1. Scope

This analysis technique covers the determination of brix, pol and sucrose in composite mixed juice samples.

2. Field of Application

The analysis method is applicable to composite mixed juice samples from South African and Southern African sugar mills. The quantifiable component concentration ranges for the analytes is shown in Table 1.

Table 1

Analyte	Range
Brix	5-19 °Bx
Pol	3-16 °Z
Sucrose	3-16 %

3. Definitions

3.1 **NIRS** – Near-infrared spectroscopy. A spectroscopic analysis method that uses the near-infrared region of the electromagnetic spectrum (from about 800 nm to 2500 nm).

3.2 **Spectrum** – a plot of the transmittance or absorption of radiation passed through a solution against the wavelength of the radiation.

3.3 **Chemometrics -** the application of mathematical, statistical, graphical or symbolic methods to maximise the information which can be extracted from data (in this case near-infrared spectra)

4. Principle

The individual NIRS transmission spectra recorded for a wide range of composite mixed juice samples (concentration, geographic location, seasonal and extraction technologies) have been correlated with the respective brix, pol and sucrose results as determined by recognised laboratory methods. Chemometric analysis was used to statistically correlate the spectral and analytical results and develop suitable prediction equations. These equations are used on subsequent samples to predict the results for any or all of these parameters without the need for conventional analytical analysis.

5. Reagents

5.1 Filter paper: Postslip medium white or Whatman No. 6

5.2 Filter Aid: Filtercel celite

6. Apparatus

6.1 Near-infrared spectrometer – a Bruker Multi Purpose Analyser (MPA) is used in transmission mode using a Hellma QX flow-through sample cell (1 mm pathlength).

6.2 Computer and software – OPUS software version 6.0 or higher (provided with the MPA by Bruker) is used for spectral interpretation. OpusLab (a subset of OPUS) provides the operating interface for sample analysis.

6.3 Sample introduction

6.3.1 Manual - a 10mL syringe can be used to manually flush the filtered sample through the sample flow cell for a single analysis.

6.3.2 Autosampler (optional) – a Metrohm 838 autosampler consisting of an automated sampling tube, peristaltic pump and 127 sample carousel for holding 11 mL vials. The autosampler is controlled by OPUS/OpusLab. The autosampler allows consecutive spectra collection from up to 127 solutions without the need for an operator.

6.4 Vials – 11mL plastic vials (optional - for use with manual sample introduction).

6.5 Test tube rack - to hold samples for manual injection.

6.6 Autofilt - pressurised filtration unit for filtration of composite mixed juice samples.

6.7 Hellma flow-through cell – SMRI matched and approved cell with 1 mm path length.

6.8 3% Formaldehyde solution - to fill the cell flow path when not in use for more than 3 hours.

6.9 2% Hellmanex® solution - to clean the flow-through cell and tubing

7. Procedure

The preparation and analysis should be carried out in a temperature controlled room at 20°C.

7.1 Composite mixed juice preparation

The composite mixed juice sample is prepared according to the standard method.

7.2 Sample Filtration

The sample contains suspended matter which could affect the prediction. The suspended matter is removed by pressure filtration. Add 6g Filtercel celite filter aid to 200 cm^3 of the sample in a 250 cm^3 Schott bottle. Shake until the filter aid is completely dispersed. Prepare the Autofilt with the filterpaper. Pour the mixture in and filter, discarding the first 50 cm^3 and collecting about 100 cm^3 of the rest of the filtrate that is clear of foam. In the event that the Autofilt is unusable, gravity filtration using the conventional method may be used.

7.3 Sample analysis.

7.3.1 Manual sample introduction.

A background spectrum is required at the start of a sequence of samples. Remove the sample flow cell from the MPA and record the background spectrum. Replace the cell and continue with sample measurements. Use a 10 mL plastic syringe to flush the sample flow cell with approximately 10 mL of the sample filtrate. Record the spectrum (approximately 30 seconds). Flush the cell twice more, each time with 10mL of the sample filtrate solution, and each time record a spectrum. Continue with the sampling process until all the samples are completed. Perform a final water rinse followed by a 2% Hellmanex® cleaning and 3% Formaldehyde rinse.

7.3.2 Autosampler introduction

A background spectrum is required at the start of a sequence of samples; remove the sample flowcell from the MPA and record the background spectrum of air. Return the cell to the measuring position before continuing with sample measurements. Pour the sample filtrate solution into 3 vials, load these onto the autosampler rack according to the sample queue on the OPUS programme. Start the sequence of tests. At the end of the sample processing, the OPUS programme is set to do final water rinse followed by a 2% Hellmanex® cleaning and 3% Formaldehyde rinse.

7.4 Sample prediction.

The OPUS method contains all the parameters for spectrum collection and interpretation. One set of results (brix, pol and sucrose) will be reported for each spectrum recorded.

8. Expression of Results

8.1 Calculation

The predicted results from each recorded spectrum test solution must be averaged for each analyte. No correction for the sample preparation step is required. The average of the three

results is calculated to two decimal places for each analyte.

8.2 Example

An example result table is shown in Table 2.

Table 2

Analyte	Result 1	Result 2	Result 3	Average	Report
Brix	13.90	13.95	13.92	13.92	13.92
Pol	11.94	12.01	11.98	11.98	11.98
Sucrose	12.02	12.09	12.07	12.06	12.06

9. Quality control - Control samples

A freshly prepared control sample is used to ensure the instrument is functioning correctly. The 10% polarimeter and refractometer control sample is suitable. First boiling sucrose (10 g) is accurately massed into a 200 ml Erlenmeyer flask and 90 g water (accurately massed) added. The sucrose is dissolved and the solution measured at the beginning of each batch of samples and measured in triplicate. A control chart should be prepared for the predicted sucrose with upper and lower warning limits and upper and lower action limits. The purpose for the warning and action limits is to show which errors are within the expected range and which are large enough to indicate a problem.

- Take action if 2 consecutive tests lie between the warning and action lines.
- Take action if 7 points in a row are on the same side of the zero line.
- Take action if a point is outside the action limits.

10. Calibration up-date

The analyte prediction calibrations will be continually monitored using the control samples and the use of random checks. The check samples will be analysed in parallel using recognised methods. Equations will be maintained and updated on an annual basis.

11. Precision

The expected precision of the method is shown in Table 3.

Table 3

Analyte (unit)	Reproducibility limits (%)
Brix (° Bx)	± 0.12
Pol (° Z)	± 0.13
Sucrose (%)	± 0.13

12. Declaration

The method is suitable for its intended use.

13. Bibliography

13.1 Walford SN (2014). Development, validation and on-site testing of NIRS calibrations for the prediction of brix, pol and sucrose in MJ samples. SMRI Technical Report 2182. 16th January 2014.