A W R I

Development of rapid analytical methods

Purpose of the RAMS project

The Australian Wine Research Institute (AWRI) has a long history of developing applications of spectroscopic techniques for the analysis of grapes and wine. Spectroscopic techniques used or developed by the AWRI includes the ultraviolet (UV), visible (Vis), near infrared (NIR) and mid-infrared (MIR) wavelength regions of the electromagnetic spectrum. These have been used to develop methods for rapid analysis and quality control in the Australian wine sector, as well as by wine scientists and researchers.

The Rapid Analytical Methods (RAMS) project at the AWRI is responsible for developing novel and less expensive rapid analytical methods that will be useful for industrial quality monitoring and/or for research purposes. The developed applications will be based on NIR, MIR, Vis and UV spectroscopy, as well as gas and liquid sensors (e.g. chemical sensors). Rapid analytical methods currently available are summarised in the table below.

	Grapes	Juice	Wine
Total anthocyanins	√		
Total soluble solids	√	√	
G-G	√	√	
Dry matter	√		
Phenolics	√	√	√
Tannins	√		√
Basic composition (ethanol, pH, VA, TA)			√

What is infrared spectroscopy?

Infrared radiation is the region of the electromagnetic spectrum between the visible and the microwave wavelengths. The nominal range of wavelengths for near-infrared (NIR) is between 750 and 2,500 nm (13,400 to 4,000 cm^{-1}), while for the mid-infrared (MIR), the spectral range is from 2,500 to 25,000 nm (4,000 to 400 cm^{-1}). Solid, liquid or gaseous samples can absorb some of the incoming infrared radiation at specific wavelengths resulting in a 'fingerprint' or spectrum. Spectral 'signatures' in the MIR result from the fundamental stretching, bending, and rotating vibrations of the sample molecules, whilst NIR spectra result from complex overtone and frequency combinations at the shorter wavelengths. Although NIR intensities are 10-1000 times lower than for the MIR range, and the peaks concomitantly smaller, highly sensitive spectrometers can be built through several means including the use of efficient detectors and brighter light sources. This allows concentrated bulk or even aqueous materials to be scanned and analysed quickly and easily.

Although spectral peaks in the MIR frequencies are often sharper and better resolved than in the NIR, all the higher overtones (1^{st} through 6^{th}) of the OH, NH, CH, and SH bands from the MIR wavelengths are still observed in the NIR region, although much more weakly. This, in addition to the existence of combination bands (e.g. CO stretch and NH bend in protein), gives rise to a crowded NIR spectrum with severely overlapping bands. A major disadvantage of this characteristic overlap and complexity in NIR spectra has been the difficulty of quantification and interpretation from NIR spectra. On the other hand, the broad

overlapping bands can diminish the need for using a large number of wavelengths in calibration and analysis routines. In recent years, new instrumentation and computer algorithms have taken advantage of this complexity and have made the technique much more powerful and simple to use. Multivariate chemometrics such as partial least-squares (PLS) regression analysis in particular have revolutionised the ability of NIR and MIR to be used for quantitative analysis.

Advantages of rapid analytical methods based on spectroscopy

NIR spectroscopy is characterised by low molar absorptivities and scattering, which permit nearly effortless evaluation of pure materials. The NIR region of the electromagnetic spectrum, once regarded as having little potential for analytical work, has now become one of the most promising for molecular spectroscopy. The advent of inexpensive and powerful computers has contributed to the surge of NIR spectrophotometric applications. NIR spectroscopy conserves time and materials in comparison to many more conventional analytical methods because:

1. analysis times under 1 second are possible
2. simultaneous multi-component analysis is the norm
3. no sample preparation is usually required for liquids, solids, or gases
4. non-invasive and non-destructive analysis is possible
5. cost per analysis is very low (no reagents are used)
6. physical properties and biological effects can be calculated from spectra of samples
7. automated correction of background and interferences can be performed in instruments using a computer algorithm
8. detection limits can be low
9. samples sizes ranging ‘from picograms to planets’ can be analysed
10. molecular structural information can be derived from spectra

Benefits of multivariate analysis

Scientists in the food and beverage industry are faced with many different quality control tasks, including: verification of products meeting required compositional and flavour standards; identifying changes in process parameters that might lead to changes in quality; detecting adulteration in raw materials and manufactured products; and identifying geographical origin of raw materials. Traditionally, much of the research into grapes and wine has been conducted in a manner that can be described as 'univariate', since it only examined the effect (response) of a single variable on the overall matrix. Multivariate analysis was developed in the late 1960s. This was embraced by a number of research groups in the analytical and physical organic chemistry fields, due to the introduction of modern instrumentation, giving multivariate responses for each sample analysed (e.g. wavelengths, ions, mass to charge ratios, chromatographic peaks) and to the availability of computers.

Univariate models do not consider the contributions of more than one variable source and can result in models that could oversimplify the system under analysis. Therefore, in the modern scientific approach, scientists need to look at the sample in its entirety and not just at a single component, if we wish to untangle all the complicated interactions between the constituents and understand their combined effects on the whole matrix.

Multivariate analysis can take into account the variation in one variable, or a group of variables, in terms of co-variation with other variables. The analysis can mathematically describe the co-variation (degree of association) between variables, or find a mathematical function (regression model), by which the values of the dependent variables are calculated from values of the measured (independent) variables.

Multivariate data analysis research spans a wide area of different methods which might be applied in grape and wine research. There are techniques for collecting data (optimization of experimental parameters, design of experiments, calibration, signal processing) and for getting information from these data (statistics, pattern recognition, modeling, structure-property-relationship estimations). The most commonly used multivariate

data analysis techniques applied to grape and wine analysis are principal component analysis (PCA) and partial least squares (PLS) regression.

Work in progress

- Investigate and develop a wide range of rapid analytical technologies for potential adaptation to the Australian grape and wine sector.
- Investigate and explore the relationships between grape and wine composition and perceived wine quality to elucidate key impact variables.
- Availability of objective data which allow producers to understand and modulate wine / varietal styles from their region, in a global context.

Instrumentation used

Hamamatsu digital camera: Hyperspectral imaging instrument capable of obtaining images in reflectance in the Vis-NIR ranges (400 – 1100 nm) (Specim Ltd).

Thermo Nicolet 380 FTIR and Multispec (UV-VIS). This spectrophotometer operates in both UV-VIS and MIR range, fitted with two transmission cells used to analyse liquid samples.

Fieldspec Vis-NIR (ASD). This is a portable Vis-NIR (500 – 1800 nm) instrument capable to obtain reflectance spectra of different samples, fitted with a fiber optic.

Foss NIRSystems6500: This is capable of reflectance and transmission mode scanning over the Vis-NIR ranges (400 nm to 2,500 nm) and has a transport module for measurement in transmission, reflectance and transflectance cells of a wide range in sizes. In addition, a fibre optic probe is available for transflectance or interactance measurement.

Zeiss Corona: This spectrophotometer provides rapid measurement in reflectance mode over the Vis and NIR ranges.

Perkin Elmer: This spectrophotometer operates in MIR range and can be fitted with a wide variety of sample presentation modules including flow through transmission cell, and attenuated total reflectance (ATR) cell for samples on a macro or microscopic scale.

Key publications

Cozzolino, D.; Cynkar, W.U.; Dambergs, R.G.; Mercurio, M; Smith, P. (2008). Measurement of condensed tannins and dry matter in red grape homogenates using near infrared spectroscopy and partial least squares. Journal of Agriculture Food Chemistry 56:7631-7636.

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