       |      |         | Laboratory method         | NIRS method  |
| <b>A-molasses</b>   |       |      |         |                           |              |
| <b>Pol (°Z)</b>     | 1.00  | 1.00 | 0.13    | ±0.37                     | ±0.37        |
| <b>Brix (°Bx)</b>   | 0.99  | 0.99 | 0.09    | ±0.18                     | ±0.18        |
| <b>Fructose (%)</b> | 0.99  | 0.99 | 0.03    | ±0.11                     | ±0.11        |
| <b>Glucose (%)</b>  | 0.99  | 0.99 | 0.02    | ±0.09                     | ±0.09        |
| <b>Sucrose (%)</b>  | 0.98  | 0.98 | 0.33    | <b>±0.70</b>              | <b>±0.69</b> |
| <b>B-molasses</b>   |       |      |         |                           |              |
| <b>Pol (°Z)</b>     | 0.93  | 0.93 | 1.55    | <b>±0.97</b>              | <b>±0.94</b> |
| <b>Brix (°Bx)</b>   | 1.00  | 1.00 | 0.09    | ±0.48                     | ±0.48        |
| <b>Fructose (%)</b> | 0.98  | 0.98 | 0.05    | ±0.10                     | ±0.10        |
| <b>Glucose (%)</b>  | 0.98  | 0.98 | 0.08    | ±0.15                     | ±0.15        |
| <b>Sucrose (%)</b>  | 0.99  | 0.99 | 0.29    | ±0.79                     | ±0.79        |

## Discussion

### *The benefits to the mill of the NIRS system*

- Figure 2 is a schematic of a sugar process flow showing areas where rapid NIRS predictions could be used. All products shown with a tick indicate where an NIRS multi-component calibration could be used to predicate product values. Comparison across unit operations could be used for rapid purity profiles, inversion losses, exhaustions and purity rise predictions.
- Work reported by Simpson and Oxley (2008) allow for mixed juice pol, Brix, conductivity ash, sucrose, fructose, and glucose predictions with no sample preparation. Cross validation of the current mixed juice calibration would enable first expressed juice to be quantified.
- Sucrose, pol and Brix are important analytes for factory control. Although fructose and glucose are two minor constituents of juices and syrup, process staff regularly examine fructose: glucose ratios before and after evaporators. Acquiring these results in minutes can quickly reveal potential inversion problems across evaporators.
- On-mill massecuite analyses for exhaustion calculations would be rapid. This would allow rapid monitoring of pans, crystallizers and centrifugals, enabling process managers in a mill to make adjustments accordingly, rather than having to wait for the weekly figures.

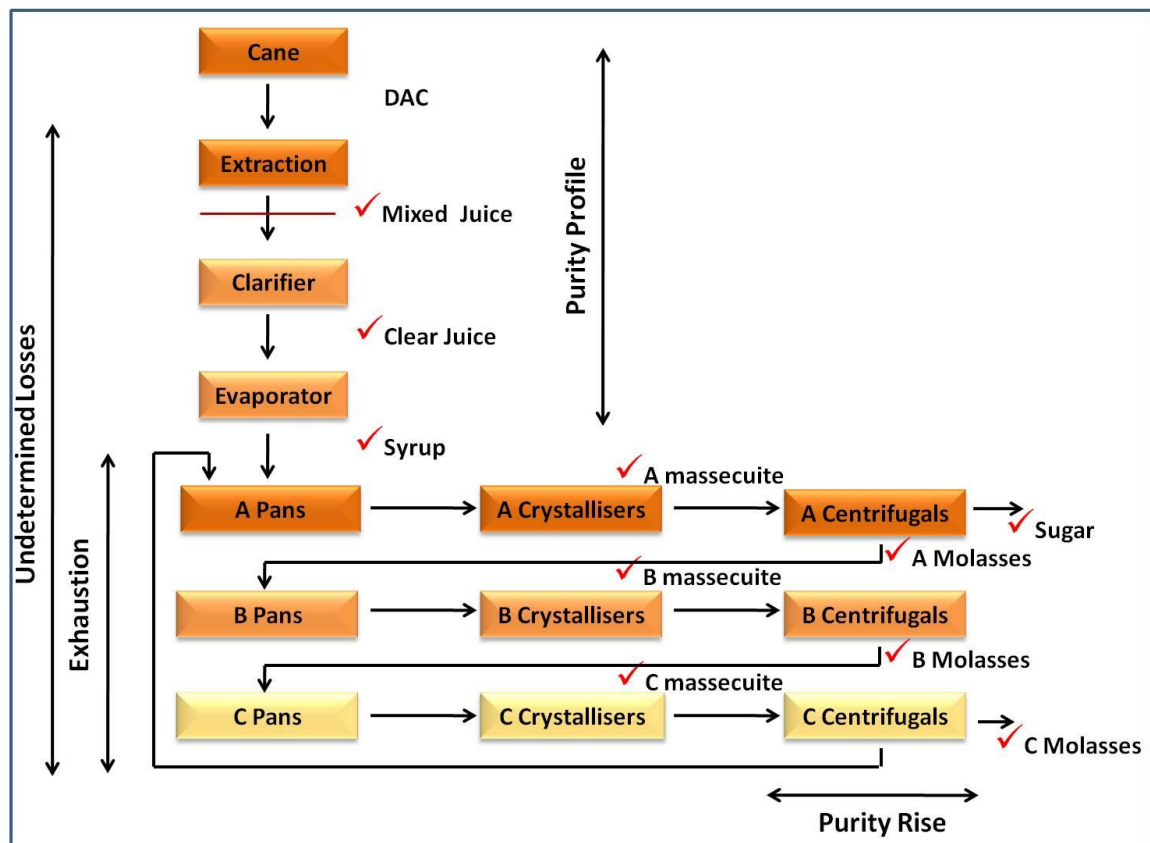


Figure 2. Schematic of a sugar process flow showing areas for rapid NIRS predictions.

- The NIRS analysis of molasses has been adopted for cane payment purposes by the South African sugar industry following the work reported by Simpson and Oxley (2008). The availability of the mixed juice and final molasses calibrations would allow process staff to frequently examine monosaccharide changes between mixed juice and final molasses as part of the undetermined loss programme. For example, if a factory is experiencing a 2.5% undetermined loss, their objective would be to achieve the benchmark of 2%. By conducting the analyses mentioned above, they could identify sources of loss. For a 300 tons cane per hour (tph) factory, reducing undetermined losses by 0.5% would result in savings as follows:
  - o 300 tph  $\approx$  30 t sugar per hour
  - o 0.5% of 30 t = 0.15 t
  - o Assume 90% of saving is sugar
  - o Sugar saved = 0.135 t sugar per hour = 3.24 t sugar per day
  - o At R3000 per tonne this equates to **savings of R9720 per day.**

- Tracking glucose and fructose values across the boiling house could also reveal the occurrence of Maillard reaction, i.e. where glucose is being destroyed.
- With the development of sucrose calibrations, factories could convert from pol to sucrose based performance/payment calculations without costly and complex GC or HPAEC analytical requirements. It would also allow benchmarking based on sucrose for the entire region and not only the South African mills.
- The SMRI analytical laboratory has experienced significant improvement in operational efficiencies following the implementation of routine molasses analysis by NIRS in the 2009/2010 season, and development of calibrations for intermediate products will certainly add to the laboratory efficiency. With a complete set of intermediate product calibrations, factory process managers will benefit immensely from reliable data produced in a fraction of the time taken for conventional analysis.

### **Conclusions**

NIRS calibrations are now available for the complete range of raw sugar factory products. The calibrations have been developed over several seasons and variations due to geographic, varietal and other effects were included in the prediction sets. This strategy has resulted in prediction results that are free generally from bias and with low SEP. Improvement in chemometric software (OPUS) has enabled robust, reliable calibration developments which retain their accuracy on lamp failure.

It is always to be borne in mind that NIRS is a secondary technique and calibration is totally dependent on the quality of the laboratory results. The SMRI has an accredited laboratory and the laboratory information obtained for this investigation was of excellent quality. The integrity of the calibrations installed with the NIRS instrumentation will be protected and, where necessary, localization of the calibrations will be performed by the SMRI. This will ensure the ongoing accuracy of the predictions. The benefits to the mills as outlined suggest that this analytical tool will be incorporated into the mill laboratories within the Southern African Development Community (SADC) countries and, where successful, will offer these mills a competitive advantage.

### **Acknowledgements**

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