

Application Note 15: The use of a Near Infrared Transmission Analyser for Grape and Wine Quality Testing



1. Introduction

1.1 Background

The use of spectroscopy in the wine industry is well established, with many routine measurements for properties such as phenolics and colour being common applications for UV-Visible instruments. NIR on the other hand has recently gained much attention based on the versatility and ease of calibration of the method.

Based on previous work in the fruit industry, properties such as total soluble solids (TSS), pH, organic acids, moisture etc have been measured using NIR techniques and in a more recent development, NIR Technology Australia (NIRTech) in conjunction with the Australian Wine Research Institute (AWRI) have developed a portable fibre optic probe analyser, primarily for the purpose of measuring red grape colour.

The instrument operates in reflectance mode and requires a homogenisation step, which in turn requires an expensive high-speed blender. The precision and accuracy of results obtained depend on the uniformity of the sample preparation, the contact of the probe with the sample and the complete removal of air bubbles. Comments from industry suggest that this method is highly user dependent and hence has prompted NIRTech to develop a transmission based system for the measurement of grape quality.

This report outlines the preliminary studies performed on a 512-element silicon diode array NIR spectrometer, operating in transmission mode for a series of raw and finished wine products. This research was carried out in conjunction with Cabonne Vintners with the following aims:

1.2 Aims

- 1) To determine if an NIR spectrometer, operating in the wavelength region 500-1050nm can be used to determine a range of grape quality parameters based on raw juice sample presentation.
- 2) To determine if the instrument can determine the alcohol content, pH, TA and sugar of both red and white wine samples.
- 3) To determine whether colour characteristics of red wine can be measured.
- 4) To make recommendations on hardware design and sampling techniques in order to develop a universal grape and wine analyser.

1.3 Instrumentation

In a previous study, the feasibility of a transmission analyser for the measurement of grape quality parameters was investigated, and from the results obtained, it was suggested that although there were good correlations between the NIR spectra of the grape samples and the quality parameters, the wavelength range of the instrument was limited and that a fibre optic based instrument could provide the most convenient method of sampling.

The instrument used for this study was a 38-element silicon diode array spectrometer (NIT-38, NIRTech), scanning the wavelength region 720-1100nm at a resolution of 10nm. Such wavelength coverage was not optimal for measuring properties such as colour and total phenolics, as these are highly correlated to the visible portion of the EM spectrum. The instrument developed for the measurement of grape quality parameters, (Fibre Optic Probe (FOP) NIRTech), hence incorporated a spectrograph covering the wavelength region 350-1050nm. A 512-element silicon diode array detector was used for this purpose and the absorption spectra obtained were the result of averaging over 4-elements of the detector giving effectively a 128-element detector scanning at a resolution of 5.47nm.

The instrument provided to Cabonne Vintners for this study was a Near Infrared Transmission, (NIT-512) Analyser (NIRTech), covering the same wavelength region as the FOP analyser. The NIT-38 instrument, mentioned previously, is the current instrument used for the alcohol analyser. The predictive ability of this instrument is shown in figure 1.

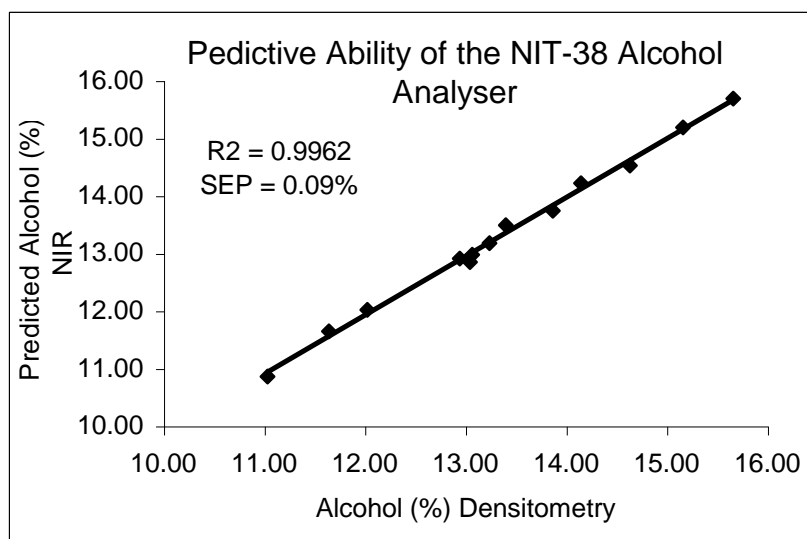


Figure 1: Performance of the NIT-38 Alcohol Analyser.

1.4 Sampling Procedures

The sample housing for the NIT-512 analyser is based around a generic design for the range of whole grain analysers produced by NIRTech and therefore is not the optimal set-up for the analysis of grape products.

Starna Cells, Inc provide a range of cuvettes suitable for the analysis of liquid samples at pathlengths of 5, 20 and 30mm. The current alcohol analyser uses a 30mm pathlength cell for analysis, and in the region 720-1100nm, provides excellent sensitivity for –OH absorption. For the analysis of highly coloured samples, such as red wine, the molar absorptivity increases by several orders of magnitude and according to the Beer-Lambert law:

$$A = \epsilon bc$$

Where: A = Absorbance
 ϵ = Molar Absorptivity
 b = Pathlength
 c = Concentration

From the above equation, Absorbance is directly proportional to Molar Absorptivity, Pathlength and Concentration. In the NIR portion of the EM spectrum, molar absorptivity is low enough to implement pathlengths as large as 30mm for liquid samples and still be in the absorbance range 0-2abs units. When measuring samples in the visible portion of the spectrum all three variables of the Beer-Lambert expression contribute to absorbance.

The sensitivity of measurement depends on the dynamic range of the detector. For the NIT-512 analyser, a study was performed to determine the limits of detection of this system using neutral density filters and it was found that the maximum absorbance reading for this instrument is 2.5abs units. This corresponds to about 0.5% light transmission.

For white grape and wine samples, pathlength considerations are not so stringent as there is no strong colour absorptions in the visible region of the spectrum measured by this instrument. The major sampling strategies to be considered in this study for red wine samples are:

- a) Pathlength Considerations.
- b) Dilution.

The sampling procedures implemented in this study are in many ways primitive and are prone to high levels of variability, but for the purposes of a preliminary study, should form the basis of any continued study. Processes such as filtration and centrifugation of samples with low clarity were compared for spectral reproducibility and will form the basis for final method development.

1.5 Spectral Results

Figure 2 shows the spectral data obtained from selected samples, chosen from the calibration set, representing both juice and wine.

The data obtained in figure 2, were all collected in a 20mm pathlength cuvette. It is obvious that red wine will require either reduced pathlength or dilution considerations if the instrumental configuration used here is to be effective, especially for colour

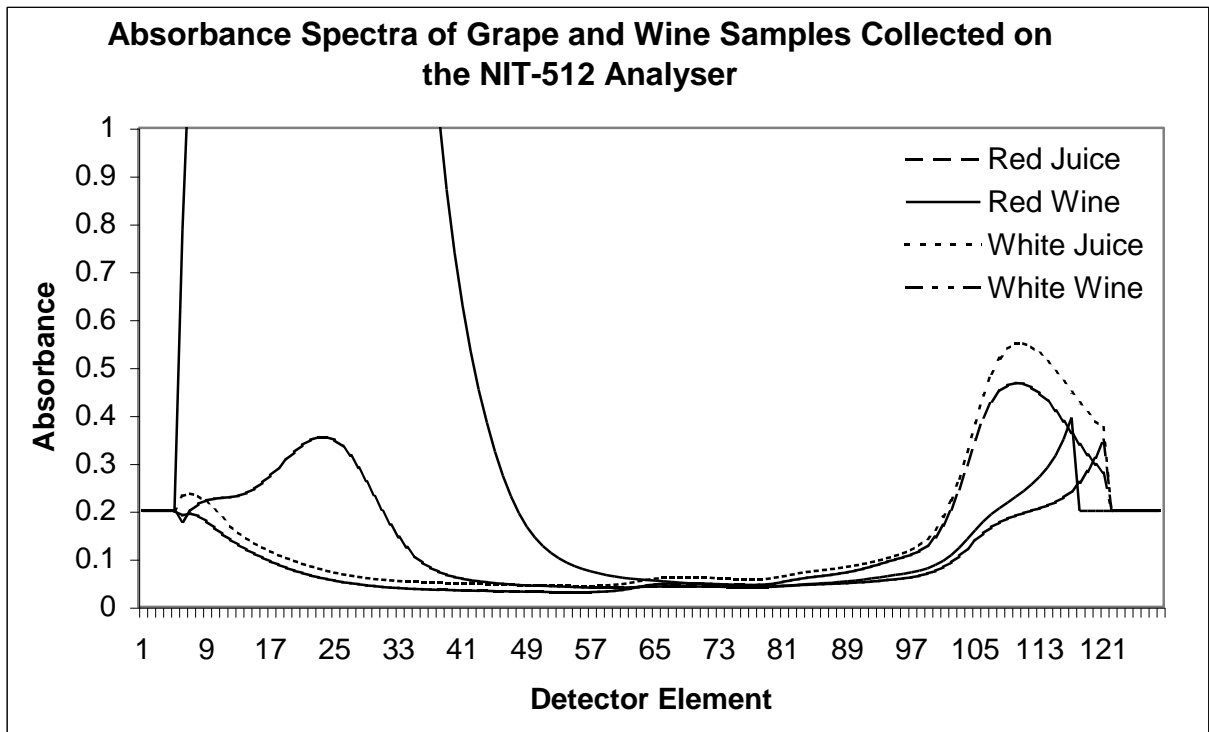


Figure 2: Spectral data for wine and juice samples.

2. Calibration

2.1 Red Juice

The parameters to be measured for red juice are as follows, pH, Total Acidity (TA) and Baume. The calibration set consisted of approximately 75 individual samples scanned three (3) times on the NIT-512 analyser. The sample presentation usually was a cloudy liquid, which was either centrifuged or filtered before spectral analysis. Partial Least Squares (PLS) regression was performed on the spectral data and reference laboratory data for all parameters measured. The calibration statistics are presented as follows.

2.1.1 pH

The calibration for pH was developed using absorbance spectra with no data pre-processing. Table 1 shows the calibration statistics for the pH calibration.

Red Juice					Calibration		Prediction	
	<i>n</i>	<i>Outliers</i>	Range	PC's	R	SEC	R	SEP
	231	35	2.8-3.3	8	0.8	0.04	0.85	0.05

Table 1: Calibration Statistics for pH determination in Red Juice.

Inspection of the spectral data obtained for these samples showed that most of the outliers occurred because of incorrect collection, i.e. the data were just noise. There are two points to note about the above data. Firstly, although the correlation for both calibration and prediction is low ($R=0.8-0.85$) the standard errors are extremely good. Secondly, the R-values may be highly effected by two variables, the laboratory data is limited and more importantly, the sampling procedure was not optimised.

2.1.2 Total Acidity (TA)

Table 2 shows the calibration statistics obtained for TA in red juice.

Red Juice	<i>n</i>	<i>Outliers</i>	<i>Range</i>	<i>PC's</i>	Calibration		Prediction	
					R	SEC	R	SEP
	231	39	7.0-12.0	8	0.90	0.23	0.96	0.24

Table 2: Calibration statistics for the determination of TA in Red Juice.

The range of laboratory values for TA is much broader, and as is the case for pH, there is a significant degree of linearity, but the low R-values, once again, may be indicating the effect of non-reproducible sampling.

2.1.3 Baume

Table 3 provides the calibration statistics for the determination of Baume in red juice.

Red Juice	<i>n</i>	<i>Outliers</i>	<i>Range</i>	<i>PC's</i>	Calibration		Prediction	
					R	SEC	R	SEP
	231	31	6.0-18.0	9	0.82	0.93	0.92	0.98

Table 3: Calibration statistics for the determination of Baume in Red Juice.

The calibration for Baume seems to show the best predictive ability of all the parameters measured for red juice. This is most likely due to the high correlation of sugar to the –OH absorption around 960nm. Better sampling should increase the predictive ability for this parameter significantly.

2.2 White Juice

White Juice	<i>n</i>	<i>Outliers</i>	<i>Range</i>	<i>PC's</i>	Calibration		Prediction	
					R	SEC	R	SEP
pH	24	20	2.7-3.8	8	0.86	0.07	0.94	0.07
TA	24	35	5.0-18.0	9	0.78	1.41	0.89	1.43
Baume	24	34	8.0-13.0	11	0.97	0.15	0.98	0.19

Table 4: Complete summary for the calibration statistics of White Juice parameters.

The sample parameters measured for red juice were also measured for white juice. Table 4 provides a summary for the calibration statistics for all parameters measured. As for red juice, calibrations were developed on absorbance spectra, with no data pre-processing, using PLS regression with the same filtration or clarification step used. Approximately 80 different samples were used for the purpose of calibration.

2.2.1pH

The predictive ability for pH is of similar quality to the red juice predictions. An SEP of 0.07 is comparable to that expected from standard laboratory measurements.

2.2.2 TA

The results obtained for TA have a much higher error associated with them compared to red juice, which is somewhat surprising as the detector operates at a higher sensitivity for less coloured samples. The calibration line showed some apparent curvature, but at this stage, it is impossible to differentiate this from actual scatter. Overall, it seems possible to determine TA based on the data obtained and more work is required to optimise this.

2.2.3 Baume

As for the case with red juice, the baume for white juice is predicted to a high level of accuracy, possibly for the same reason presented for red juice.

2.3 White Wine

The parameters of interest to be measured in white wine are alcohol, pH, TA and sugar. White wine samples are optimally measured in a 30mm pathlength cell to obtain the best sensitivity from the alcohol absorbance, but for this study, a 20mm pathlength cuvette was used. PLS regression was applied to the data as usual and table 5 presents the calibration statistics for all parameters.

2.3.1 Alcohol

White Wine	<i>n</i>	<i>Outliers</i>	Range	PC's	Calibration		Prediction	
					R	SEC	R	SEP
Alcohol	13	18	12.0-16.0	11	0.96	0.13	0.99	0.13
pH	13	19	3.0-3.5	10	0.95	0.02	0.98	0.02
TA	13	21	6.0-9.0	8	0.85	0.18	0.93	0.18
Sugar	13	48	0.0-25.0	7	0.94	0.93	0.98	0.95

Table 5: Summary of calibration statistics for white wine parameters.

The results presented in table 5 for white wine for alcohol content are consistent with the accuracy expected for the NIT-38 alcohol analyser.

2.3.2 pH

The pH results obtained are also to a high level of accuracy. The sample set is limited in range of laboratory data but both calibration and prediction have a high correlation to this reference data.

2.3.3 TA

The determination of TA in white wine shows a much better correlation than to white juice. The low correlation $R=0.85$ for the calibration data may be indicative of the limited range of sample data (6.0-9.0), but at the sample time, the SEC and SEP correspond to good predictive ability. The scatter observed in the white juice may also be indicative of sample clarity and hence these may require more careful filtration.

2.3.4 Sugar

The results of table 5 show that there is a high degree of linearity associated with the calibration of sugar in white wine. This sample set was prepared to give a large range of sugar readings, as the actual results expected for wine samples is very limited. This sample set had a range of 0-25% sugar and an SEC of 0.93. The propagation of errors may inflate the actual error based on the extended calibration set. Application of the model to those samples below 6% sugar reduces the SEP.

For the samples measured, there seems to be quite a large scatter of the data. This may also be due to inconsistent sampling, but overall, the determination of residual sugar looks promising.

2.4 Red Wine

Based on the spectral data obtained so far, even at 5mm pathlength, determination of colour in red wine is way outside the limits of sensitivity of the detector (see figure 2, red wine spectrum and figure 3 below). Without considering the visible portion of the spectral data, the quality of the NIR portion is also relatively poor compared to the data obtained for the white wine samples, which were collected at a much larger pathlength. Figure 3 shows the spectral results for red wine samples successfully diluted to get the visible portion of the spectrum in range.

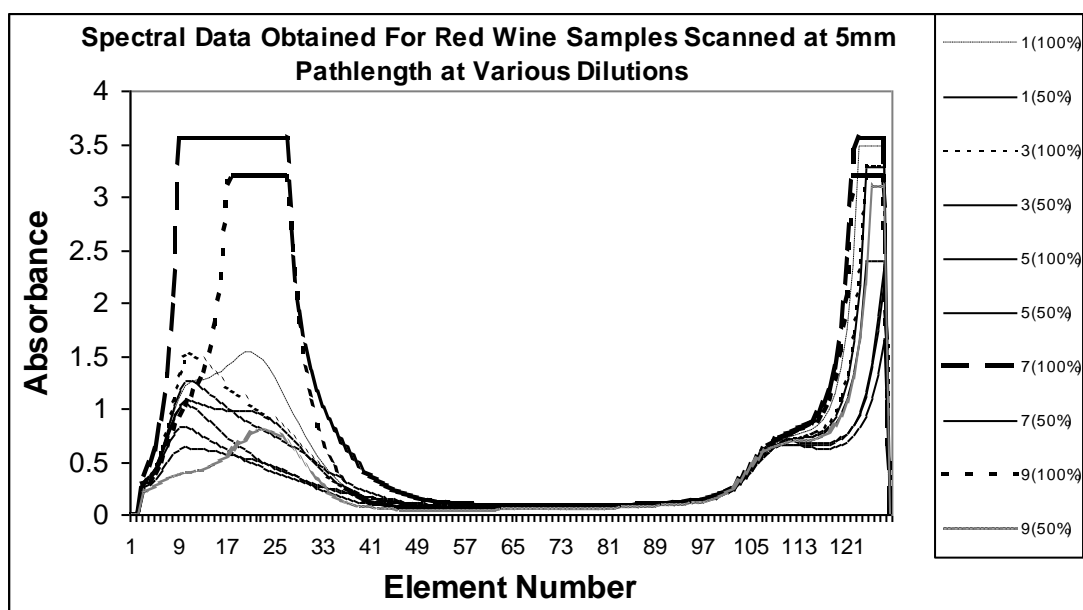


Figure 3: Spectral data obtained for red wine samples collected in a 5mm pathlength cuvette at both 100% concentration and diluted to 50%.

The spectral data of figure 3 shows that at 50% dilution in a 5mm pathlength cuvette, the visible portion of the spectrum is now measurable by the detector. Although it is feasible to do this, it is not the overall objective of a rapid analyser to add a dilution step. Inspection of the NIR region of

the spectrum also shows some deterioration around the alcohol –OH absorbance (at about 960nm or element 113 onwards). This was not observed in the white wine spectral data and this effect seems to be caused by highly coloured samples. The next section of this report proposes a new system based on the current technology used in the alcohol analyser. The proposed system will measure a wider spectral region and although some loss in resolution will result, this system should provide a much wider dynamic range for the measurement of absorbance. Table 6 shows the calibration statistics obtained for the data obtained for red wine. The parameters to be measured for red wine are the same as for white wine, with colour to be measured at a future time.

Red Wine	<i>n</i>	<i>Outliers</i>	<i>Range</i>	<i>PC's</i>	Calibration		Prediction	
					R	SEC	R	SEP
Alcohol	160	50	11.0-14.0	5	0.86	0.19	No	
pH	160	55	3.4-3.7	8	0.92	0.02	Prediction	
TA	160	58	6.0-6.8	6	0.76	0.16	Data	
Sugar	160	54	0.3-1.7	9	0.87	0.22		

Table 6: Summary of calibration statistics for red wine analysis.

Although the statistics look reasonable for the parameters measured, some required too many principal components (PC's) to describe them and the profile of the regression equation was quite "jagged" indicating over prediction was occurring.

3 . Conclusion

From the data collected in this first phase of the project, it is apparent that calibrations can be developed to measure the quality parameters associated with both white and red grape and wine products.

Preliminary calibrations show good linearity for all parameters measured, but are affected by scatter due to the sampling systems used. Another factor affecting the performance of the calibrations was the instrument itself, not being optimised for transmission measurements.

The development of a new instrument, using technology well established by NIRTech should provide a solution to the instrumental problems and the development of standard sampling techniques, possibly incorporating minimal sample pre-treatment (i.e. centrifugation, filtration etc) will lead to a high level of reproducibility, required for any analytical method.