The Evaluation of the Applicability of Fourier Transform Near-Infrared (FT-NIR) Spectroscopy in the Measurement of Analytical Parameters in Must and Wine

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Fourier transform near-infrared (FT-NIR) spectroscopy can be used as a rapid method to measure the percentage of sugar and to discriminate between different must samples in terms of their free amino nitrogen (FAN) values. It can also be used as a rapid method to discriminate between Chardonnay wine samples in terms of their malolactic fermentation (MLF) status. By monitoring the conversion of malic to lactic acid, the samples could be classified on the basis of whether MLF has started, is in progress or has been completed. Furthermore, FT-NIR spectroscopy can be used as a rapid method to discriminate between table wine samples in terms of their ethyl carbamate (EC) content. It is claimed that high concentrations of ethyl carbamate in wine can pose a health threat and has to be monitored by determining the EC content in relation to the regulatory limits set by authorities. For each of the above-mentioned parameters QUANT+™ methods were built and calibrations were derived and it was found that a very strong correlation existed in the sample set for the FT-NIR spectroscopic predictions of the percentage of sugar (r = 0.99, SEP = 0.31 °Brix). However, the correlation for the FAN predictions (r = 0.602, SEP = 272.1 g.L^{-1}), malic acid (r = 0.64, SEP = 1.02 g.L^{-1}), lactic acid (r = 0.61, SEP = 1.35 g.L^{-1}) and EC predictions (r = 0.47, SEP = 3.6 µg.kg^{-1}) were not good. The must samples could be classified in terms of their FAN values when Soft Independent Modelling by Class Analogy (SIMCA) diagnostics and validation were applied as a discriminative method, with recognition rates exceeding 80% in all cases. When SIMCA diagnostics and validation were applied to the Chardonnay and EC wine samples, recognition rates exceeding 88% and 80% respectively were obtained. These results therefore confirm that this method is successful in discriminating between samples.

Near-infrared spectroscopy

The NIR spectroscopy method of analysis is an instrumental method for rapid and reproducible measurement of the chemical composition of samples, requiring little or no sample preparation (Norris, 1989). Each of the major chemical components of a food sample has NIR absorption properties, which can be used to differentiate one component from the other. By using NIR Spectrophotometers and Fourier transform interferometers, FT-NIR diffuse reflectance signals are formed that contain information about the composition of the sample (Willard *et al.*, 1988). Such information can be extracted by the appropriate mathematical treatment of the data (Willard *et al.*, 1988). NIR spectroscopy is being used for the determination of the alcohol content of wine and preliminary investigations have been carried out to determine the glycosyl-glucose content of grapes (quality indicator) and the methanol concentration in spirits (Gishen & Dambergs, 1998). This means that a calibration or learning set of samples is analysed by standard laboratory methods for reference and that the same samples are then scanned by the NIR spectrophotometer. The data obtained by the reference method are correlated with the large amount of spectral data, using sophisticated multivariate statistical data analysis techniques, in order to find a correlation that can predict the analytical results from the spectral data. The NIR spectroscopic instrument can then be used to scan new samples to obtain analytical data (Gishen & Dambergs, 1998). A measurement can be made in as few as 10 seconds, although the average would be between 30 seconds and three minutes. Little or no sample preparation is needed and the technique can be used by employees without extensive training. It is also applicable to on-line measuring systems (Willard *et al.*, 1988; Wehling, 1994).

FT-NIR spectroscopy can also be used for the classification and verification of raw materials (Downey & Beauschêne, 1997). In many cases in which sample classification is applied, it is only necessary to know whether a sample belongs to a specific class or not, or whether it is above or below a specific cut-off point. In such cases the data sets are divided into classes to differentiate between the specified properties. FT-NIR was used to discriminate between pure Arabica and pure Robusta coffees and blends of these two (Downey *et al.*, 1994). If the materials to be identified are spectroscopically dissimilar, it is often only necessary to use a simple distance measure, such as a spectral difference. If the spectra are similar, such as must and wine spectra (Fig. 1), it may be necessary to include slightly more sophisticated techniques that take both the variability of the spectra of interest and the dif-

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ferences between the spectra into consideration. The Soft Independent Modelling by Class Analogy (SIMCA) technique provides such a method (Downey & Beauschêne, 1997).

In the SIMCA method, a principal component model is created for each class. An envelope is constructed to contain the standards of a class. If one principal component is used, the class mean and a line are needed to model the data. If two principal components are used, the class mean and a plane are required, and with three principal components, the class mean and a volume are required. If there are more than three components, the envelope can be thought of as a box with more than three dimensions (a hyperbox) (Anonymous, 1997). In a SIMCA classification, the unknown spectrum is classified according to whether it lies inside or outside the hyperbox (this is the model residual).

An advantage of SIMCA is the use of an objective statistical test, the F-test, to establish the probability of a sample belonging to any given class (Anonymous, 1997). SIMCA is thus a method that provides a set of parameters that characterises each class and forms the basis for other quantities that describe the data. The procedure checks every standard spectrum to ensure that the ones from a single class fit that class (recognition) and that those from other classes that were selected are rejected (rejection).

The recognition rate, also known as the sensitivity, is the number of spectra that are assigned to the class as a percentage of the number of spectra that should have been assigned to the class (Anonymous, 1997). The rejection rate, also known as the specificity, is the number of spectra that are rejected, thus not assigned to the class, as a percentage of the number of spectra that should have been rejected (Anonymous, 1997).

Fermentation and the optimal nitrogen balance of must

The nitrogen content of grapes affects the production of yeast biomass, the fermentation rate and the time taken to complete a fermentation and can influence the spectrum of end products of yeast metabolism (Bisson, 1991). A value of 500 mg.L^{-1} of nitrogen in must was reported as being necessary to achieve maximal yeast biomass production (Agenbach, 1978). In addition to the impact of the nitrogen content on cell production, nitrogen also affects the fermentation rate. At least 140 mg.L^{-1} of assimilable nitrogen is needed in juice or must in order for the yeast to complete fermentation to dryness (Agenbach, 1978).

Free amino (or alpha) nitrogen (FAN) has often been utilised as an indicator of the nitrogen richness or nitrogen availability for yeast growth and fermentation (Amerine & Ough, 1980). Statistical analyses established the FAN/°Brix ratio as the most reliable means of determining optimal nitrogen balances in must (Vos *et al.*, 1980). The natural FAN content of musts from mature grapes of most cultivars (Pinotage is the exception) ranges from approximately 400 to 1000 mg.L^{-1} N when ammonium sulphate is used as reference standard. With must samples at lower levels the addition of a maximum of 500 mg.L^{-1} N would thus ensure a total FAN content of at least 800 mg.L^{-1} N, the minimum concentration required for maximum fermentation rates (Vos *et al.*, 1980).

The FAN content should be an accurate index of the nitrogen requirements of yeast and hence of fermentation rates. The FAN/°Brix ratio is now established as a superior index and indicates that the FAN requirement of yeast is influenced by the sugar content of the musts (Vos *et al.*, 1980).

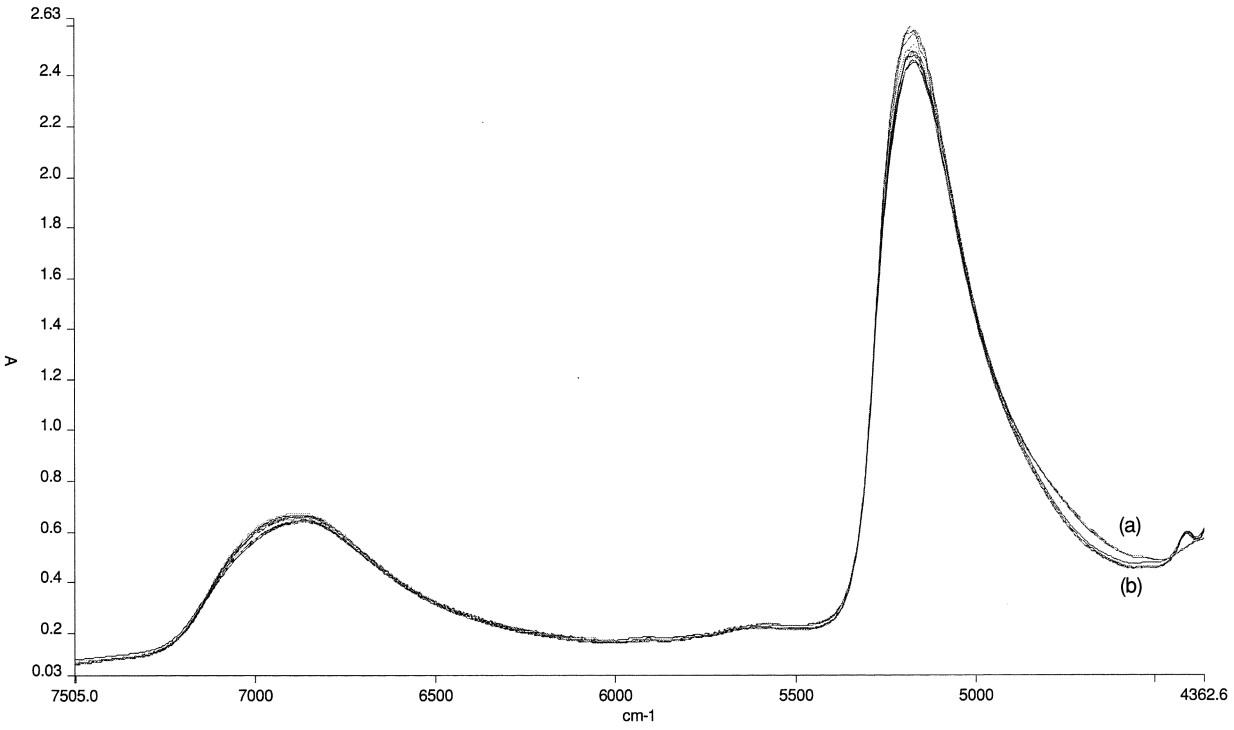

FIGURE 1

FT-NIR spectra of must (a) and wine (b) samples. Absorption (A) vs. wave number (cm^{-1}).

Malolactic fermentation in wine

Most red wines and some white wines in colder wine regions are subjected to the secondary malolactic fermentation (MLF) during or soon after alcoholic fermentation (Volschenk *et al.*, 1997). Malolactic fermentation in wine is caused by the metabolic activity of certain lactic acid bacteria (LAB), the most important aspect being the microbial deacidification that results from the decarboxylation of malic acid to lactic acid (Nielsen *et al.*, 1996). The total acidity decreases and the pH increases, resulting in wine with a softer palate (mouthfeel). Malolactic fermentation also contributes to the flavour and complexity, and it increases the microbiological stability of the wine (Nielsen *et al.*, 1996).

However, malolactic fermentation can also be considered a spoilage factor if it takes place under the wrong conditions, such as in wines to which microbial stabilisation agents have not been added carefully (Kunkee, 1991).

The implication of ethyl carbamate for the wine industry

Ethyl carbamate (EC) or urethane occurs naturally in all fermented foods and beverages. Because EC has been shown to be a potential carcinogenic when administered in high doses in animal tests and some countries (e.g. Canada) have set rather strict limits, the wine industries have to monitor the EC levels in their products (Butzke & Bisson, 1997). Ethyl carbamate is not an added substance, but forms during the fermentation of alcoholic beverages. If the fermented product is heated, such as in "baking" sherry or distilling spirits, its levels can increase (Segal, 1988).

Urea, a natural by-product of yeast metabolism, is the main precursor of ethyl carbamate in wines (Monteiro *et al.*, 1989). Arginine and proline are generally the major amino acids found in grape juice. The enzyme arginase catalyses the cleavage of arginine to ornithine and urea. The resulting urea can also be used as a nitrogen source and is further broken down to ammonia and carbon dioxide by the yeast *Saccharomyces cerevisiae*. This takes place via a degradative enzyme complex, composed of urea carboxylase and allophanate hydrolase (Henschke & Ough, 1991). However, this process may not be complete before the end of fermentation if the must originally contained high levels of nitrogenous compounds (i.e. high alpha-amino acids), which are metabolised by yeast before arginine and urea. Residual levels of urea remaining after fermentation can react with ethyl alcohol to form EC. This reaction is dependent on temperature and time (Henschke & Ough, 1991).

No regulatory limits for EC levels in wines exist in South Africa, but wines that are exported to countries with regulatory limits have to show the EC content (M. Waldner, ARC Infruitec-Nietvoorbij, personal communication). It is therefore necessary to monitor the EC content in some export wines to determine whether they fall within the regulatory boundaries of these countries.

Currently the FAN, malic and lactic acid and EC measurements are monitored using expensive, quantitative, time-consuming analytical methods, such as GC-MS and HPLC. FT-NIR spectroscopy, on the other hand, can be used as a rapid, alternative method that requires no sample preparation. Although the measurement of the sugar content only by use of a Balling meter is a simple and fast method, simultaneous determination of the FAN together with the percentage of sugar will save time.

MATERIALS AND METHODS

Wine samples

A selection of 97 must samples of white grape varieties, representative of the wine regions of the Western Cape, was drawn from settling tanks at the cellars of Distell in Stellenbosch, South Africa to carry out sugar and FAN determinations. The set included the following samples: 46 Chenin blanc, 29 Sauvignon blanc, 9 Chardonnay, 9 South African Riesling; 5 Pinot noir and 2 Gewürztraminer. The must samples were collected during the harvest period over two consecutive seasons (1999 & 2000) after one day in the settling tanks.

For the MLF determinations 65 Chardonnay wine samples were drawn from barrels at the cellars of Distillers Corporation and another 43 Chardonnay samples were received from the Institute for Wine Biotechnology at the University of Stellenbosch, South Africa. The samples, stored at 4°C, were collected over a three-month period.

A selection of 200 wine samples was drawn from barrels at the cellars of the ARC Infruitec-Nietvoorbij in Stellenbosch, South Africa for EC determinations. The samples were collected over a period of two months (February 1999 and January 2000). All the above samples were analysed on receipt at the Department of Food Science, University of Stellenbosch by FT-NIR.

Reference analyses

The FAN content of the must samples was determined spectrophotometrically by means of an auto-analyser (Vos, 1977-1980). The sugar content (°Brix) of the must samples was determined by means of a Balling meter. Determinations of the malic and lactic acid content of the wine samples were done by means of high-pressure liquid chromatography (HPLC) (Schneider *et al.*, 1987), while the EC content of the wine samples was determined by means of gas chromatography with mass selective detection (GC/MS) according to the OIV method (Canas *et al.*, 1994).

Fourier transform near-infrared (FT-NIR) spectroscopy measurements

Fourier transform near-infrared (FT-NIR) spectroscopy analyses of the must and wine samples were carried out in transmission mode. The spectra were recorded in a 0.5 mm quartz cuvette at 4 cm^{-1} intervals with an 8-scan sequence for the must samples and a 16-scan sequence for the wine samples, using a Perkin Elmer Spectrum IdentiCheck™ 2.0 FT-NIR System. The wavelength region for all calibrations was 1000 to 2500 nm (10 000 to 4000 cm^{-1}), resulting in a total of 1501 data points per spectrum.

Data manipulation

Multiplicative scatter correction (MSC) was applied to the spectra to remove unwanted variability due to variations in particle size or path length. Without the application of MSC spectral noise would have influenced the goodness of the calibrations. The spectra were further transformed with second derivative processing. Pretreatment and calibration model development was performed using Perkin Elmer's QUANT+™ 4.1 software. The partial least squares (PLS) algorithm was used to derive calibrations for predictions in must and wine samples. Partial least squares regression can be described as a projection of the NIR spectral data and the chemical data onto a few latent orthogonal factors, retaining the main part of the information for both spectral and chemical data

(Garcia-Jares & Medina, 1997). This results in reduced spectral data without discarding useful information (Osborne *et al.*, 1993). In the PLS model both the independent (spectral data) and dependent (chemical data) variables participate in the construction of the latent variables. The latent variables of the independent set of data not only represent the original data, but are also correlated with the dependent set of data by their latent variables. Partial least square regression achieves a compromise between the explanation of the spectral variables and the prediction of the chemical variables.

Upon completion of the respective calibrations, the models were validated with independent sample sets. The spectra were randomly divided into two sets: ca. 70% of the samples were used for the calibration set and ca. 30% for the validation set. The accuracy of the calibrations was expressed as the standard error of prediction (SEP) of the bias-corrected residuals (equation 1). The bias (equation 2) is interpreted as the average difference between y and y_i in the prediction set. If the bias is near a value of zero, the overall error of validation can be interpreted as the standard deviation (SD) of the NIR prediction. Alternatively, the accuracy of the calibrations when predicting an independent set of samples was expressed as the root mean square error of prediction (RMSEP) (equation 3). RMSEP is an estimate of the accuracy of the calibration against the reference method and is calculated using an independent test set.

$$SEP = \sqrt{\frac{\sum_{i=1}^{n} (y_i - \hat{y}_i - BIAS)^2}{n-1}} \quad .....1$$

$$BIAS = \frac{1}{n}\sum_{i=1}^{N} (y_i - \hat{y}_i) \quad .....2$$

$$\text{and } RMSEP = \sqrt{\frac{\sum_{i=1}^{n} (y_i - \hat{y}_i)^2}{n}} \quad .....3$$

Where: y_i = the reference value for the i^{th} sample

$\hat{y}_i$ = the predicted value of the i^{th} sample

n = the number of samples.

The standard deviation of the reference data (SD) divided by the SEP is called the standard deviation of reference data (RPD) (equation 4) (Williams, 1991). The RPD is an indication of the efficiency of a calibration (Table 1).

$$RPD = \frac{SD}{SEP} \quad .....4$$

SIMCA classification

The spectra of the must samples for FAN classifications were divided into Class 1 (1 – 800 mg.L^{-1} N), where it might be necessary to add extra nitrogen for a complete fermentation, and Class 2 (800 – 2000 mg.L^{-1} N), where enough nitrogen is present to complete the fermentation. Principal component analyses (PCA) models were derived for the two classes and SIMCA models were created to allow differentiation between the classes. The validation set was constituted by selecting samples from the two classes prior to SIMCA model building and consisted of 12 samples. The diagnostic procedure checks every standard spectrum to ensure that the spectra from a single class fit that class (recognition) and that those from other classes selected are rejected (rejection). After diagnostics was performed on the SIMCA models, the validation set was predicted by each of the models and decisions on their affiliation were made based on their distance from the nearest cluster model. This validation procedure validates the methods (data divided into classes) that have been constructed, using test spectra that were removed from the data sets before the PCA models were built, i.e. a validation of the independent spectra.

TABLE 1

Interpretation of RPD statistics (P.C. Williams, Canadian Grain Commission, personal communication).

RPD value	Classification	Application
0.0 – 2.3	not recommended	–
2.4 – 3.0	poor	very rough screening
3.1 – 4.9	fair	screening
5.0 – 6.4	good	quality control
6.5 – 8.0	very good	process control
8.1+	excellent	any application

The three respective classes used for the three SIMCA models from the MLF wine samples were: Class 1 (0 - 0.3 g.L^{-1}), representing the samples where MLF has not started; Class 2 (0.3 - 2 g.L^{-1}), where MLF is underway; and Class 3 (> 3 g.L^{-1}), where MLF has been completed. The accuracy of the SIMCA models, derived from PCA models, was determined using an independent validation set (n = 22) to perform the future classification of unknown samples.

Principal component analyses models were also derived for the samples on which EC determinations were done. The spectra were classified into Class 1 (0 - 10 µg.kg^{-1}), Class 2 (10 - 15 µg.kg^{-1}) and Class 3 (>15 µg.kg^{-1}), based on the EC values of the samples. Class 1 represents the samples where the EC content has no regulatory or legal threat. Class 2 consists of those samples in which the EC content is close to the restricted value and should be tested to determine the exact EC value. Class 3 contains the samples where the EC content is above the restricted values. The three SIMCA models were validated by the validation set, consisting of 10 samples, after diagnostics have been performed on the SIMCA models.

RESULTS AND DISCUSSION

Sugar content and FAN

It was found that a very strong correlation existed in the sample set (combined seasons: 1999 & 2000) for the FT-NIR spectroscopic predictions of the percentage of sugar (measured in °Brix) in the must (r = 0.99, SEP = 0.31 °Brix) (Table 2, Fig. 2). The strong correlation for the percentage of sugar was expected, given that the measurement of °Brix in grape juices by NIR spectroscopy has been well established (Gishen & Dambergs, 1998). A good RPD value of 5.95 was obtained for the validation set.

TABLE 2

Summary of the results obtained from the independently validated calibrations on the FAN, percentage sugar, MLF and EC data sets.

	Independent validation				
	% Sugar (°Brix)	**FAN** ($g.L^{-1}$)	**Malic acid** ($g.L^{-1}$)	**Lactic acid** ($g.L^{-1}$)	**EC** ($\mu g.kg^{-1}$)
Range	17 – 27	590 – 2100	0 – 4.78	0 – 5.62	0.41 – 19.30
Mean	21.54	1217	1.158	1.856	5.85
SEP	0.31	294	1.024	1.345	3.6
RMSEP	0.343	351	0.967	1.102	3.51
BIAS	0.128	52.73	0.027	0.243	–
r	0.99	0.405	0.636	0.608	0.47
n (calibr.)	84	100	73	73	115
n (indep.)	43	52	36	36	56
No of PLS factors	5	1	4	4	4
SD	2.04	324.3	1.217	1.292	3.79
RPD	5.95	1.1	1.188	0.961	1.06

FAN = free amino nitrogen.
MLF = malolactic fermentation.
EC = ethyl carbamate.
SEP = standard error of prediction of the bias-corrected residual.
RMSEP = root mean square error of prediction.
BIAS = average residuals.
n = number of samples.

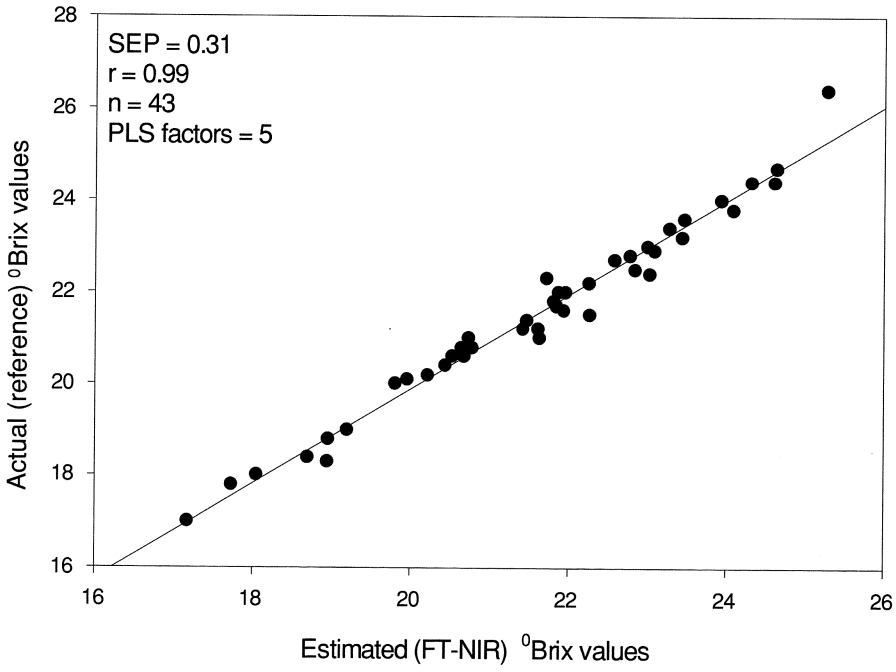

FIGURE 2

A plot of the estimated percentage sugar values (by FT-NIR) versus actual percentage sugar values (by means of ballingmeter) of the validation of must samples.

The calibrations that were established for the FT-NIR spectroscopic prediction of the FAN content in the must were not as accurate (r = 0.405, SEP = 294 $g.L^{-1}$) (Table 2, Fig. 3). The RPD value was 1.1 for the validation set, which is not recommended in terms of classification.

As a result of the poor calibration obtained with the FAN values, SIMCA classification was applied to the FAN data. The two models that were created (Class 1 with FAN values between 1 and 800 $mg.L^{-1}$ N and Class 2 with FAN values between 800 and 2000 $mg.L^{-1}$ N) showed good classification possibilities. The recognition rates were above 87% for both the data sets (Class 1 = 100%, Class 2 = 87%), indicating good separation of each class (Fig. 4).

The two models were validated on the results from the diagnostic procedure. Good results were obtained again, with recognition rates above 88% (Class 1 = 88%, Class 2 = 100%), indicating that the classification had been successful (Fig. 4).

Malolactic fermentation

The calibrations obtained for the prediction of malic acid (r = 0.64, SEP = 1.024 $g.L^{-1}$) (Table 2) and for lactic acid (r = 0.61,

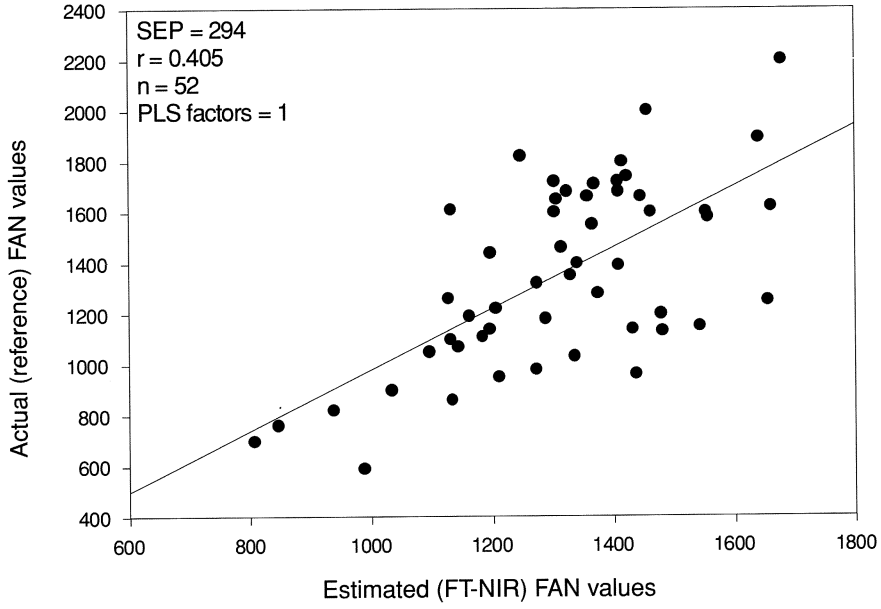

FIGURE 3

A plot of the estimated FAN values (by FT-NIR) versus actual FAN values (by means of physical analyses) of the validation must samples.

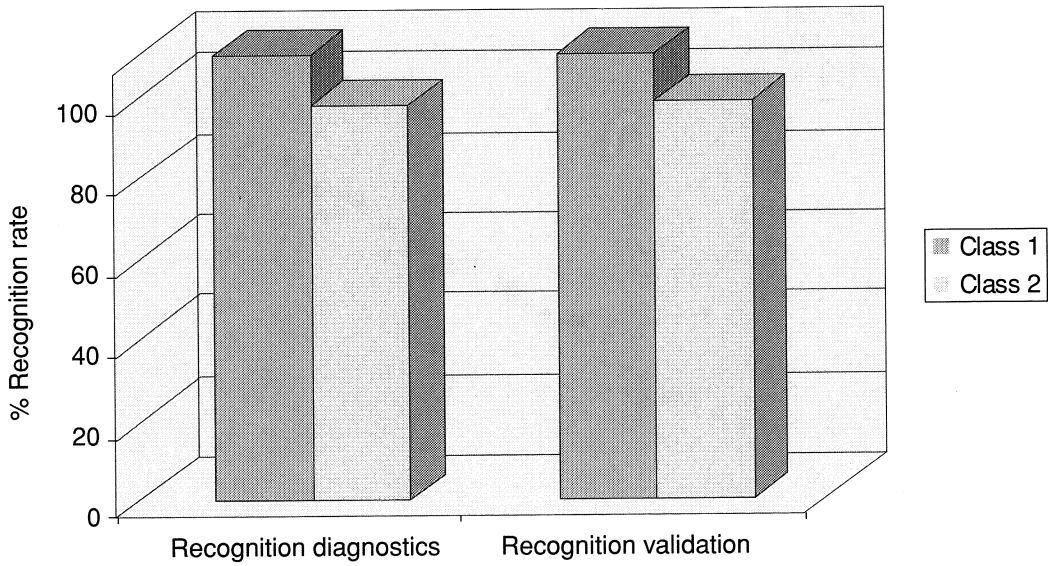

FIGURE 4

Graphic representation of SIMCA diagnostic and validation results for FAN classification. Class 1 representing the samples with a FAN value between 1 and 800 $mg.L^{-1}$ N, indicating the need to add extra nitrogen, and Class 2 representing the samples with a FAN value between 800 and 2000 $mg.L^{-1}$ N, showing sufficient nitrogen for a complete fermentation.

SEP = 1.35 g.L^{-1}) (Table 2) were not acceptably accurate. The RPD values were 1.13 for both the malic and lactic acid calibrations, confirming the inaccuracy of the quantitative calibrations.

Currently the status of the MLF is determined by means of quantitative analysis (HPLC) or qualitative analysis (paper chromatography). As it is only necessary to know whether the MLF has started, is in progress or has been completed, SIMCA methods have been constructed. Accurate classifications were possible with the three models that were created (Class 1 with lactic acid values between 0 and 0.3 g.L^{-1}, Class 2 with values between 0.3 and 2 g.L^{-1} and Class 3 with values above 3 g.L^{-1}).

Recognition rates of above 95% were reported, indicating good classification of each class (Fig. 5). Following the diagnostic procedure, the three data sets were validated using a validation set with independent spectra. Once again good results were obtained, with the recognition rates of above 88% being reported (Classes 1 & 2 = 100%, Class 3 = 88%), indicating good separation (Fig. 5).

Ethyl carbamate

Correlation that was not accurate enough for quantitative predictions occurred in the sample sets for the FT-NIR spectroscopic predictions of EC (r = 0.47, SEP = 3.60 µg.kg^{-1}) (Table 2). A RPD

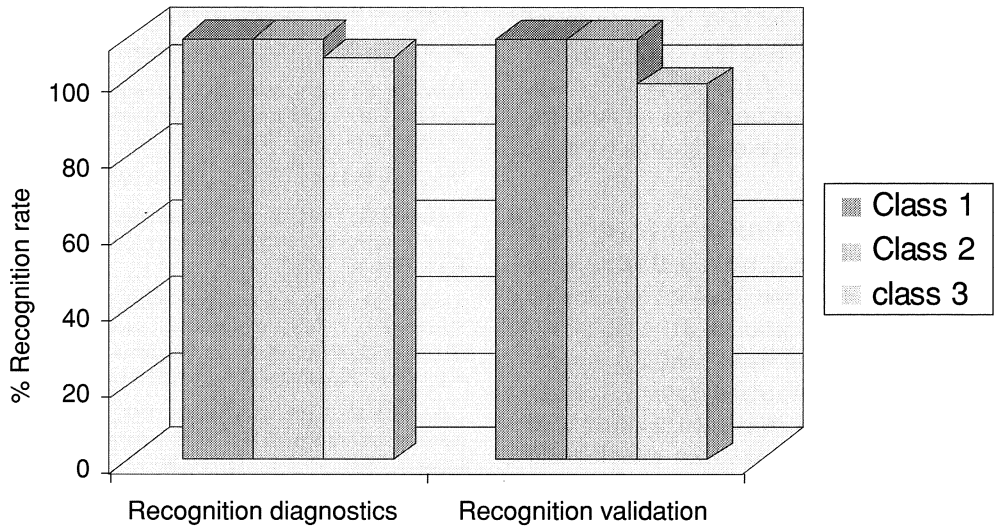

FIGURE 5

Graphic representation of SIMCA diagnostic and validation results for MLF classification. Class 1, representing the samples where MLF have not started (lactic acid values between 0 and 0.3 g.L^{-1}), Class 2 representing the samples where MLF is underway (lactic acid values between 0.3 and 2 g.L^{-1}) and Class 3 representing the samples where MLF has been completed (lactic acid values above 3 g.L^{-1}).

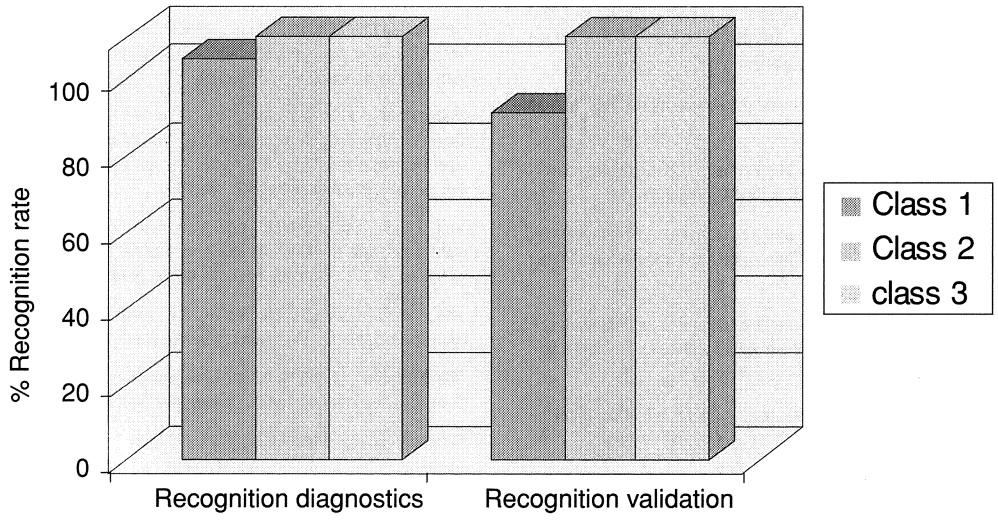

FIGURE 6

Graphic representation of SIMCA diagnostic and validation results for EC classification. Class 1 representing the samples where the EC content possess no regulatory or legal threat (EC values between 0 and 10 µg.kg^{-1}), Class 2 where the EC content is close to the restricted value and should be tested to determine the exact EC value (EC values between 10 and 15 µg.kg^{-1}) and Class 3 representing the samples where the EC content is above the restricted values (EC values above 15 µg.kg^{-1}).

value of 1.06 for the independent validation set confirmed the inaccuracy of the quantitative calibrations (Table 1).

As a result of the poor calibration obtained with the EC data sets, SIMCA classification diagnostics were applied. The three models that were created (Class 1 with EC values between 0 and 9.99 $\mu g.kg^{-1}$, Class 2 with values between 10 and 15 $\mu g.kg^{-1}$ and Class 3 with values above 15 $\mu g.kg^{-1}$) showed good classification possibilities. The recognition rate columns reported 94% for Class 1 and 100% for Classes 2 and 3 respectively, indicating that excellent separation of each class had been achieved. A summary of the verification diagnostic report is shown graphically in Fig. 6. The three data sets were consequently tested using the validation procedure in the SIMCA analysis. This procedure validates the methods (data divided into classes) that have been constructed using test spectra that were removed from the data sets before the models were built, i.e. a validation of independent spectra. Good results were obtained, with recognition rates of 80% for Class 1 and 100% for Classes 2 and 3 respectively (Fig. 6).

CONCLUSIONS

This evaluation of the applicability of FT-NIR spectroscopy in the measurement of FAN and percentage sugar, malic and lactic acids and EC classifications in must and wine shows considerable promise and may have immediate application in the wine industry. The conventional calibration method was tested, but inaccurate results were obtained, causing a shift in focal point. A classification chemometric method, SIMCA, was then applied with considerable success; it can discriminate between samples and has the potential to reduce the analysis times considerably for a range of measurements commonly used to determine the composition of samples. For many processes it is only necessary to know whether a specified cut-off point has been reached or not, and this method can therefore replace expensive, time-consuming quantitative analytical methods either completely or partially.

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