

Optimisation of the Quantification of Total Soluble Solids, pH and Titratable Acidity in South African Grape Must using Fourier Transform Mid-infrared Spectroscopy

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Date of submission for publication: March 2007

Date of acceptance for publication: May 2007

Key words: wine grape chemical analysis, Fourier transform infrared spectroscopy, chemometrics.

Calibration models for Fourier transform mid-infrared (FT-MIR) spectroscopy were developed for the simultaneous quantification of total soluble solids (TSS, measured as °Brix), pH and titratable acidity (TA, expressed as g/L tartaric acid) in South African (SA) grape must. An exploratory data analysis of the FT-MIR spectra of 1170 grape must samples (647 for °Brix, 252 for pH and 271 for TA) was done by principal component analysis, and partial least squares regression was used for the computation of the regression models. The prediction errors for TSS (0.34 °Brix), pH (0.04 units) and TA (0.51 g/L) provided analytical data of satisfactory accuracy. The evaluation of ready-to-use global calibrations to quantify these three parameters in SA samples presented standard error of prediction (SEP) values of 0.46°Brix, 0.10 pH units and 3.13 g/L for TA. After slope and intercept adjustments of the original global calibration algorithms, the SEP values were reduced to 0.38 °Brix, 0.05 pH units and 0.49 g/L for TA. These results show the necessity for optimisation of the global FT-MIR WineScan calibrations to provide a better fit to samples of South African origin. The results demonstrate that FT-MIR spectroscopy is a useful technique for the rapid quantification of major grape must parameters and for quality control purposes in an industrial cellar.

INTRODUCTION

Extensive research has been done to identify the physical and chemical characteristics of grapes that serve as quality measures (Du Plessis, 1984; Callao *et al.*, 1991; Damberg *et al.*, 2003; Gishen *et al.*, 2004; Tardaguila & Martinez de Toda, 2005). Total soluble solids (TSS, predominantly consisting of sugars and measured as °Brix or Balling) and acidity, measured as pH and titratable acidity (TA), are widely accepted as broad indicators of grape maturity (Zoecklein, 2001). Initial °Brix conditions in Chardonnay musts were also correlated, among other factors, to changes in ester formation during fermentation (Lee *et al.*, 2004). The measurement of other grape must parameters, such as red grape colour, phenolic composition and phenolic maturity (Francis *et al.*, 2004; Herderich *et al.*, 2004), yeast-assimilable nitrogen (Sinton *et al.*, 1978), flavour components such as terpenes in floral grape varieties (Mateo & Jiménez, 2000) and glucosylated flavour precursors (Iland *et al.*, 1996), provides information related to specific grape characteristics that are also considered important quality indicators. Although the analytical methods for the quantification of many grape must components are well established, several of the methods are complex and require extensive sample preparation and time-consuming experimental procedures. These factors impair the implementation of such tests for routine analysis. The optimisation of a rapid analytical method, such as Fourier transform mid-infrared (FT-MIR) spectroscopy for a single-step comprehensive analysis of grape must, is clearly of great importance for the assessment and monitoring of grape quality.

FT-MIR spectroscopy is an indirect analytical method whereby the concentrations of analytes of interest in a sample are predicted on the basis of a predetermined calibration algorithm developed for each respective analyte (Skoog *et al.*, 1997). The technology is based on the measurement of the frequencies of fundamental vibrations of covalent bonds in functional groups such as C-C, C-H, O-H, C=O and N-H in the mid-infrared region of the electromagnetic spectrum. This region is usually defined as ranging from 400 to 4000 cm^{-1} or, in terms of nanometres, from 25000 to 2500 nm (Smith, 1999). The characteristic wave numbers at which molecules absorb infrared light depend on the bond itself and its immediate molecular environment. Absorbance is directly proportional to the concentration of a particular component (Skoog *et al.*, 1997; Smith, 1999). This relationship is established during a calibration process through the application of chemometric techniques that include partial least squares (PLS) regression (Eriksson *et al.*, 1999; Esbensen, 2002). In a matrix with the chemical complexity of grape juice or wine, the calibration process is extensive and large numbers of samples typically are needed to meet the important requirement that all possible sources of physical and chemical variation to be expected in future unknown samples are accounted for in the calibration model (Nieuwoudt *et al.*, 2004).

The first purpose-built FT-MIR spectrometer dedicated to wine analysis was marketed in 1998 and the instrument featured ready-to-use, or so-called global, calibrations for the quantification of major wine components (<http://www.foss.dk>). These calibrations were developed using samples mostly of European origin.

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Acknowledgements: Technical assistance from the laboratory staff of Westcorp International, Vredendal, South Africa and from Mark van der Walt of Rhine Ruhr, South Africa is acknowledged.

The application of this technology for wine analysis was first evaluated by Patz *et al.* (1999) in a comprehensive comparison between FT-MIR-predicted values and the corresponding reference values obtained with standard chemical methods. The global FT-MIR calibrations for wine analysis were recently evaluated in a large-scale investigation using wines selected from 13 different winemaking regions in Germany, and the outcomes of the investigation were used to adjust the global calibration algorithms and optimise the FT-MIR prediction accuracies, among others for alcohol, TA, residual sugar and relative density in German wines (Patz *et al.*, 2004). At present, FT-MIR spectroscopy is well established worldwide for routine wine analysis (Palma & Barroso, 2002; Kupina & Shrikhande, 2003; Moreira & Santos, 2004). Recently, the technology was also evaluated to analyse wine components that traditionally require lengthier and complicated analytical techniques, including anthocyanins (Soriano *et al.*, 2006) and wine polysaccharides (Boulet *et al.*, 2006).

In contrast to wine analysis, spectroscopic applications for grape analysis have mostly focussed on the use of visible and near-infrared spectroscopy (Manley *et al.*, 2001; Jarén *et al.*, 2001; Damberg *et al.*, 2003; Herrera *et al.*, 2003; Arana *et al.*, 2005), and the evaluation of FT-MIR spectroscopy for comprehensive grape analysis has only been reported in a very limited number of publications (Dubernet *et al.*, 2000). Furthermore, the prediction accuracies of the global calibrations, when used for the quantification of grape samples from non-European origin, have not been evaluated thoroughly.

The reference methods for TSS, pH and TA determinations in grape must are routine and easily performed, but the ability of FT-MIR spectroscopy to predict the concentration of multiple components from one infrared spectrum of filtered grape must gives it an advantage in terms of speed and convenience. However, since TSS, pH and TA are considered important first-stage quality indicators and play an important role in decision making regarding optimal harvest time and the quality grading of grapes, it is essential that accurate analytical data are generated, particularly when values are used in schemes employing discrete intervals for classification.

The aim of this work was to implement and optimise FT-MIR spectroscopy for the quantification of TSS (measured as °Brix), TA and pH in grape must. For the purpose of this article, must refers to juice obtained from freshly pressed grapes (*i.e.* prior to fermentation). The specific objectives of the study were: (i) to evaluate the prediction accuracies of the global calibrations of the WineScan FT120 spectrometer when used for the quantification of °Brix, TA and pH in South African (SA) grape must samples; and (ii) to compare the prediction accuracies of the global calibrations with those of new calibration algorithms developed in this study for these three parameters. This work forms part of a larger program at Westcorp International, Vredendal, SA that is aimed at implementing and optimising FT-MIR spectroscopy to its full potential as a rapid analytical technique for quality control in the vineyard, at the weighbridge during grape intake, for process monitoring during fermentation and for the quantification of chemical components in finished wine.

MATERIALS AND METHODS

Grape samples

A total of 1170 grape samples of the cultivars Sauvignon blanc, Chardonnay, Colombar, Chenin blanc, Merlot, Pinotage, Caber-

net Sauvignon and Shiraz were collected over three consecutive vintages (2003 to 2005) from three grape-producing areas, Lutzville, Vredendal and Spruitdrift, in the Olifants River valley, SA. Vineyards were sampled (three to four bunches per sample) representatively from early ripening (~11°Brix) to late in the ripening process (~26°Brix). Bunches were placed and sealed in plastic bags after collection and transported at 15°C to the winery laboratory. Grapes were usually delivered to the laboratory within two hours after sampling.

Samples were also taken from incoming grape loads (six tonne loads) delivered at the winery weighbridge. These samples were collected with a sampling auger system in use at many large wineries. Upon arrival at the winery laboratory, the grape bunches were immediately manually pressed using a kitchen masher. The freshly pressed grape musts were filtered with a filtration unit (type 79500, FOSS Analytical, Denmark) connected to a vacuum pump. Filter paper disks graded with pore size 20 to 25 µm and with a diameter of 185 mm (Schleicher & Schuell, Germany, catalogue no. 10312714) were used for filtration. Individual filtered must samples were mixed thoroughly to ensure homogeneity, and aliquots were used for FT-MIR spectroscopy and reference analyses. A total of 647 samples were analysed for °Brix, 252 for pH and 271 for TA.

Reference analyses

Reference analyses were done in duplicate using methods recommended by the Office International de la Vigne et du Vin (<http://www.oiv.com>). TSS was measured as °Brix, which represents gram sucrose per 100 g of solution (Zoecklein *et al.*, 1999) and was assayed by refractometry using an automated digital refractometer (Atago Palette model PR-32a, catalogue No. 3405, Japan) with temperature compensation and an accuracy of 0.1% °Brix, calibrated against a 20°Brix sucrose solution. pH was determined using an automatic titrator equipped with a combination electrode (Crison, catalogue no. 4473624, LASEC, SA) and a temperature probe. Certified buffers (pH 7.00 and pH 4.00, LASEC, SA) were used to calibrate the electrode. TA was expressed as g/L tartaric acid and measured by potentiometric titration (Crison Compact Titrator D, SN 01714, Spain, software version 5.6) using standardised 0.33 N sodium hydroxide (LASEC, Cape Town, SA) to the end point of pH 7.00, as described by Zoecklein *et al.* (1999).

The accuracy of the reference methods was expressed as the standard error of laboratory (SEL) and calculated as:

$$SEL = \sqrt{\frac{\sum (y_1 - y_2)^2}{2n}}$$

where y_1 and y_2 are the values from duplicate determinations and n is the number of samples.

FT-MIR spectral measurements

The FT-MIR spectrum of each must sample was obtained immediately after filtration using a WineScan FT120 spectrometer equipped with a Michelson interferometer and CaF₂-lined cuvette with a path length of 37 µm (FOSS Analytical, Denmark). Because the WineScan is a specialised instrument designed specially for quantification in wine- and grape-derived matrices, some instrument settings are pre-set by the manufacturer and cannot be changed by the user. These include the sample temperature, which is set at 40°C, the scanning interval set from 930 to 5011 cm⁻¹ at

4 cm⁻¹ intervals, and the number of repeated scans of each sample, which is set at 20. Zero liquid S-6060 (Foss Analytical, Denmark) was scanned hourly prior to the addition of the samples to obtain a background scan. Repeated scans of each sample were averaged and processed to a single beam transmittance spectrum through a series of mathematical procedures, including Fourier transformation (WineScan FT120 Type 77110 and 77310 Reference Manual, Foss Analytical, Denmark, 2001). Background absorbance was corrected for by division of the sample spectrum by the zero liquid spectrum, at each recorded wave number. Finally, the corrected transmittance spectrum was converted to a linearised absorbance spectrum (WineScan FT120 Type 77110 and 77310 Reference Manual, Foss Analytical, Denmark, 2001).

Principal component analysis (PCA)

The FT-MIR spectra of the grape must samples were exported to Unscrambler software (version 9.2, Camo Process ASA, Oslo, Norway). The complete data matrix was defined by variables (1056 wave numbers) in the columns and samples in the rows. Duplicate spectra were averaged and the data matrix was mean-centred by column (*i.e.* subtracting the average value for a particular variable from each data point in that column). In order to make all variable variances comparable, the data matrix was also scaled or weighted by column (*i.e.* dividing each data point in a specific column by the inverse of the standard deviation of that particular variable). Mean-centring and weighting of data matrices are standard procedures for PCA and have been described (Esbensen, 2002).

PCA models the maximum directions of variation in a data set and provides an overview of the data structure by revealing relationships (similarities and differences) between the samples (Eriksson *et al.*, 1999; Esbensen, 2002). The FT-MIR spectra of the grape must samples (also referred to as objects) were projected as data points in a new multi-dimensional space defined by principal components (PCs). PCs are constructed to capture, in decreasing order, the maximum variation in the data set and the first few PCs (PC1 and PC2) therefore often describe the largest proportion of variation in the data. Because PCs are calculated to be orthogonal to one another, they can be interpreted independently. In order to identify these sources of variation in the samples, the original data matrix, defined by $X(n,m)$, is decomposed into the object space, the variable space and the error matrix as described by the algorithm:

$$X(n,m) = T(n,k)P(k,m)T + E(n,m)$$

where X is the independent variable matrix, T the scores matrix, P the loadings matrix, E the error matrix, n the number of objects, m the number of variables and k the number of PCs used (Eriksson *et al.*, 1999; Esbensen, 2002). The E matrix represents the variation not explained by the extracted PCs and is dependent on the definition of the problem.

Establishment of new FT-MIR calibrations

Wave number selection

The wave numbers at which the highest correlations between the reference values and measured absorbance of the sample were obtained were selected using the Advanced Performance software module version 2.2.2 (WineScan FT120 Type 77110 and 77310 Reference Manual, Foss Analytical, Denmark, 2001). In this way, the 15 most important “filters” (consisting of a single wave

number or a small group of wave numbers) for each chemical component were identified.

Partial least squares regression (PLS-R)

The establishment of new calibration models was done with PLS-R, using the Advanced Performance module version 2.2.2 (WineScan FT120 Type 77110 and 77310 Reference Manual, Foss Electric, Denmark, 2001) and Unscrambler software (version 9.2, Camo ASA, Trondheim, Norway). PLS-R is a bilinear modelling method whereby the original X data (in this study the absorbance of must samples at the respective wave numbers) are projected onto a small number of underlying variables called partial least squares (PLS) components. The computation of PLS components actively uses the Y data (in this study the reference values for °Brix, pH and TA respectively) to ensure that the first PLS components are most relevant for predicting the Y variables (Næs *et al.*, 2002). The interpretation of the relationship between the X data and Y data is therefore simplified, since the information is concentrated in the smallest number of PLS components. The relationship between the X data and the Y data are described in a linear algorithm in the format:

$$y = b_0 + b_1x_1 + b_2x_2 + \dots + b_nx_n$$

where y is the dependent variable, b_0 to b_n are the regression coefficients, b_0 is the intercept and x_1 to x_n represent the absorbance at the selected wave numbers.

Samples that were poorly predicted by the calibration models were identified in X - Y relation regression plots generated with Unscrambler software. In these regression plots, poorly predicted or so-called “outlier” samples protrude orthogonally from the regression line and can be identified easily (Esbensen, 2002).

Statistical indicators

Statistical indicators used to evaluate the performance of the calibration models included bias, standard error of cross validation (SECV) and standard error of prediction (SEP), and were calculated with the Advanced Performance software module version 2.2.2 as described (WineScan FT120 Type 77110 and 77310 Reference Manual, Foss Analytical, Denmark, 2001). Bias gives an indication of the systematic error in the predicted values and is calculated as the average of the difference between the reference values and the corresponding predicted values, also referred to as residual values. SECV describes the predictive accuracy of the calibration model in relation to the reference data and SEP describes the bias-corrected prediction error of the calibration model in relation to an independent validation set. The equations used for the calculation of bias, SECV and SEP were:

$$\text{bias} = \frac{1}{n} \sum_{i=1}^n (y_i - \hat{y}_i)$$

$$\text{SECV or SEP} = \sqrt{\frac{\sum_{i=1}^n (y_i - \hat{y}_i - \text{Bias})^2}{n-1}}$$

where y_i is the reference value for the i th sample; \hat{y}_i is the predicted value for the i th sample; and n is the number of samples. R^2 , referring to the coefficient of correlation, was also used as an indicator of the performance of the calibrations. Cross validation

was done by testing the calibration model on a subset of 25% of the total number of samples that was not included in the PLS computation of the model. Successive sets of 25% of the samples were used until all samples had been left out of the calibration computation once.

The residual predictive deviation (RPD) was used as a broad indicator of the performance of the calibration models when using independent validation (Williams, 1995). RPD is defined as the ratio of the standard deviation of the reference values to the standard error of the predicted values. It has been proposed that an RPD value of less than three serves as an indication that the calibration model is unsuitable for quantification, a value between three and five indicates that the model is suitable for screening, and a value greater than five indicates that the model is suitable for quantification (Williams, 1995).

Evaluation of global calibrations

Global calibrations refer to ready-to-use algorithms provided with the WineScan instrument and were developed by the manufacturer using reference data collected from samples analysed by a number of independent laboratories in Europe (<http://www.foss.dk>). The prediction accuracies of the global calibrations for °Brix (WineScan FT120 Application Note 175, 2001), pH (WineScan FT120 Application Note 177, 2001) and TA (WineScan FT120 Application Note 178, 2001) on SA must samples used in this study were evaluated using the PLS-R function of the Advanced Performance software module version 2.2.2 (WineScan FT120 Type 77110 and 77310 Reference Manual, Foss Electric, Denmark, 2001). Samples used for the evaluation of the global calibrations were selected randomly from all three vintages. Prediction accuracies were evaluated by the statistical indicators bias, SEP and coefficient of correlation R^2 . The latter describes the correlation between the predicted and corresponding reference values. In some instances, the option of adjusting the slope and intercept of the global algorithms to provide a better fit to the SA sample sets was evaluated by using the calculated adjustments suggested by the Advanced Performance software. These adjustments can be described as:

final predicted result = (predicted result obtained from original global calibration) * C_1 + I_1

where C_1 is the slope and I_1 is the intercept calculated for the adjusted global calibration.

RESULTS AND DISCUSSION

Grape must samples

The development of FT-MIR spectroscopy calibrations for viticultural and oenological applications is usually aimed at achieving sufficient accuracy for a particular application, while at the same time building so-called "robustness" into the calibration models (*i.e.* developing the calibration so that one model can be used for the maximum number of different sample types). The selection of calibration samples is therefore very important and, ideally, all the sources of variation to be expected in future unknown samples should be built into the calibration model. Major sources of variation that can affect the accuracy of analytical data generated with infrared spectroscopy if they are not accounted for sufficiently in the calibration model include the chemical composition of samples, the grape cultivar and the geographic origin of the grapes (Gishen *et al.*, 2004; Arana *et al.*, 2005). In the present study, grape samples were selected to be representative of white cultivars (Sauvignon

blanc, Chardonnay, Colombar and Chenin blanc) and red cultivars (Merlot, Pinotage, Cabernet Sauvignon and Shiraz) grown in the three major viticultural regions in the Olifants River Region, SA. These regions, namely Lutzville, Spruitdrift and Vredendal, have very different climatic conditions. Lutzville is situated close to the cold Atlantic Ocean (five to ten km) and average day temperatures in summer are 3°C lower than those of the inland Vredendal and Spruitdrift areas (J. Joubert, Vinpro, Vredendal, SA, personal communication, 2006). Grape samples were also collected at various stages during grape ripening in order to include the widest possible range in the values for °Brix, TA and pH in the sample set. The descriptive statistics (average, minimum, maximum and standard deviation) for these three parameters, using 1 170 grape must samples prepared in the winery laboratory for the purpose of this study, are shown in Table 1.

The maximum values for TSS (25.60°Brix) and TA (14.90 g/L) of the SA samples fell outside the corresponding value ranges of samples used in the establishment of the global calibrations (23.10°Brix and 12.99 g/L for TA), while the minimum value for pH of the SA samples (pH 3.23) was lower than that of the sample set used in the establishment of the global pH calibration (pH 3.27). The ranges for pH and TA in the SA sample sets are normal for the Olifants River valley region, while the high sugar concentrations in the SA samples are typical of grapes ripening under the hot climatic conditions prevailing during grape ripening in the southern hemisphere. These results clearly indicate that the global calibrations for °Brix, pH and TA in grape must require an extension of the ranges of the values and redevelopment for application to samples originating from SA.

FT-MIR spectra

The FT-MIR spectra of grape must represent the collective absorbance of all IR-active components present in the sample. Distinct variation between the FT-MIR spectra of grape must samples of different ripeness levels were observed in the region 935 to ~3700 cm^{-1} (Fig. 1). Water absorbs strongly in the wave number regions 1543 to 1716 cm^{-1} and 2970 to 3626 cm^{-1} (Smith, 1999), and these bands were prominent features of the must FT-MIR spectra. The region ~3700 to 5009 cm^{-1} appeared to contain very little useful information (Fig. 1). To exclude noise being introduced in the calibration model, only the following regions of the FT-MIR spectra of the must samples were considered for wave number selection: 964 to 1542 cm^{-1} and 1717 to 2969 cm^{-1} . The wave numbers selected for the establishment of new calibrations for °Brix, pH and TA in this study were all selected in the region 1474 to 2685 cm^{-1} . This region includes the so-called "fingerprint region" (930 to 1600 cm^{-1}), since absorption bands in this area are characteristic of specific molecules (Smith, 1999). The region 1713 to 2300 cm^{-1} is referred to as the "functional group region" and -COOH (carboxylic acid groups) and -C=O (carbonyl groups) absorb strongly in this area (Coates, 2000).

Principal component analysis of FT-MIR spectra

Principal component analysis was done to identify the main sources of variation in the FT-MIR spectra of the grape must samples. In the exploratory stages of data analysis, six samples with sugar concentrations lower than 11°Brix were included in the data set. These musts were obtained from grapes sampled very early in the ripening process. For PCA, the wave numbers where water absorbs and the area in the FT-MIR spectra that showed very little interesting infor-

TABLE 1

Value ranges of SA grape must samples used in this study and of samples used by Foss to establish the global WineScan FT-MIR calibrations for TSS (measured as °Brix), pH and TA, respectively.

| Parameter | Value ranges of SA grape must samples | | | | | Value ranges of samples used to establish global WineScan FT-MIR calibrations ^a | | | | |
|-----------------------|---------------------------------------|---------|---------|---------|-----------------|--|---------|---------|---------|-----------------|
| | Sample No. ^b | Average | Minimum | Maximum | SD ^c | Sample No. ^b | Average | Minimum | Maximum | SD ^c |
| °Brix | 653 | 18.50 | 6.0 | 25.60 | 2.89 | 261 | 15.88 | 8.34 | 23.10 | na ^d |
| pH | 252 | 3.23 | 2.77 | 3.78 | 0.19 | 1759 | 3.27 | 2.64 | 4.07 | na ^d |
| TA (g/L) ^e | 271 | 8.52 | 4.26 | 14.90 | 2.33 | 1422 | 5.59 | 1.60 | 12.99 | na ^d |

^aApplication notes 175, 177, 178 for WineScan FT 120 Type 77110 and 77310, Foss Analytical, Denmark. <http://www.foss.dk>

^bsample number; ^cstandard deviation; ^dnot available; ^eexpressed as g/L tartaric acid

TABLE 2

Validation of the prediction accuracies of global WineScan FT-MIR calibrations for quantification of TSS (measured as °Brix), pH and TA in SA grape must samples.

| Parameter (No.) ^a | Descriptive statistics of SA sample sets ^b | | | | | Validation statistics of global FT-MIR calibrations | | | | |
|------------------------------|---|-------------------|-------------------|-------------------|-----------------|---|-------|---|-------|-----------------|
| | | | | | | Before slope and intercept adjustment ^c | | After slope and intercept adjustment ^c | | |
| | SEL ^d | Ave. ^e | Min. ^f | Max. ^g | SD ^h | SEP ⁱ | bias | SEP | bias | R ^{2j} |
| °Brix (540) | 0.10 | 19.31 | 9.80 | 23.10 | 2.54 | 0.46 | 0.23 | 0.38 | 0.01 | 0.98 |
| pH (133) | 0.05 | 3.43 | 2.77 | 3.78 | 0.12 | 0.10 | -0.08 | 0.05 | -0.06 | 0.84 |
| TA ^k (158) | 0.25 | 5.59 | 4.26 | 12.91 | 2.19 | 3.13 | -3.05 | 0.49 | 0.01 | 0.97 |

^anumber of samples; ^bSA grape must samples used in this study; ^cas described in the text; ^dstandard error of laboratory; ^eaverage; ^fminimum; ^gmaximum; ^hstandard deviation; ⁱstandard error of prediction; ^jcorrelation coefficient; ^ktitratable acidity measured as g/L tartaric acid

mation (Fig. 1) were excluded (in total $n = 555$ wave numbers). The complete data matrix, consisting of 1176 samples and 501 wave numbers, was mean centred and weighted as described. In the resultant score plot (Fig. 2), six extremely deviating samples (designated by symbol A) located diagonally towards the negative ends of PC1 and PC2 were identified as samples with sugar concentrations lower than 11°Brix. In the explorative stages of data analysis it was already evident that higher calibration prediction accuracy could be obtained if these samples were excluded from the calibration sample set (data not shown). PC1 and PC2 collectively explained 96% of the variance in the sample set, which indicates that the variation in the FT-MIR spectra of the must samples was modelled satisfactorily by the 501 selected wave numbers. PC1 explained 91% of the variance in the sample set and could be interpreted as the relationship between the sugar and acid concentrations of the samples, based on the respective reference values. Samples with low °Brix and high TA concentrations (collected early in the ripening process) were located towards the negative end of PC1, while those with high °Brix and low TA concentrations (collected late in the ripening process and designated by symbol B) were located towards the positive end of PC1. No clear clustering of samples on the basis of grape cultivar or geographic origin could be identified in the score plot defined by PC1 and PC2.

Evaluation of global WineScan FT-MIR calibrations for quantification of TSS, pH and TA in SA grape must

The prediction accuracies of the global WineScan calibrations for the quantification of TSS (measured as °Brix), pH and TA

were validated using SA grape must samples that were selected so that the parameter ranges fell inside the calibration ranges of the corresponding global calibrations. Validation statistics of the analytical data generated with the unadjusted global calibrations (Table 2) showed substantial systematic errors for TSS (bias = 0.23, SEP = 0.46°Brix), pH (bias = -0.08, SEP = 0.10) and TA (bias = -3.05, SEP = 3.13 g/L). The SEP values for all three parameters were also significantly higher than the corresponding errors of the laboratory methods (SEL = 0.10°Brix, 0.05 pH units and 0.25 g/L for TA respectively).

The regression plot for reference TA values *vs.* values predicted by FT-MIR spectroscopy (Fig. 3) showed a significant lack of fit between the target regression line and the true regression line, and samples with reference TA values higher than ~8 g/L had particularly large prediction errors. Under ideal conditions, where the reference values and corresponding predicted values are identical, the regression line has a slope and regression coefficient (R^2) equal to 1 and a bias equal to zero. In practice it is normal for slight deviations from these ideal values to occur. Bias gives an indication of the magnitude of the systematic error in the prediction data and, ideally, this error should be very small. The adjustments of the slope and intercept of the respective algorithms for TSS, pH and TA (as automatically calculated and proposed by the Advanced Performance software of the instrument) resulted in a significant correction of bias and a lowering in the SEP values (Table 2). An excellent prediction accuracy for must samples from SA was obtained with the adjusted global calibration for pH

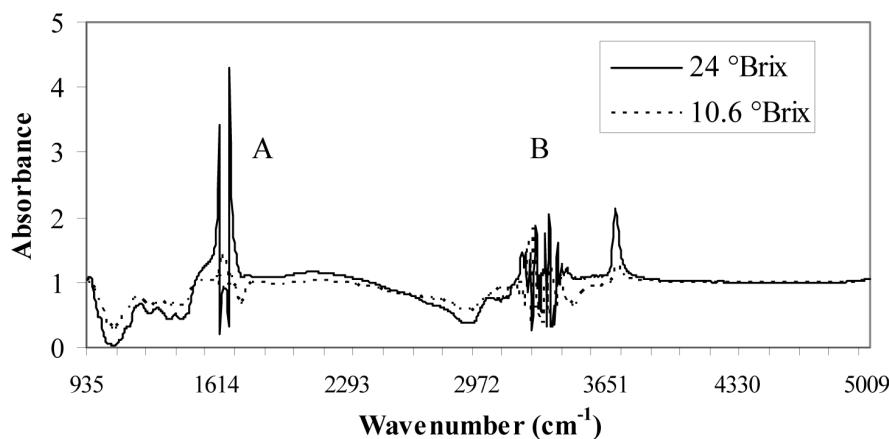


FIGURE 1

FT-MIR spectra of two grape must samples of different ripeness levels. The areas where water absorbs strongly, 1543 to 1716 cm^{-1} and 2970 to 3626 cm^{-1} are designated by the symbols A and B respectively.

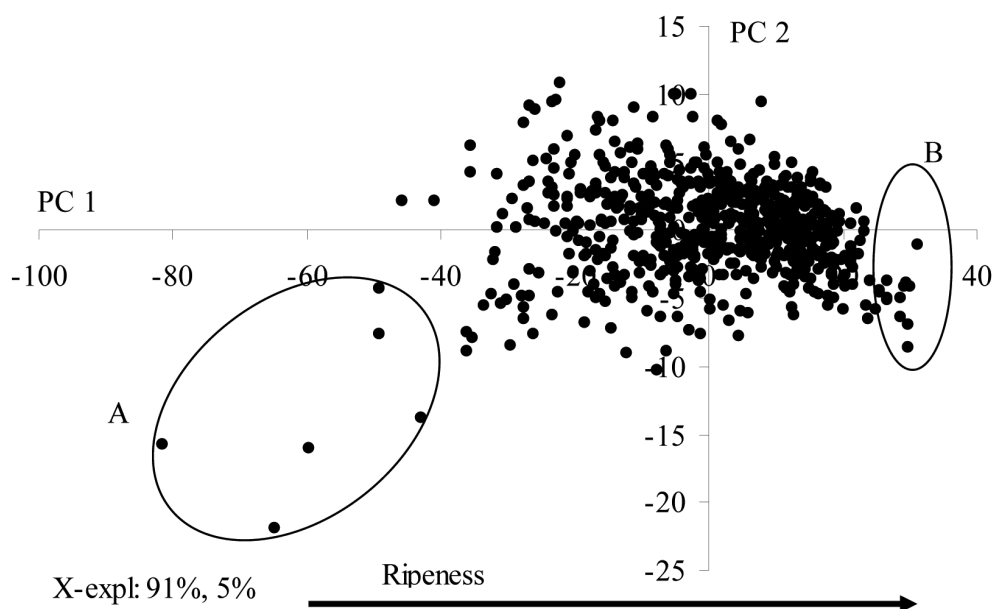


FIGURE 2

PCA score plot of the data matrix consisting of 1176 grape must samples and 501 wavenumbers. PC1 and PC2 explained 91% and 5% of the variance in the X -data, respectively. The arrow indicates the degree of ripeness of the samples. A: samples with sugar concentrations lower than 11°Brix; B: samples with sugar concentrations higher than 24°Brix.

(SEP = 0.05), while the adjusted TSS (SEP = 0.38°Brix) and TA (SEP = 0.49 g/L) calibrations also yielded accuracies sufficient for rapid screening purposes.

The results showed that each of the global algorithms provided a better fit to the SA samples after the adjustment of the original algorithms (as evaluated by both the correction of bias and the lowering of prediction errors). The potential sources of error due to so-called matrix effects when using FT-MIR spectroscopy are well known (Smith, 1999), and the results of this study showed that the SA samples had interfering sources of variation that were not sufficiently accounted for by the global WineScan calibrations. Nevertheless, the results obtained confirmed the usefulness of the global calibra-

tions, provided that prediction accuracies are first evaluated on sample types that have not been included in the original computation of the algorithms. In this study, relatively large sample sets were used for the evaluation in order to assure that samples originating from the Vredendal area in SA were well represented. It is quite possible that validation can be done with fewer samples in some instances, but that will depend on the specific application.

Establishment of new calibration models for TSS, pH and TA for SA grape must

The parameter range for TSS, pH and TA for the SA samples fell outside the calibration ranges of the global WineScan calibrations and it therefore was necessary to develop new calibration models

for these parameters. The objective of the work was to establish robust calibrations and the specific aims were to develop models that yielded analytical data of acceptable accuracy, while at the same time being applicable to many different sample types. A characteristic feature of infrared spectroscopy calibrations is that they are dependent on the data sets used for their development and, in this study, it therefore was important to use sample sets representative of different cultivars, ripeness levels and geographic origins. In the design of the calibration models, two thirds of the samples were used for calibration and one third for independent validation of the model. Calibration and validation samples were selected randomly and the various cultivars and geographic origins were represented well in both sets. Statistics used to describe the performance of the calibration models included SECV, while SEP, bias and R^2 were used to describe the validation sets.

The new TSS calibration model was initially computed using 15 PLS components and these collectively explained 96.9% of the accumulated variance in the sample set. The last seven PLS components (components 9 to 15) only explained 0.06% of the total variation in the sample set and deselection of these resulted in an improvement in the SECV value. The final model for the quantification of TSS in SA must was established with eight PLS components and the calibration error (SEC) was 0.31°Brix (Table 3). An independent validation set using 215 must samples was used to test the predictive accuracy of the calibration model and the regression plot is shown in Figure 4. The SEP value (0.34°Brix) showed an improvement on the SEP value obtained with the adjusted global calibration (SEP = 0.38°Brix, Table 2), although the error was higher than the corresponding error of the laboratory method (SEL = 0.10°Brix). The RPD value of 9 indicated that the calibration model was suitable for quantification purposes (Williams, 1995). Using near-infrared spectroscopy for TSS determination in grapes, Manley *et al.* (2001) reported a prediction error of 0.31°Brix and Jarén *et al.* (2001) reported an error of ~1°Brix.

For the present study, the interpretation of the prediction error in terms of the distribution of the residual values (residual = reference value minus FT-MIR predicted values) showed that 92% of the samples had a prediction error smaller than $\pm 0.5^\circ\text{Brix}$, while 99% of the samples had a prediction error smaller than $\pm 1.0^\circ\text{Brix}$ (residual plot not shown). Only 1.4% of the samples had predic-

tion errors larger than $\pm 1.0^\circ\text{Brix}$. The distribution of the residual values was centred around zero, pointing to the bias being negligible in this case. These results indicated excellent prediction statistics and the analytical data generated by FT-MIR spectroscopy for TSS quantification is of sufficient accuracy to be used in grape classification schemes.

A new calibration model for pH in SA must samples was developed using 162 samples and 15 PLS components (Table 3). In total, 93% of the explained variance in the sample set was accumulated by these factors and the calibration error (SECV) was 0.04 pH units. The predictive accuracy of the calibration model was tested using an independent sample set ($n = 81$). The regression plot of the validation data is shown in Figure 5. The resulting SEP value of 0.04 pH units was an improvement on the laboratory error for pH (SEL = 0.05 pH units) and the bias of 0.004 was negligible when compared to the SEP value. In total, 77% of the samples had a prediction error lower than ± 0.05 units, which is the desired accuracy for pH determination in must, while 98% of the samples had a prediction error lower than ± 0.1 for pH (residual plot not shown). The RPD value of 5 (Table 3) indicated that the calibration model was suitable for quantification purposes. It is not uncommon for results predicted by infrared to have better accuracy than laboratory methods (Næs *et al.*, 2002) and, for pH determination in particular, the performance of FT-MIR spectroscopy surpassed that of the reference method. Using near-infrared spectroscopy, Damberg *et al.* (2003) reported prediction errors of 0.05 to 0.08 units for pH determination in grape juice.

The new TA calibration for South African must samples was computed using 15 PLS factors and these explained 84% of the accumulated variance in the calibration sample set ($n = 180$). The SECV for the calibration model was 0.41 g/L (Table 3). When applied to an independent validation set consisting of 90 samples, the prediction error (SEP = 0.51 g/L) of the new TA calibration model was comparable to the SEP value obtained with the adjusted global calibration for TA (0.49 g/L, Table 2). The RPD value of 5 indicated that the new calibration model was suitable for quantification purposes. TA values predicted with FT-MIR spectroscopy were higher (for both the global and new TA calibration) than the laboratory error (SEL = 0.25 g/L). Bias in the new TA calibration (0.12 g/L) was higher than that obtained with the adjusted global

TABLE 3

Validation statistics of the newly developed FT-MIR spectroscopy calibrations for the quantification of TSS (measured as °Brix), pH and TA in SA grape must.

| Para-meter | Calibration statistics | | | | Validation statistics | | | | | | |
|---------------------|-------------------------|-------------------------------------|-------------|-------------------|-------------------------|-------------------------------------|------------------|------------------|-----------------|-------|------------------|
| | Sample No. ^a | Range average \pm SD ^e | PLS factors | SECV ^b | Sample No. ^a | Range average \pm SD | SEL ^c | SEP ^d | R ^{2c} | bias | RPD ^f |
| °Brix | 379 | 12.7 \pm 24.7 19.38 \pm 2.67 | 8 | 0.31 | 215 | 12.8 \pm 24.5 18.8 \pm 2.46 | 0.10 | 0.34 | 0.99 | 0.07 | 9 |
| pH | 162 | 2.77 \pm 3.78 3.26 \pm 0.19 | 15 | 0.04 | 81 | 2.78 \pm 3.59 3.19 \pm 0.18 | 0.05 | 0.04 | 0.95 | 0.004 | 5 |
| TA g/L ^h | 180 | 4.42 \pm 16.40 8.58 \pm 2.48 | 15 | 0.41 | 90 | 4.63 \pm 15.33 8.58 \pm 2.30 | 0.25 | 0.51 | 0.96 | 0.12 | 5 |

^anumber of samples; ^bstandard error of cross validation; ^cstandard error of laboratory; ^dstandard error of prediction; ^ecorrelation coefficient; ^fresidual predictive deviation; ^gstandard deviation; ^hmeasured as g/L tartaric acid

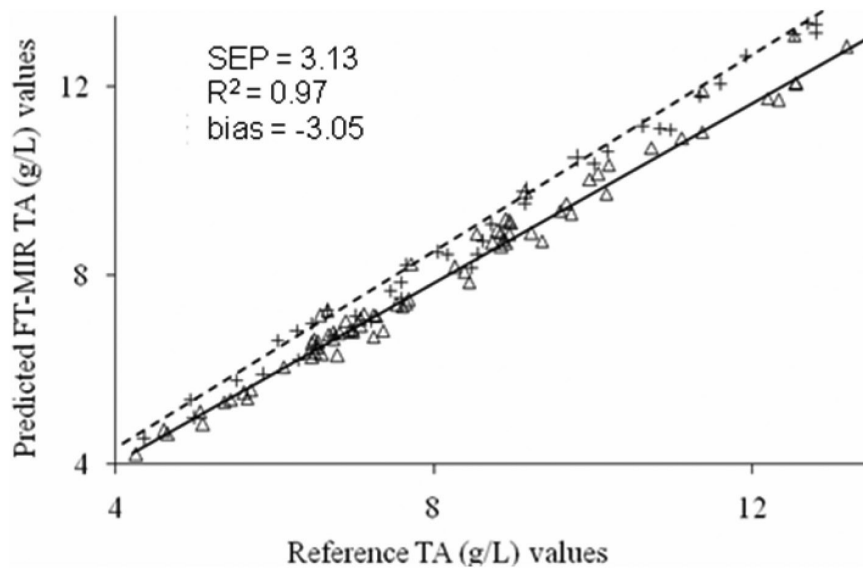


FIGURE 3

Regression plot of FT-MIR predicted values for TA (marked +) using the global WineScan calibrations before slope and/or intercept adjustments vs. reference TA values (marked Δ). The dashed line represents the target regression line of the unadjusted global calibration and the solid line the proposed regression line for the adjusted algorithm. TA was measured as g/L tartaric acid.

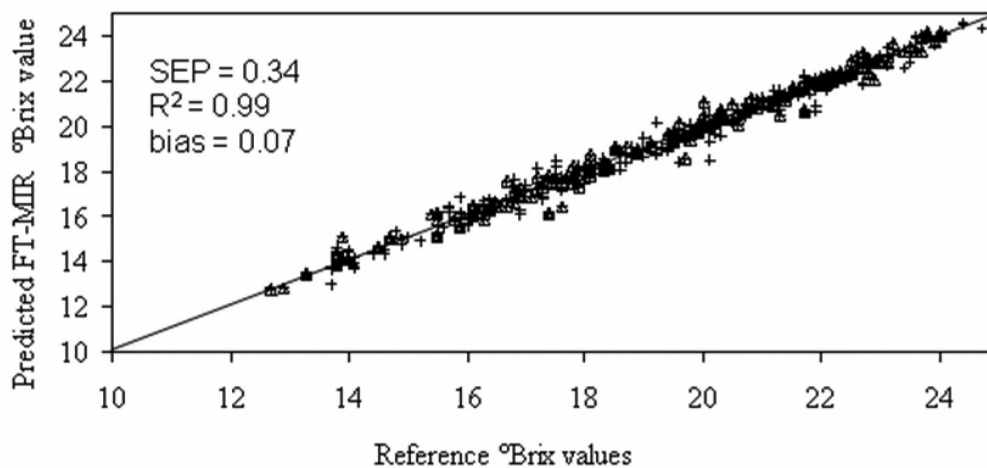


FIGURE 4

Regression plot of FT-MIR predicted values for TSS (measured as $^{\circ}$ Brix, marked +) vs. reference $^{\circ}$ Brix values (marked Δ).

calibration (bias = 0.01 g/L). The regression plot of the predicted values of TA is shown in Figure 6.

The determination of acidity in grapes by the reference method is done by a method whereby the hydrogen ions consumed by titration with a standard base to an endpoint are measured and expressed as g/L tartaric acid (Zoecklein *et al.*, 1999). This is an indirect measurement of grape acidity and could possibly explain why the prediction error for TA is higher with infrared spectroscopy than with the laboratory method. The high throughput of grape samples using FT-MIR analysis compensates to a certain extent for the higher prediction error. The new TA calibration model will be developed further by incorporating more samples in consecutive harvest seasons.

CONCLUSIONS

Particularly attractive features of FT-MIR spectroscopy include the speed of analysis (less than one minute per sample), the low

individual analysis cost, very little sample preparation, simultaneous quantification of several parameters in one analysis, and no generation of chemical waste. The analytical accuracies obtained for the measurement of $^{\circ}$ Brix, pH and TA in grape must using FT-MIR spectroscopy showed very good potential for quality control purposes in an industrial cellar. Prediction errors for TSS (0.34 $^{\circ}$ Brix), pH (0.04 pH units) and TA (0.51 g/L) of the newly developed calibration models using South African must samples proved satisfactorily low for quantification purposes. More accurate analytical data were obtained for pH determination using FT-MIR spectroscopy than with the conventional reference method. The global WineScan calibrations provided a convenient, ready-to-use option with the technology, but the prediction accuracies when applied to South African must samples had to be improved by adjusting the slopes and intercepts of the original algorithms for all three parameters tested. One of the ambitions is to collabo-

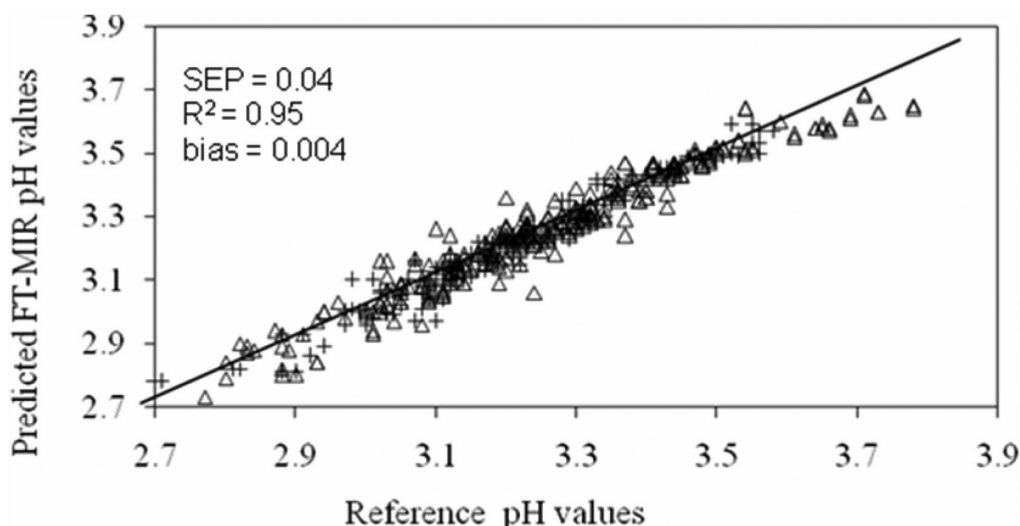


FIGURE 5

Regression plot of FT-MIR predicted values for pH (marked +) vs. reference pH values (marked Δ).

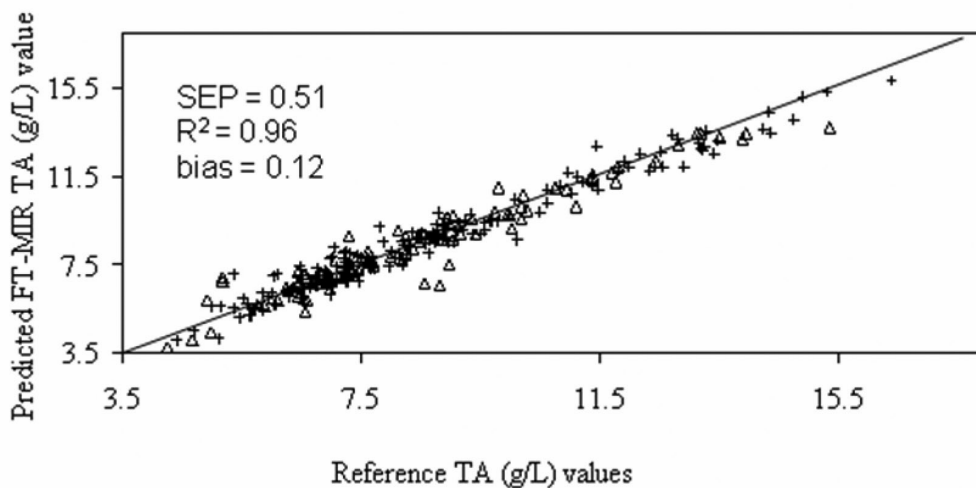


FIGURE 6

Regression plot of FT-MIR predicted values for TA (measured as g/L tartaric acid, marked +) vs. reference TA values (marked Δ).

rate with other laboratories in SA that use FT-MIR spectroscopy for wine grape analysis and expand the number of samples to be truly representative of all major viticultural regions in the country. Further extensions to calibrations will be made for other grape components, such as free amino nitrogen, alpha amino nitrogen, grape colour and polyphenols. The rapid analysis of these parameters will lead to higher throughput of grape must samples in the laboratory, and will realise the full potential of the advantages offered by FT-MIR spectroscopy.

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