Cooperative Research Centre for Viticulture

Australian Government
Grape and Wine Research and Development Corporation

RAPID INSTRUMENTAL METHODS

FINAL REPORT to
GRAPE AND WINE RESEARCH & DEVELOPMENT CORPORATION

Project Number: **CRCV 1.4 1.4.1**
(CRV 98/2 AWRNS)
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Research Organisation: Cooperative Research Centre for Viticulture

Date: **26 May 2006**

Project Title:	Rapid instrumental Methods At-vineyard grape quality measurement: rapid, non-destructive measures of grape quality for potential "at vineyard" applications
CRCV Project Number:	1.4 CRCV Supplementary Project 1.4.1
Period Report Covers:	July 1999 to June 2006
Author Details:	Mark Gishen AWRI
Date report completed:	June 2006
Publisher:	Cooperative Research Centre for Viticulture
ISBN OR ISSN:	

The Cooperative Research Centre for Viticulture is a joint venture between the following core participants, working with a wide range of supporting participants.

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Table of Contents:

1 Abstract **5**
2 Executive summary **6**
3 Background **8**
4 Project Aims and Performance targets **10**
4.1 Milestones from original project application **11**
4.2 Revised milestones **11**
4.2.1 Original project *11*
4.2.2 Supplementary bid project *11*
4.3 Outputs and performance targets **13**
4.3.1 Outputs and performance targets for 1999/2000. ..... *13*
4.3.2 Outputs and performance targets for 2000/2001. ..... *13*
4.3.3 Outputs and performance targets for 2001/2002. ..... *14*
4.3.4 Outputs and performance targets for 2002/2003. ..... *14*
4.3.5 Outputs and performance targets for 2003/2004. ..... *15*
4.3.6 Outputs and performance targets for 2004/2005 ..... *16*
4.3.7 Outputs and performance targets for 2005/2006 ..... *17*
5 Method **18**
5.1 Methanol in grape spirits **18**
5.2 NIR methods for determining colour, total soluble solids and pH in grapes **18**
5.3 Standardisation of analytical methods **19**
5.4 Other parameters in grapes and wine **19**
5.5 Tannin and phenolics **19**
5.6 Process monitoring applications **19**
5.7 Accelerate the uptake of NIR technology in the wine industry **20**
6 Results/Discussion **20**
6.1 NIR as a laboratory technique **20**
6.1.1 Methanol *20*
6.1.2 Grape analysis *21*
6.1.2.1 Colour, pH, TSS 21
6.1.2.2 Grape phenolics 23
6.1.2.3 Glycosyl-glucose in grapes 24
6.1.2.4 Yeast assimilable nitrogen (YAN) in white grape juice 24
6.1.2.5 Moulds — Botrytis and powdery mildew (*U. Necator*) 25
6.1.3 Wine analysis *26*
6.1.3.1 Wine colour measurement 26
6.1.3.2 Wine quality grading 27
6.1.3.3 Wine authentication and discrimination 28
6.1.3.4 Fermentation monitoring 29
6.1.3.5 Wine sensory attributes 29
6.1.3.5.1 Panel sensory scores 29
6.1.3.5.2 Wine spoilage 29
6.1.4 Other applications *29*
6.1.4.1 Yeast strain identification 29
6.1.4.2 Grapevine sap composition 30
6.1.5 Protocols *30*
6.1.5.1 Sample presentation 30
6.1.5.2 Standardising reference methods 32
6.1.5.2.1 Reference laboratory method 32
6.1.5.2.2 NIR method 33
6.1.5.3 Developing reference standards 34
6.1.5.4 Industry inter-laboratory proficiency-testing 35
6.1.5.5 IR spectroscopy users group 35
6.2 Practical application of NIR **35**
6.2.1 Commercialisation *36*
6.2.1.1 Calibrations and instruments 36
6.2.1.2 Provision of training and education programs about NIR technology 37
6.2.2 Instrumentation *37*
6.2.2.1 Custom made NIR instruments 37
6.2.2.2 Generally commercially available NIR instruments 38
6.2.3 Calibration transfer *38*

6.2.4 Process applications — On- or at-line (harvester, weighbridge, crusher, packaging line) 39
6.3 Other rapid analytical technologies 40
6.3.1 Fourier transform (FT) mid-infrared spectroscopy (MIR) 40
6.3.2 Chemical sensors—mass spectrometry-based 'Electronic nose' instruments 41
6.3.3 Combining instrumentation — the 'hybrid' approach 41
6.4 Multivariate statistical techniques 42
7 Outcome/Conclusion 42
7.1 Performance against planned outputs 44
7.2 Practical implications for industry 44
7.3 Benefits of the research 46
8 Recommendation 47
9 Appendix 1: Communication 50
9.1 Public relations and communication — Media reports (radio, newspapers, television 50
9.2 Publications and written technical communications 50
9.2.1 Scientific publications (refereed) 50
9.2.2 Technical reports and industry journal articles (non-refereed) 51
9.2.3 Draft papers in preparation (as at 26/05/2006) 52
9.3 Presentations 52
9.3.1 Conference and meeting addresses 52
9.3.2 Conference posters 55
9.3.3 Seminar and workshop presentations 57
9.3.4 Public Presentations 59
9.3.5 Other communications 60
10 Appendix 2: Intellectual Property 62
10.1 Patents 62
10.2 Calibration database 62
10.3 Know-how 62
11 Appendix 3: References 63
12 Appendix 4: Staff 63
13 Appendix 5: Data 64
14 Appendix 6: Budget reconciliation 64
CRCV Attachment 1: Project Summary & Impact Form 65
CRCV Attachment 2: CRCV Annual Report Requirements 70

1 Abstract

The project has successfully evaluated the feasibility of infrared spectroscopy technology for rapid determination of several constituents of grapes, wines and spirits. In response to the wine industry's need for rapid analytical methods for the determination of objective indicators of grape quality, the project has delivered a practical standardised methodology and collaboratively developed prototype near infrared instruments for determining colour, total soluble solids (TSS) and pH in red grapes, culminating in four patent applications being granted. A wide range of other applications, some using other technologies or combinations thereof, were also investigated for feasibility as rapid instrumental techniques.

2 Executive summary

NIR has been used routinely since the early 1990s for the determination of the ethanol content of wine. This project has demonstrated that the NIR technique shows promise as a useful tool for the wine industry for rapid determinations of several constituents of grapes, wines and spirits and, as a result, several companies have adopted the technology into their business activities (for example, more than 20% of the red grapes harvested in the 2003 vintage were analysed by means of NIR). Benefits have also been demonstrated from the use of NIR in the research environment to significantly reduce the cost of analysis and the necessity for wet chemistry in relation to research projects.

The project concentrated its efforts on the development of an NIR method for determining colour, total soluble solids (TSS), pH and total phenolics in red grape homogenates that was suitable for practical industry use. Significant challenges as to the robustness of the calibrations developed were identified and in the main resolved, as was the issue of ease of technology transfer to the broader industry. Since such measurements appear likely to be used for trade purposes (i.e. grape payment), there was a need for strict standardisation and validation of the methods of determination being used by the industry. Standard operating procedures for both the NIR and reference laboratory methods for colour have been prepared and we have also developed stable reference standards for these procedures.

The NIR technique also shows considerable promise for the semi-quantitative determinations of yeast assimilable nitrogen (YAN) and glycosyl-glucose (G-G, a potential indicator of flavour) in white grape juices, and also of the extent of fungal infection of grapes.

The NIR technique has also been shown to be able to quantify compositional changes in phenolics (colour, tannin and pigmented polymer) during red wine fermentation — a difficult challenge for any analytical technique due to the constantly changing matrix — and furthermore, qualitatively track the progress of fermentation in general.

It appears to be useful for rapid determination of the Somers' spectral measures in red wines, and for the classification of white wines (e.g. Chardonnay and Riesling) by variety.

A range of novel multivariate techniques were required for these developments and the skills developed by the project team have been successfully applied to other projects in the Australian wine research and industry technical community through collaboration and direct assistance.

Investigations into the practical aspects of implementation of the technology highlighted several significant challenges that required further detailed investigation. The range of different instrument types available, each with their own particular relative strengths and weaknesses, means that no single type is universally applicable for all applications. Calibration transfer both within- and between- instrument types appear feasible but requires further research and validation.

Whilst the development of rapid laboratory methods has been a primary aim of the project, it was also recognised that the techniques developed must also be suitable for practical use in the industry. The Cooperative Research Centre for Viticulture managed the commercialisation of the intellectual property developed by the project through their commercialisation agent, CRCV Technologies. Development of education and training material (e.g. workshops and industry standard laboratory procedures) to effect this uptake was undertaken and completed. In addition, industry trials with relatively cheap prototype instruments were conducted with some success. Early trials were limited by instrumentation that appeared not as robust as might be required for successful uptake by industry. However, more recent trials established following the injection of additional funding resources through the supplementary bid, and the dedicated efforts of the Technical Applications Manager, have shown far more promise. So much so, that a licensing and commercialisation agreement was reached and implemented. The commercialisation partner in this instance was Integrated Spectronics, a small to medium sized enterprise based in Sydney, who are continue to commercialise and develop the instruments in accordance with the plan.

Several innovations developed during the project have led to the lodging of four patents. These include patents describing stable reference materials for use in standardising NIR instruments

used for red grape analysis, spectral imaging analysis, non-destructive application of NIR spectroscopy in bottled wine, and novel chemometric calibration algorithms.

Whilst the project has focussed mainly on the use of NIR technology, it maintains a watching brief over other rapid techniques in general. Investigations into the potential of a rapid 'electronic nose' chemical sensor system showed some promise for classification, identification or blending of wines (Chardonnay and Riesling). We have also investigated during the last three years of the project, new sensors such as electronic nose devices that were investigated as rapid methods to analyse juices and wine. In particular an 'electronic nose' based on mass spectrometry (i.e. E-Nose) was evaluated for its ability to discriminate white wines of two different varieties, and also to discriminate red wines (Tempranillo) from around the world by geographical origin. However, combining data from such a sensor with that from NIR, also proved to be a powerful tool for predicting sensory attribute scores in white wines. Preliminary trials with FT-MIR (Fourier Transform Mid-Infrared) have provided more accurate measurement of some compounds that are present in relatively low concentration in grapes, such as amino acids, than NIR.

The project was collaborated extensively with some Australian Universities through co-supervision of PhD and Masters degree students: for example, James Cook University, School of Mathematics (Queensland) in areas related to novel multivariate tools to interpret spectra, and with the University of Adelaide, Department of Chemical Engineering (South Australia) in areas related to wine spectra.

Other collaborations with organisations around the country have permitted the opportunity to investigate other novel applications. For example, collaboration with Serve-AG Research in Tasmania allowed an investigation of the potential use of NIR to predict mineral composition in grapevine sap samples as part of a GWRDC-funded RITA project investigating the production of Pinot Noir at several sites across Tasmania.

The project team have also established proficiency testing programs for red grape colour testing in the Australian wine industry for both reference and NIR methods. It has also been instrumental in assisting and contributing to an industry-based 'Infrared Spectroscopy Users Group' which is attended by participants from wineries in Australia and New Zealand.

3 Background

Although Australia is recognised as being a technologically advanced wine producer, the wine industry is still seen by many of the more established chemical and food processing industries as still slightly 'cottage' in nature. The wine industry in general has not yet embraced many of the modern process engineering principles that were developed for the bulk manufacturing industries in response to a requirement for economic efficiency. Further, whilst the wine industry lags behind, most manufacturing industries have now moved even further to include environmental and social aspects to their monitoring and control systems: the so-called 'triple bottom line.'

It is clear however, that the Australian wine industry has also recognised many of these drivers for change. Many of its competitors are now developing and adopting quality and market driven industry strategies for growth that are echoes of Australia's Strategy 2025 which has been instrumental in driving the successful performance of the national industry. Continuation of Australia's advantage in the future will depend in part on efficiency of implementation of fundamental science and research outcomes. As the industry continues to grow, it becomes even more essential to focus on relatively small improvement in efficiencies and the resulting cost savings which contribute greatly to improved national industry performance and competitiveness.

To enhance further the quality of its products, the wine industry has a clear need for simple, rapid and cheap techniques for objectively evaluating the quality of grapes. The measurement of grape characteristics which impact on product quality is a basic requirement for vineyard improvement and for optimum production of desired wine styles. Therefore rapid, objective measurement of grape quality attributes is a whole-of-industry priority.

It is common industry practice for quality assessment to be achieved by total soluble solids and acidity measurement, visual assessment, and also by tasting assessment of fruit and of wines following vinification. Acidity and soluble solids measures are insufficient as quality indicators, and it is not possible to adequately assess quality by tasting alone. There is a strong need in the modern wine industry for timely information that can be used for grape berry maturity assessment, identification of vineyard blocks or sections of a vineyard that should be segregated, and load quality assessment.

Significant competitive advantage in terms of Australian wine value is expected to derive from the provision of sound information to grape growers concerning the quality and value of grapes leading to the confirmation and adoption of quality-enhancing vineyard management.

To further enhance the quality of its products, the wine industry has a clear need for simple, rapid, and cheap techniques for objectively evaluating the quality of grapes, wine and spirits. Some analytical methods for analytes used for the evaluation of quality are both time consuming and costly and this can to a certain extent hamper the pursuit of excellence in the Australian wine industry. Existing analytical methods for measurement of grape composition are not appropriate for the demands of production, where the two factors of rapidity and low costs of analysis are of paramount importance. Even simple analyses currently require samples to be sent to a geographically separate laboratory, with inherent delays in achieving results. Near infrared spectroscopy (NIR) is a correlative technique that offers the potential to reduce analytical times from hours to less than seconds in a range of measurements. The technique has been successfully used worldwide in the wine industry for the measurement of alcoholic content of wines, and for sugar in grape juice.

There is strong demand among grape and wine producers for cheap robust equipment for the routine monitoring of grape development and quality attributes. NIR appears to have the ability to provide data used for assessing quality with the capacity and speed the industry requires. The potential exists to extend the application of both NIR and other technologies to meet the requirements of grape producers. It is proposed that the most expedient means to accelerate the uptake of such technology is through commercialisation activities conducted under the management of CRCV Technologies Pty Ltd. These activities will include development of training and educational materials, but will primarily attempt to pursue the development of cheap robust field type instruments in collaboration with a commercial instrument manufacturer.

The NIR project has been, from commencement in 1997, a highly collaborative one involving corporate wine industry partners. The project was initially developed from within the AWRI's Industry Services extension activities that clearly have an excellent record in providing industry with relevant

solutions to technical challenges. Initially, BRL Hardy became involved in the preliminary trials and then joined with the AWRI to develop the project application to GWRDC in 1998. Even at that stage BRL Hardy were sufficiently interested in the potential of the technology that they purchased a research grade spectrophotometer to commence the project experimentation. As the project evolved, several other wine industry companies, including Orlando Wyndham, Southcorp/Rosemount, Mildara Blass and Simeon Wines, became involved during 1998/9 in the evaluation of project results and planning and discussion of future directions. Additional links have been established with several other companies including Yalumba Wines, McWilliam's Wines and Brown Brothers, all of whom are testing the technology and seeking assistance from the AWRI in evaluating the applicability to their own situations. In addition, all of these and other potential collaborations are being maintained and fostered through the CRCV mechanism of industry review of programs by an industry reference group. In 1999, the project was moved to the CRCV portfolio with expanded scope and objectives.

The objective of this project was:

To develop rapid instrumental techniques to improve the measurement of attributes of grapes (pre- and post- harvest), wines and spirits, specifically:

- Develop an NIR method for determining methanol in spirits
- Develop an NIR method for determining colour, total soluble solids and pH in grapes suitable for practical industry use
- Investigate the ability of NIR to measure other parameters in grapes and wine: G-G, yeast assimilable nitrogen, phenolic compounds and botrytis infection.
- Investigate the ability of NIR as a direct quality grading tool and possible means of authentication of grapes or wine

The proposed project outcomes were identified as:

- A means of objectively, rapidly and cheaply assessing vineyard and harvest load quality
- Ability to identify and segregate grape loads on the basis of measurable parameters
- Improved control of processes such as distillation, juice nutrient addition, and wine quality grading.

It was recognized very early on that this project would develop some intellectual property (IP) that might be suitable for commercialisation. The AWRI proactively and independently sought advice from a commercial consultant, and took exhaustive steps to document the chronology of the development of the IP, including an audit of the background IP, so as to ensure smooth transition in the future when developing agreements with potential commercialisation collaborators as well as supporting participants and industry partners. Preliminary discussions with several international instrument companies that had approached AWRI indicated that commercialisation issues needed to be resolved promptly as the potential value of the IP would diminish rapidly if delayed. The AWRI negotiated extensively with the CRCV in this regard to secure a viable and reasonable outcome, but unfortunately differing views of the value of the IP and the prerogatives of the AWRI and CRCV slowed progress. As a result, in August 2001, CRCV Technologies assumed full responsibility for all activities of commercialisation of the technology arising from this project. With the assistance of CRCV Technologies, a bid for extra funding — a 'supplementary bid' to the CRC program — was launched and was successful in securing more resources to assist in commercialisation activities.

Following the securing of additional funds through the 'supplementary bid' to the CRC program, the project proceeded with some new aims and objectives. The new goals were clearly stated as given below.

To develop rapid instrumental techniques to improve the pre- and post-harvest measurement of grape attributes to assist the grape and wine industry in defining and objectively evaluating the quality of its products in support of the principles of continual improvement.

Specifically, the objectives were:

- To continue the development and accelerate the uptake of cost-effective near infrared (NIR) methods for determining colour, total soluble solids and pH in grapes and transfer calibrations to instruments suitable for practical industry use. The preferred method is by the means of commercialisation activities, managed by CRCV, by means including (i) the development of a low cost instrument for rapid, non-destructive in-vineyard measurement of key grape quality attributes including colour, sugar, acid and pH, and (ii) the preparation of training and educational materials and courses about implementation of the technology;

- Standardisation of methods (NIR and relevant laboratory reference) used in the industry for grape compositional analysis;
- Investigate the ability of vibrational spectroscopy techniques including NIR and FT-MIR (Fourier Transform mid-infrared) to measure other parameters in grapes and wine: e.g. G-G in white fruit, yeast assimilable nitrogen (YAN), and phenolic compounds.
- Investigate the feasibility of application of vibrational spectroscopy techniques including NIR and FT-MIR for process monitoring (crushing, fermentation, blending and maturation);
- Investigate the potential of vibrational spectroscopy techniques including NIR and FT-MIR for wine and juice analysis (minerals, amino acids, grading, origin).

4 Project Aims and Performance targets

The aims of the initial project were:

To develop rapid instrumental techniques to improve the measurement of attributes of grapes (pre- and post- harvest), wines and spirits, specifically:

- Develop an NIR method for determining methanol in spirits
- Develop an NIR method for determining colour, total soluble solids and pH in grapes suitable for practical industry use
- Investigate the ability of NIR to measure other parameters in grapes and wine: G-G, yeast assimilable nitrogen, phenolic compounds and botrytis infection.
- Investigate the ability of NIR as a direct quality grading tool and possible means of authentication of grapes or wine

These aims were expanded following securing of additional funds from the CRC program in 2003 though a 'supplementary bid':

To develop rapid instrumental techniques to improve the pre- and post-harvest measurement of grape attributes to assist the grape and wine industry in defining and objectively evaluating the quality of its products in support of the principles of continual improvement.

Specifically, the objectives were:

- To continue the development and accelerate the uptake of cost-effective near infrared (NIR) methods for determining colour, total soluble solids and pH in grapes and transfer calibrations to instruments suitable for practical industry use. The preferred method is by the means of commercialisation activities, managed by CRCV, by means including (i) the development of a low cost instrument for rapid, non-destructive in-vineyard measurement of key grape quality attributes including colour, sugar, acid and pH, and (ii) the preparation of training and educational materials and courses about implementation of the technology;
- Standardisation of methods (NIR and relevant laboratory reference) used in the industry for grape compositional analysis;
- Investigate the ability of vibrational spectroscopy techniques including NIR and FT-MIR (Fourier Transform mid-infrared) to measure other parameters in grapes and wine: e.g. G-G in white fruit, yeast assimilable nitrogen (YAN), and phenolic compounds.
- Investigate the feasibility of application of vibrational spectroscopy techniques including NIR and FT-MIR for process monitoring (crushing, fermentation, blending and maturation);
- Investigate the potential of vibrational spectroscopy techniques including NIR and FT-MIR for wine and juice analysis (minerals, amino acids, grading, origin).

4.1 Milestones from original project application

The milestones for the project as submitted in the original application are shown below.

	Year						
	1	2	3	4	5	6	7
1. Development of calibration for measurement of methanol in spirits							
2. Development of calibration for measurement of total soluble solids and pH in grapes							
3. Development of calibration for measurement of colour in grapes							
4. Development of instrument suitable for colour, pH and TSS measurement *in situ*							
5. Development of calibration for measurement of G-G in grapes							
6. Use of NIR for quality grading of wine							
7. Development of calibration for yeast assimilable nitrogen in grape juice							
8. Development of calibration for grape and wine phenolics							
9. NIR screening of grape loads for botrytis							

4.2 Revised milestones

The project milestones have been amended twice throughout the life of the project. The original project was revised with some delays due to technical difficulties encountered quite early in the project. Secondly, a 'supplementary bid' for extra funds was mounted in 2003 to boost the funding resources for commercialisation activities. This was required as it was becoming apparent that the project had generated significant intellectual property of potential commercial value, and that the opportunities presented could be missed without adequate resource allocation. Therefore, all the milestones were reviewed and amended in line with the new direction of the project. Each of these revisions are set out below.

4.2.1 Original project

The milestones were modified and consolidated in the final version of the original project that was accepted and those modifications are given below.

Progress Against Milestones								
Milestone	Year							Revised Date for Achievement of Milestone
	1	2	3	4	5	6	7	
Development of calibration for yeast assimilable nitrogen in grape juice	----------							December 2004
Investigate use of NIR for screening of grape lots for Botrytis and other negative quality parameters	----------							June 2006

4.2.2 Supplementary bid project

After inclusion of the successful 'supplementary bid' proposal submitted in 2003 all the milestones were amended as given below. The project was defined as two 'streams' to reflect the differing emphasis, with the new commercialisation milestones being listed as a sub-project.

Project 1.4 Rapid instrumental measurement techniques

	Milestone	Year							Progress	Revised Date for Achievement of Milestone
		1	2	3	4	5	6	7		
1	Development of NIR calibration for measurement of methanol in spirits.	›	›						Achieved	
2	Development of NIR calibration for measurement of total soluble solids, pH, colour in grapes.	›	›	›	›	›	›		Achieved	
3	Investigate NIR and electronic nose system for predicting wine sensory attributes/quality grading using grape and wine samples.	›	›	›	›	›	›		Achieved	
4	Development of NIR instrumentation and sampling protocols.	›	›	›	›	›	›	›	Achieved	
	Sub project S1.4.1 Rapid, non-destructive measures of grape quality for potential "at vineyard" applications									
1	Development, if possible, of robust portable NIR devices for in-vineyard measurement of red grape colour, TSS, pH and total acid					›	›	›	Achieved	
2	Determine the potential of on-line and on-harvester application of NIR techniques for red colour of grapes					›	›	›	Achieved	
3	Development of training and course material for colour measurement of grapes					›	›	›	Achieved	
4	Investigate use of NIR for screening of grape lots for Botrytis and other negative quality parameters.					›	›	›	Achieved	
5	Assessment of the feasibility of FT-MIR for the determination of G-G, YAN, phenolics and wine quality grading.					›	›	›	Achieved	

4.3 Outputs and performance targets

Outputs and performance targets for each year are given in the following sections.

4.3.1 Outputs and performance targets for 1999/2000.

Output	Performance Target
1. Publication in international conference proceedings describing preliminary NIR studies.	• Submission by September 1999.
2. A method for measurement of methanol in spirits by NIR.	• Submit a manuscript to a refereed journal on the methanol application by July 2000.
3. A robust method for determination of total soluble solids and colour in red grapes by NIR.	• Have analysed by both NIR and lab methods >400 berry samples of a number of red varieties from each of several regions, at a range of maturity levels, and generate a calibration equation by July 2000.
4. Information on glycosyl-glucose (G-G) determination by NIR. An evaluation of the feasibility of NIR for measurement of G-G.	• Have scanned samples of known glycoside composition, plus >100 berry samples of red and white varieties at a range of maturity levels, and develop a calibration equation to determine the feasibility of NIR for this application, by July 2000.

All targets were met with the exception of output 2 which was delayed until late 2001.

4.3.2 Outputs and performance targets for 2000/2001.

Output	Performance target
1. Have produced a laboratory method for analysing grape samples for colour, pH and total soluble solids	• Have published a paper in a refereed journal and also an industry journal article disclosing the colour, pH and TSS NIR method for the laboratory, and potentially the G-G analysis also, by July 2001.
2. Have produced a method for analysing grape samples for colour, pH and total soluble solids with minimal sample preparation and using a relatively cheap fibre optic probe equipped instrument suitable for broad industry use.	• Have analysed by NIR and by laboratory reference methods greater than 400 berry samples using minimal sample preparation, ideally in a harvester bin, and generate a calibration equation suitable for transfer between instruments by July 2001.
3. Information on the applicability of NIR for quality assessment, varietal and regional discrimination of wines.	• Have obtained and scanned >500 wines of a range of styles and varieties, obtain tasting data, and have investigated the suitability of classification models, by December 2000.
4. Information on phenolics determination by NIR. An evaluation of the feasibility of NIR for measurement of grape seed and wine phenolic composition.	• Have scanned >50 red wine samples that have also been analysed for phenolic composition using UV-Vis spectrophotometric methods and HPLC, plus >50 Shiraz seed samples at a range of maturity levels, and develop calibration equations to determine the feasibility of NIR for this application, by July 2001.
5. Information on determination of degree of botrytis infection in a grape lot by NIR. An evaluation of the feasibility of NIR for measurement of botrytis.	• Have carried out mixture experiments with heavily infected grapes and healthy, uninfected grape lots, by December 2000.

All targets were achieved with the following exceptions:

- Output 1 — The publication of the NIR method for colour, pH and total soluble solids was delayed due to matters surrounding intellectual property until April 2002.

- Output 3 — This was delayed in order to further pursue the potential of the G-G assay for the prediction of wine quality.
- Output 5 — The lack of availability of a reliable reference method meant that this was delayed until June 2001.

4.3.3 Outputs and performance targets for 2001/2002.

Output	Performance target
1. Have produced a laboratory method for analysing grape samples for colour, pH and total soluble solids	• Have published a paper in a refereed journal and also an industry journal article disclosing the colour, pH and TSS NIR method for the laboratory, and potentially the G-G analysis also, by April 2002. • Have evaluated calibration transfer issues and determined if a universal calibration can be developed, by having scanned and analysed greater than 1000 homogenised berry samples from the 2001 season, by December 2001. • Have carried out two workshops as part of 11th Australian Wine Industry Technical Conference (October 2001) to an audience of at least 40 conference attendees (chemometrics/NIR calibration and measurement of grape quality parameters)
2. Have produced a method for analysing grape samples for colour, pH and total soluble solids with minimal sample preparation and using a relatively cheap instrument suitable for broad industry use.	• Have analysed by NIR and by laboratory reference methods greater than 200 whole berry samples by December 2001
3. Information on the applicability of NIR for quality assessment, varietal and regional discrimination of wines.	• Have obtained and scanned >30 Riesling juices and wines, obtain terpene and G-G analyses, tasting data, and have investigated the suitability of prediction models, by June 2002.
4. Information on determination of degree of botrytis infection in a grape lot by NIR. An evaluation of the feasibility of NIR for measurement of botrytis.	• Have carried out experiments with heavily infected, moderately infected, and uninfected grapes, by March 2002.
5. Assess the feasibility of using NIR for the prediction of yeast assimilable nitrogen in white grape juice.	• Evaluate and optimise sample presentation and scanning options using synthetic media by December 2001.

All targets were achieved with the following additions:
- Output 2 — Field trials with beta-version prototype instruments were conducted, and extended to include in-situ measurement.
- Output 5 — Experiments were extended to include evaluation of the FT-MIR technique.

4.3.4 Outputs and performance targets for 2002/2003.

Outputs	Performance Target
1. Information on glycosyl-glucose (G-G) determination by NIR. An evaluation of the feasibility of NIR for measurement of G-G.	• Developing calibrations for red (>400 samples) and white (>300 samples) grapes and evaluate accuracy for usefulness by July 2003.

Outputs	Performance Target
2. Assess the feasibility of using NIR for the prediction of yeast assimilable nitrogen in white grape juice	• Evaluate and optimise sample presentation and scanning options using synthetic media (2001). If feasible, test on juice samples from vintage 2002 and evaluate calibration accuracy by December 2002.
3. Information on determination of degree of mould infection in a grape lot by NIR. An evaluation of the feasibility of NIR for measurement of botrytis and powdery mildew (in collaboration with project 1.5.2).	• Have carried out mixture experiments with heavily infected grapes and healthy, uninfected grape lots, by July 2002. Comparison between DNA probe (UA), ELISA (CSIRO PI) and NIRS (AWRI) methods underway by June 2003.
4. Evaluate the potential feasibility of fast diode array type (Vis/NIR) spectrometers for use in on-line (harvester, weighbridge, crusher) applications and purchase suitable hardware for trials*	• Trial alternative rapid spectrometers in the laboratory to determine sample presentation requirements in collaboration with other CRCV projects (Bramley/Lamb).
5. Investigate the options to effecting calibration transfer both between and across laboratory-based research grade instruments and robust affordable field types.	• Evaluate advanced chemometrics routines and software (starting with WinISI) and advancing to other software packages where appropriate.

All targets were achieved with the following additions:

- An invited chapter on "Application in analysis of beverages and brewing products" was submitted to a monograph on "Near infrared spectroscopy in agriculture", published by the American Crop Science, Agronomy and Soil Science Societies.
- Testing and evaluation of the ARRM Bioprep system was completed.
- As a result of industry feedback, a detailed experiment was established to investigate the possible influence of different homogenisers and sample storage status (i.e fresh/frozen) before homogenisation on the determination of total anthocyanins by the reference laboratory method.

4.3.5 Outputs and performance targets for 2003/2004.

Output	Performance Targets	Date
1. Commercialisation (CRCVT) — develop, if possible, robust portable NIR devices for in-vineyard measurement of red grape colour, TSS, pH and total acid.	• Select the most appropriate commercial instrument manufacturer (company X) and establish a collaborative research agreement with appropriate milestones and performance targets • Provide technical support to manufacturer to assist in the preparation of prototype instruments • Assist in the conduct of industry trials of prototype instruments • Evaluate performance of prototype instruments and prepare report	**July 2003** **July 2003 onwards** **As reqd.** **June 2004**
2. Commercialisation (CRCVT) — process monitoring applications	• Conduct laboratory simulations of process conditions to determine the potential of on-line and on-harvester application of NIR techniques for red colour of grapes	**Dec 2004**
3. Commercialisation (CRCVT) — existing IP including calibration sets	• Establish agreements with commercial instrument manufacturers that wish to commercialise existing IP	**July 2003**

Output	Performance Targets	Date
4. Commercialisation (CRCVT) — training and course materials; Standardisation of analytical methods used by the industry in determining parameters used for payment purposes	• Prepare technical notes on the application of reference laboratory techniques for colour measurement of grapes with details on standard procedures for processing and sample handling • Prepare technical notes on the application of NIR techniques for colour measurement of grapes with details on standard procedures for processing and sample handling • Develop a standard or reference material for colour analysis by NIR and reference laboratory methods	**Dec 2003** **Dec 2003** **June 2004**
5. Information on the effect of vintage on the robustness of NIR calibrations for colour, TSS and pH	• Publish in a refereed journal, a paper describing the application of NIR for measurement on colour in red grapes	**June 2004**
6. Evaluation of appropriate methods of transfer of NIR calibrations both within- and across-platform of instrument type	• Assess the efficacy of Shenk's instrument standardisation technique • Purchase artificial neural network software and assess its application to the spectral dataset	**Dec 2003** **June 2004**
7. Evaluate the potential of application of NIR and FT-MIR for wine quality grading, classification and origin	• Assess the feasibility and compare the application of NIR and FT-MIR for prediction of wine quality grading	**June 2004**

All targets were achieved with the following exception and additions:

- Outputs 1 and 3 — Delays in establishing a commercialisation agreement with a partner meant that this could not be achieved.
- Materials that could potentially be used for instrument matching to enable transfer of calibrations for the determination of the concentration of total anthocyanin were developed and investigated.
- The ability of NIR to measure heat-unstable protein in wine was examined although the results were inconclusive.
- Through collaboration with a study of sensory/chemical properties of Chardonnay and Riesling wines (PhD student, Heather Smyth) it was demonstrated that NIR could be used to discriminate between the varieties.

4.3.6 Outputs and performance targets for 2004/2005

Output	Performance Targets	Date
1. Commercialisation (CRCVT) — develop, if possible, robust portable NIR devices for in-vineyard measurement of red grape colour, TSS, pH and total acid.	• Provide technical support to manufacturer to assist in the preparation of prototype instruments • Assist in the conduct of industry trials of prototype instruments • Evaluate performance of prototype instruments and prepare report	**July 2003 onwards** **As reqd.** **June 2004**
2. Commercialisation (CRCVT) — process monitoring applications	• Conduct laboratory simulations of process conditions to determine the potential of on-line and on-harvester application of NIR techniques for red colour of grapes	**Dec 2004**

Output	Performance Targets	Date
3. Commercialisation (CRCVT) — training and course materials; Standardisation of analytical methods used by the industry in determining parameters used for payment purposes	• Prepare and conduct workshops at 12 AWITC on (i) introduction to IR spectroscopy, (ii) applications of IR spectroscopy in the wine industry, (iii) chemometrics and calibration of NIR instruments • Survey industry for their interest in workshops on the implementation of NIR analytical techniques for grape quality monitoring	**July 2004** **June 2005**
4. Evaluate the feasibility of spectroscopic techniques (NIR and FT-MIR) for measurement of grape seed, skin and wine phenolic composition	• Scan and analyse by the reference methods samples from up to 50 different fermentations (in collaboration with AWR 6) and develop a calibration.	**Dec 2004**
5. Evaluation of the potential application of spectroscopic techniques for determination of other parameters in grapes	• Assess the feasibility of NIRS and FT-MIR for measurement of G-G in white grapes (juice)	**June 2005**
6. Evaluation of the potential of spectroscopic techniques for fermentation monitoring applications	• Assess the feasibility of monitoring red wine fermentations for the evolution of colour	**June 2005**
7. Evaluate the potential of application of the CIELab colour measurements for grape colour specification	• Acquire and apply calculation software to predict CIELab values directly from spectra of grape samples	**June 2005**
8. Evaluate the potential of application of NIR and FT-MIR for wine quality grading, classification and origin	• Assess the feasibility and compare the application of NIR and FT-MIR for prediction of wine quality grading	**June 2005**

All targets were achieved with the following exception and additions:

- Ouput 5 — The feasibility assessment for the G-G application was not completed, however, instead a calibration for the prediction of yeast assimilable nitrogen in white grape juice was developed for the FOSS *WineScan* instrument.
- Some preliminary experiments examining if Brettanomyces-spoilage could be detected and quantified using IR spectroscopy was undertaken and showed considerable promise.

4.3.7 Outputs and performance targets for 2005/2006

Output	Performance Targets	Date
1. Commercialisation — develop, if possible, robust portable NIR devices for in-vineyard measurement of red grape colour, TSS, pH and total acid.	• Provide technical support to manufacturer to assist in the preparation of prototype instruments • Assist in the conduct of laboratory trials of concept (i.e. 'bread board') prototype instruments, including trials in sample presentation options • If feasible, refine prototype design (Integrated Spectronics) • Evaluate performance of 2nd generation prototype instruments and prepare report (Integrated Spectronics) • Review any agreements made to ensure continuity beyond the lifetime of CRCV2; renegotiate where necessary	**March 2004 onwards** **As required** **Dec 2005** **April 2006** **May 2006**

Output	Performance Targets	Date
2. Commercialisation — process monitoring applications	• Conduct field trials of those applications determined to be feasible for on-line application	**June 2006**
3. Commercialisation — existing IP including calibration sets	• Review any agreements made to ensure continuity beyond the lifetime of CRCV2; renegotiate where necessary	**Feb 2006**
4. Commercialisation — training and course materials	• If sufficient interest exists, develop and conduct at least one 'research-to-practice' style workshop	**June 2006**
5. Information on the effect of vintage on the robustness of NIR calibrations for colour, TSS and pH	• Submit for publication in a refereed journal, a paper describing the application of appropriate techniques to ameliorate the effects of non-linearity and matrix effects in red grape analysis by NIR • Determine the relative change in error of prediction on new season's grape samples using a cumulative calibration dataset over the years 1999 to 2006	**June 2006** **June 2006**
6. Evaluation of the potential application of spectroscopic techniques for determination of other parameters in grapes.	• Assess the feasibility of FT-MIR for the determination of YAN and, total phenolics and botrytis	**June 2006**

All targets were achieved with the following exception:

- Output 6 — This output had been achieved ahead of schedule in 2004/2005.

5 Method

The research methodologies used in the project are outlined below under the various headings of the broad objectives.

5.1 Methanol in grape spirits

Development of calibration for measurement of methanol in grape spirits:

A range of spirit samples from distillation houses around the country were analysed for methanol content by traditional means (i.e. gas chromatography) and the reference data correlated with NIR spectra for the samples. In addition, some samples from operating plants during distillation (i.e. in-process samples) were also analysed. Standard additions of methanol were made to some samples to ascertain the recovery by both the reference and NIR methods. The NIR calibration was developed using about 100 samples, and then validated by testing another 20 different samples.

5.2 NIR methods for determining colour, total soluble solids and pH in grapes

To develop cost-effective NIR methods for determining colour, total soluble solids and pH in grapes and transfer calibrations to instruments suitable for practical industry use (in the laboratory and in-situ):

The aim was to produce a robust, accurate calibration with the simplest possible sample preparation. This was targeted at the various end user sectors in the wine making process chain - in-field, on-harvester, at-line, at-crusher, on-line, in-laboratory. Assessment of a range of instruments including simplified cheaper instruments as well as the more sophisticated laboratory-based instruments was undertaken in order to assess the appropriateness of available technology. A most important variable in the robustness of the calibration appears to be the between-season variation, and it is also recognised that it will be necessary to assess between-region and between-variety effects. This was tackled using statistical techniques to ensure that the main effects are identified and targeted for efficient use of research resources. The various established and emerging techniques of calibration transfer were investigated to assess the practicality of within- and cross-platform transfer between instruments of a similar type (i.e. *FOSS NIRSystem6500*). These may include assessment of various software techniques including artificial neural networks, genetic algorithms, locally weighted regression, and database matching (constituent and spectral) techniques.

5.3 Standardisation of analytical methods

Standardisation of methods (NIR and relevant laboratory reference) used in the industry for grape compositional analysis:

The increasing use of colour measurement (by NIR and standard chemical techniques) as a means of modulating payments made to growers by wineries for grape deliveries has meant an increased need for standardisation in line with other 'for trade' measurements used in agriculture. The reference laboratory measurement techniques and procedures will need to be standardised in order to ensure that the industry is full conversant with all aspects of uncertainty and error in the measurements. A complete evaluation of all processing steps and procedures was undertaken to quantify the effects of variations from the standard protocols. This applies equally to correlative techniques that are developed, such as NIRS methods. Any instrumentation used in measurement by correlative techniques will also need to be capable of standardisation in order to maintain a level playing field in 'for trade' measurements.

5.4 Other parameters in grapes and wine

Investigate the ability of NIR and other vibrational spectroscopy techniques to measure other parameters in grapes and wine: e.g. G-G, yeast assimilable nitrogen, and fungal infection:

Glycosyl-glucose: Consolidation of spectral and reference data allowed evaluation of the feasibility of the NIR technique. In addition, the technique of FT-MIR (Fourier transform infrared) spectroscopy was attempted using a novel attenuated reflectance (ATR) sample presentation device.

Yeast assimilable nitrogen (YAN) in grape juice: Preliminary work with NIR technique had shown promise but the accuracy appeared to be somewhat limited to an indicative type of calibration. Therefore, the application of the FT-MIR technique was attempted and the feasibility assessed. The development of an acceptable calibration will mean that routine screening of every tank of juice before inoculation will be possible at minimal cost, and allow optimal control of juice nitrogen content.

Botrytis In the first instance, blending of infected, sporulating berries with clean, disease free fruit of the same variety was carried out to assess the degree to which NIR can detect botrytis infection. Further work in collaboration with researchers involved in Project 1.5.2 of the CRCV investigated the development of an adequate system for reliable botrytis assessment.

Powdery Mildew Grapes with varying degrees of infection were scanned and correlated with reference data obtained by molecular methods in collaboration with Project 1.5.2 of the CRCV. Commercial samples with various degrees of infection were also examined.

Quality grading: Some preliminary work has shown that NIR might have potential for rapidly grading of wines in commercial situations. Using commercial samples and winery sensory data or product allocation scores, the technique was assessed in the first instance for semi-quantitative use (i.e. classify LOW, MEDIUM and HIGH grades of wines). This approach was extended to include FT-MIR and investigation of critical wavelength loadings in order to further understand and identify the major impact constituents of the wine. In a further extension to the work, sensory data were used to investigate the feasibility of the technique for predicting 'panel scores' of sensory attributes.

Other parameters: A range of other applications had been proposed including those in microbiology — prediction of propensity for fermentations to 'stick', yeast culture physiology, colour and phenolic adsorption to yeast lees, culture identification, and hybrid yeast screening. Some of these have been shown to be very promising.

5.5 Tannin and phenolics

Investigate the suitability of NIR and other vibrational spectroscopy techniques to measure tannin and phenolics in grapes, fermentations and finished wines:

In collaboration with the tannin research project team (project 1.2), the potential of spectroscopic techniques was investigated. The initial focus was on the monitoring of fermentation for the evolution of colour during the process, but also several other compositional parameters of interest including seed phenolics composition in seeds, seed homogenates and seed extracts, the concentration of catechin and other monomeric species, colour, pigmented polymers and tannins. Grape, juice and fermentation samples were analysed using both standard spectrophotometric and HPLC methods for a study as part of the tannin research project were scanned using NIR and FT-MIR. Development of calibrations was undertaken to evaluate the feasibility and practicality of the technique for fermentation monitoring. Further studies involving collaboration with industry partners were undertaken to examine blending and maturation operations for potential application of the technology.

5.6 Process monitoring applications

Technology evaluations—investigate the feasibility of application of NIR and other vibrational spectroscopy techniques for process monitoring (crushing, fermentation, blending and maturation):

An obvious extension to the developed laboratory techniques is the application of the methods at the crushing operation where in-line monitoring of grape quality composition can influence processing decisions in real time. The feasibility of this approach was attempted in practical situations. The initial work tested a range of different instrument types and configurations in order to find the most appropriate technology for each specific application. This allowed the targeting of particular technologies to the best niche end-use as appropriate. Extending this application to on-harvester situations was attempted through collaborative efforts with Rob Bramley (CSIRO Land and Water) initially, and later through collaborations with Provisor and a commercial entity (Computronics).

5.7 Accelerate the uptake of NIR technology in the wine industry

To accelerate the uptake of NIR technology in the wine industry and to develop, if appropriate and viable, robust, low cost, portable NIR devices for in-vineyard measurement of red grape colour, total soluble solids, pH and total acidity:

These commercialisation activities were directed and managed by CRCV Technologies Pty Ltd — the commercialisation vehicle of the CRCV. In particular, the expertise and experience of the Technical Applications Manager, Mr Ian Atkinson, was instrumental in securing appropriate commercial arrangements. An appropriate commercial partner (Integrated Spectronics was selected as 'Company X') helped develop the technology to meet the aims of the project — to apply the technology for direct in-vineyard use. Integrated Spectronics have experience and expertise in commercialisation of IR technology for laboratory as well as remote sensing applications and provided hardware and software design and engineering expertise, marketing, and project management. They also provided their own manufacturing and testing facilities to produce and validate prototype instruments prior to implementation in field trials. Field-testing of prototype instruments were conducted in collaboration with corporate wine industry participants in the project.

In addition, there was the potential to more fully exploit the intellectual property that has been generated in the project previously especially in relation to datasets that were collected using instruments other than the *FOSS NIRSystem6500*. However, it is clear that any such agreements with any other companies will need to be viable in their own right and not jeopardise the existence and functioning of the primary collaboration with Integrated Spectronics.

Another aspect of commercialisation is the 'softer' approach of the development of a training course and guidance materials on the use of NIR. These have included the preparation of technical notes on the application of both NIR and reference laboratory techniques for colour measurement of grapes with details on standard procedures for processing and sample handling, developing and conducting workshops at 12 AWITC on (i) introduction to IR spectroscopy, (ii) applications of IR spectroscopy in the wine industry, (iii) chemometrics and calibration of NIR instruments, and the development and delivery of 'hands-on' style workshop on the implementation of NIR in the wine industry.

6 Results/Discussion

6.1 NIR as a laboratory technique

6.1.1 Methanol

In the production of fortified wines, Australian legislation demands the use of only grape-derived ethanol (Australian Food Standards Code, Regulation P4, 2001, Australia and New Zealand Food Authority). Wine fortifying spirit, known as SVR (from the Latin *spiritus vini rectificatissimus),* is generally produced in commercial facilities from the distillation of by-products of the winemaking process, rather than from finished wine. SVR commonly contains around 96% v/v ethanol with low concentrations of other volatile compounds. A major source of grape alcohol is from grape pomace – this is waste from a pressing step in wine production and consists of predominantly skins, seeds and stems, but contains sufficient ethanol or fermentable sugar to warrant recovery. Methanol is one of the major undesirable contaminants in SVR. As a result of the storage conditions before distillation, bacterial and fungal activity can result in production of methanol in grape pomace. The use of this type of material for the production of wine fortifying spirit is therefore one of the major sources of methanol.

In the commercial production of SVR, the distillation process must be carefully monitored to minimise methanol carry over in the ethanol fractions, particularly when distilling material with high methanol concentration. Monitoring methanol concentrations has become particularly critical after the lowering of the permissible levels of methanol in wine fortifying spirit from 8 g/litre of alcohol to 3 g/litre of alcohol (Australian New Zealand Food Authority Code).

A common method for measuring methanol in wine and fortifying spirit is gas chromatography (2, 3, 4, 5, 6). This is a relatively complex and slow procedure, requiring an experienced analyst. Most large commercial spirit production facilities use continuous distillation equipment that requires careful adjustment to achieve a steady state producing spirit of the required low methanol content. Rapid analysis of the spirit composition is therefore required to expedite distillation process monitoring.
Gas chromatography can be slow (up to 40 samples per day) and expensive (e.g. $94 per sample, AWRI Analytical Service 2002-2003). NIR instruments using a reliable calibration could reduce the time of analysis to less than a minute per sample and reduce the operating (i.e. quality control) costs of distillation dramatically.

An NIR calibration for the determination of the concentration of methanol in spirits has been developed. This was achieved by applying the technique to a large set of distillate (SVR) samples obtained from a large commercial continuous still and was validated using spirit samples from three vintages and from a further two commercial stills. The outcome of this investigation is that companies involved in distillation can achieve substantial savings in analysis cost and time due to the speed and simplicity of the NIR approach. Having immediate availability of compositional data on still fractions will also provide an improvement in the control and optimisation of continuous stills and there is potential to perform online monitoring.

- ***The ability of NIR to measure methanol in spirits has been established.***

6.1.2 Grape analysis

The suitability for NIR as a method for industry uptake will be dependent on careful consideration of the level of accuracy required. Considering the speed of analysis, it is likely that a slightly reduced degree of accuracy is more than compensated for by the ability to measure many more samples in a given period, so that for example during routine grape maturity testing an improved assessment of the whole vineyard can be made.

6.1.2.1 Colour, pH, TSS

It is evident from extensive practical experience by wineries, as well as from research studies (see Institute publications of Somers and co-workers i.e. reprint numbers 127, 151, 233, 283, 285, 333, and Francis et al 1999), that red grape colour measurement (i.e. the concentration of total anthocyanins) is a practically useful indicator of wine quality for some specific wine styles. There is growing interest in setting payment levels by using colour as a contributing objective specification parameter.

The conventional method for colour analysis of red grape berries requires several steps (Iland et al 1996):

1. homogenisation of the berry sample, followed by
2. a one hour solvent extraction step;
3. centrifugation;
4. adjustment of the extract to low pH with acid; and
5. a three hour waiting period before a spectrophotometric reading at 520 nm is taken.

The NIR method developed to date requires the sample to be homogenised and then directly scanned in reflectance mode in spectrophotometer. The largest part of the work has been done using a research-grade instrument however several other (cheaper) types have also been used. From the analysis of a wide range of samples collected each year since 1999, a comprehensive suite of partial least squares (PLS) calibrations has been developed for the measurement of colour, pH, and TSS in red grapes on several distinctly different types of NIR instruments. These calibrations range in specificity from general 'pan-Australian' to more accurate region and variety specific. It appears that matrix effects are important and it is necessary to encompass sources of variation in a developed calibration. A calibration can be developed that will give acceptable results for samples from a wide range of regions or from different varieties, but best accuracy is obtained from restricted variable data sets. It was observed, however, that samples from the 'unusual' vintage of 2002, were not well predicted by calibration models developed from combinations of samples from the other years. However, the inclusion of these samples in the 'pan Australian' calibration only marginally increased the calibration errors due to the very large size of the overall dataset.

- ***Calibrations have been developed for the measurement of colour, pH, and TSS in red grape homogenates for several distinctly different types of NIR instruments.***

- ***The accuracy of the calibrations varied depending on the nature of the dataset used — from general 'pan-Australian' to more accurate vintage, region and variety specific. It appears that matrix effects are important and it is necessary to encompass sources of variation in a developed calibration.***

- ***This work was a world first and was presented at the 11th Australian Wine Industry technical conference (2001) and at 9th International Near Infrared Spectroscopy Conference (1999)***

As the size of the dataset grew, it became clear that the calibrations for red grape colour and pH were not linear. This had the effect of giving higher error than the average at the extreme ends of the range of the calibration, indicating an important practical limitation in the commercial use of the technique. An alternative calibration technique of locally weighted regression ('LOCAL'), available in the *WinISI* software package, was investigated to determine its usefulness in overcoming this effect. Preliminary work clearly showed that this approach could alleviate the non-linearity observed as shown in the example in Figure 1.

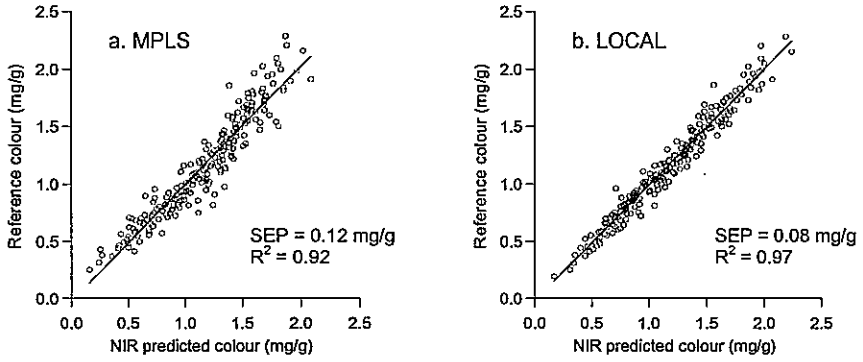

Figure 1. Comparison of calibration performance of (a) modified partial least squares (MPLS) and (b) LOCAL regression, using a 10% subset of the main sample set as a validation set. SEP is the standard error of prediction. Note the higher R^2 and lower SEP with the LOCAL calibration.

An important finding of this study was that selection of calibration sets with the LOCAL algorithm, which is done on the basis of spectral similarity, coincidentally resulted in selection of samples with similar analyte concentrations i.e. with this particular analyte/matrix combination it effectively operated as "LOCALxy" and gave superior prediction performance. Examination of the LOCAL sets also showed that vintage, variety and growing region were not necessarily barriers to spectral matching, provided that the database contained samples with analyte values similar to the unknown sample. The implication of this observation is that an effective spectral database for measurement of total anthocyanins in red grapes may not necessarily require samples from all growing regions and varieties, but must be representative of the full expected range of colour values and with respect to this, the database may require careful monitoring with regard to seasonal variations. The LOCAL calibration algorithm has been shown to give optimal performance in the face of such sample origin variation. Further validation work was performed with samples from the 2003 vintage. Again, LOCAL performed well but required refreshing of the database, a standard procedure with such methods. A validation sample set was collected from the 2006 vintage to test the efficacy of the WinISI algorithm in prediction of colour in red grape homogenates. The WinISI LOCAL calibration algorithm, using the now very large CRCV database, performed very well on new vintage samples, although some re-biasing may be advantageous. A paper describing this application was published in the Journal of Near Infrared Spectroscopy.

- ***The LOCAL algorithm can reduce matrix-related error in colour measurements by NIR with large, diverse sample sets but requires an appropriate spectral and reference colour database. This finding has been published in the Journal of Near Infrared Spectroscopy***

- ***At least one major Australian winery is now using LOCAL regression as part of its NIR testing procedure for routine grape quality measurements.***

As a direct consequence of the abnormal 2002 vintage, information including data up to the 2002 vintage was published in The Australian and New Zealand Grapegrower and Winemaker, 2003 Technical Issue. The influence of variety, region and vintage, and calibration strategies were discussed. This publication also outlines the extent that the Australian grape and wine industry has implemented the NIR technique for this application, thereby demonstrating the commercial relevance and benefits of this project. Most industry practitioners of this technology have opted to use 'closed' calibrations (i.e. single vintage), as outlined in our 2003 publication.

- ***A paper describing the use of laboratory based NIRS methods to quantify colour, pH and total soluble solids in grapes has been published in Australian Grapegrower and Winemaker (2003 Annual Technical Issue). The main points of the paper relate to issues of matrix variation (effects of variety, region and vintage), calibration transfer and calibration strategies.***

The consolidation of NIR calibrations developed for the analysis of grape berry colour, total soluble solids and pH has continued with the ongoing cooperation of industry partners so that more than 3500 berry samples from the 1999, 2000, 2001, 2002, 2003, 2004, 2005 and 2006 seasons, and from a wide range of growing regions and red varieties (but predominantly Shiraz and Cabernet Sauvignon), have been analysed by the conventional laboratory method and scanned with a research grade NIR instrument. It is clear that 'refreshing' is required, however, only a relatively small number of samples are required each year in order to account for sample variability between vintages.

- ***The calibration database for the determination of red grape colour (total anthocyanins), total soluble solids and pH appears to be quite robust and mature for predictive purposes.***

- ***These outputs have been presented to the industry through the development of workshops and also through publications.***

6.1.2.2 Grape phenolics

Early studies using NIR for investigating grape tannins also showed promise. Data with a set of samples, which included four different varieties, showed promise for prediction of individual anthocyanins from spectra of grape homogenate ethanol extracts. There was also good varietal discrimination using spectra of grape homogenate extracts; particularly for the so-called 'non-required' variety, Ruby Cabernet, which could be discriminated from premium red varieties. These tannin calibrations appeared to suffer from lack of robustness, possibly reflecting inconsistencies in the tannin reference assays.

Since the 2001 vintage, the NIR team collaborated with the Tannin Project (CRCV 1.2) team in their investigations of process scale fermentations. The main objective was to examine the potential of NIR spectroscopy to predict the concentration of and monitor the extraction and evolution of phenolic compounds during red wine fermentation. If successful, NIR spectroscopy techniques would offer potential as a rapid, low cost and non-invasive tool for monitoring the fermentation process. The results from the 2001, 2002 and 2003 winemaking trials conducted at the Hickinbotham Roseworthy Wine Science Laboratory showed that NIR spectroscopy could predict the concentration of malvidin-3-glucoside (the major anthocyanin in grapes), pigmented polymers and tannins in Cabernet Sauvignon and Shiraz wines during fermentation. Results from a calibration for malvidin-3-glucoside in red wines from 2001, 2002 and 2003 fermentation trials are shown in Figure 2. However the specificity of the calibrations developed remains to be confirmed, as there are many simultaneous changes occurring during fermentation.

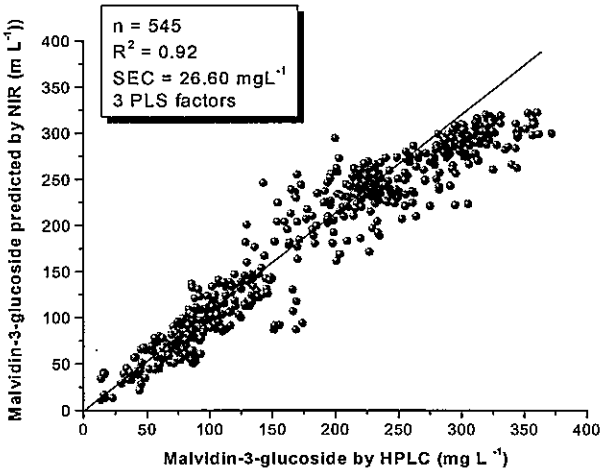

Figure 2. The relationship between NIR-predicted and reference analysis (HPLC) values for malvidin-3-glucoside in red wine ferments (n: number of samples; SEC: standard error of calibration; PLS: partial least squares).

- ***The prediction of phenolics by NIR appears feasible and offers potential for monitoring the progress of red wine fermentation.***

6.1.2.3 Glycosyl-glucose in grapes

The use of NIR for determination of G-G in homogenates of both red and white grapes has been investigated. In the case of red grape homogenates, the correlation does not appear to be robust enough for practical application, and may simply be a function of inter-correlation with colour. Since colour can be readily measured by NIR or standard laboratory techniques, the measurement of G-G in red grapes by NIR does not seem to be worth pursuing further.

- ***Development of an NIR calibration to quantitatively measure G-G in grape homogenates does not appear to be feasible.***

The work with white grape homogenates led to the investigation of sources of error in the G-G assay reference method and refinement of the analytical technique to be more relevant to real winemaking practices. Work with commercial juice samples and juices prepared in the laboratory with gentle methods rather than total homogenisation, showed that a reasonably robust NIR calibration is feasible, albeit with a degree of accuracy that would be more suited to only classifying broad concentration categories (e.g. high, medium, low) — this must be further validated. Additional studies using FT-IR have been undertaken to determine if this technique has any advantages over NIR for this application.

- ***An NIR calibration for G-G in white grape juices appears to be feasible but with limited accuracy and offers potential as a qualitative analytical tool.***

6.1.2.4 Yeast assimilable nitrogen (YAN) in white grape juice

The development of a rapid technique to predict yeast assimilable nitrogen (YAN) in grape juice might be very useful to winemakers in terms of improved fermentation control and efficiency. Whilst recognising that accuracy might not be paramount and that a semi-quantitative calibration might suffice for practical purposes, the project has nonetheless encountered significant technical challenges. Preliminary work showed some promise but proved difficult to refine and optimise due to the nature of the matrix. It appeared that high absorption by water in the relevant wavelength regions reduced the sensitivity of the assay. Further work with synthetic juices concentrated on ammonia and arginine (the most relevant amino acid in grapes), analysed in transmission mode with various path lengths or dried onto glass-fibre filters then scanned in reflectance mode. This work indicated that ammonia and amino acids produce very weak signals in the NIR region at concentration ranges normally encountered. These difficulties meant that the achievement of this

milestone was delayed from Year 5 to Year 7. Additional studies using FT-MIR have shown good correlation with peaks expected to occur in the spectra for these compounds.

- ***Using NIR to measure yeast assimilable nitrogen shows some promise as a semi-quantitative method. Prediction from FT-MIR spectra appears to be of quantitative accuracy.***

6.1.2.5 Moulds — *Botrytis* and powdery mildew (*U. Necator*)

Unavailability of a prototype commercial ELISA test kit for *Botrytis* antigen (the manufacturer delayed the release of the kit due to stability problems with the reference antigen and an assay being developed by collaborators at CSIRO Plant industry was still at a development stage), as well as staffing limitations delayed this aspect of the project. Therefore, it was decided to investigate the feasibility of NIRS for measurement of Botrytis by linking with CRCV project 1.5.2 (Eileen Scott). Work is under way with laboratory infected fruit – this removes the problem of opportunistic secondary infection encountered with field collected samples and will allow classification on a visual infection level basis, without the need for a quantitative ELISA test.

Homogenates of powdery mildew *(U. Necator)* infected fruit were scanned in the Vis/NIR wavelength range in reflectance mode with the FOSS *NIRSystems6500*. Distinct spectral differences could be observed, correlating strongly with the level of infection. Using non-linear discriminant analysis on principal component scores of spectra, infection levels of at least 10% could be detected. Discrimination appeared to be independent of the general matrix variables, TSS (°Brix) and pH, which can be altered with infected fruit. Samples were originally selected based on visual assessment of the degree of infection. The overall classification was 92% correct, with the only incorrectly classified samples belonging to the lowest infection level (1-10% infected). The same samples were analysed for powdery mildew DNA content by the University of Adelaide Fungal Pathology Laboratory (Eileen Scott, Belinda Stummer, CRCV project 1.5.2) to confirm the levels of infection. The fungal DNA content correlated with the visual grading and an NIR calibration model was developed to predict DNA content (Figure 3).

It was also shown that the classification of infection level was robust across two vintages and with two different experimental plots, but further work with field samples is required. Further work should also focus on attempting to identify the compounds contributing to the spectral features and distinguishing spectral changes due to the fungal mass, fungal metabolites and fungal- induced grape tissue changes.

The implication of this work was that it may be possible to discriminate fungal infected fruit at the weighbridge to provide a 'go/no-go' test to highlight suspect fruit for further detailed analysis to determine suitability for winemaking. Grape assessment for fungal infection at the weighbridge would normally be done by visual inspection, but this can be difficult with mechanically harvested fruit, particularly red grapes.

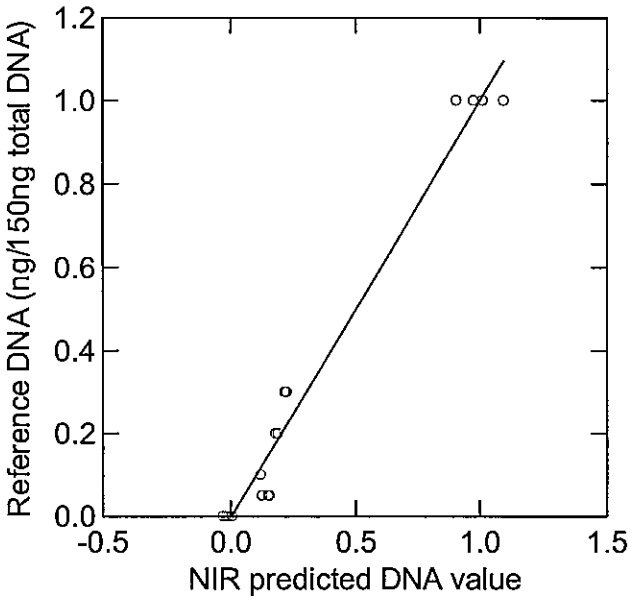

Figure 3. The correlation of NIR predicted and reference values for powdery mildew DNA value (ng/150 ng total DNA), using a PLS calibration with 1st derivative spectra using a wavelength range of 450 – 1900 nm (2003 vintage). $R^2 = 0.98$, standard error of calibration (SEC) = 0.07 ng, standard error of cross validation (SECV) = 0.09 ng, 2 PLS factors used. *Note: calibration statistics when using 450 – 1098 nm wavelength range were: $R^2 = 0.98$, SEC = 0.07 ng , SECV = 0.08 ng, 2 PLS factors used.*

- ***NIR has been demonstrated to be capable of discriminating different levels of fungal infection in grapes***

- ***A preliminary patent application on the use of spectroscopy to measure and classify fungal infections in grapes has been accepted.***

6.1.3 Wine analysis

6.1.3.1 Wine colour measurement

It has been well accepted that the spectral measurements of red wine have a profound influence on the grade or value of wine (Somers and Evans, 1974). Although the methods for these measurements are simple, they are nonetheless tedious to perform, and therefore the availability of a rapid technique was seen to be of great value.

Preliminary work showed that the NIR technique could be useful for rapid determination of the Somers' spectral measurements. Samples of young, commercially-made red wine (145 samples, mainly Shiraz, Cabernet sauvignon and Merlot) from the Riverland and Sunraysia regions were analysed for these measures and scanned with the *FossNIRSystems6500.* This work was extended by an Honour's student from Adelaide University who investigated this application under the supervision of Dr Leigh Francis, with Dr Bob Dambergs as a co-supervisor. Samples included 113 commercial red wines of various varieties, vintages and country of origin (38 foreign wines) and 124 samples from a large fermentation trial conducted by the Tannin and Microbiology groups. With these diverse sample sets some of the basic Somers' measures could be predicted well, but results in general were not as good as with the more restricted sets. However, an important outcome of this work was the validation of modifications to the Somers' methods against the methods. These modifications significantly simplify the assay, account for matrix variability such as pH and ethanol content of the wine and open the way to the adoption of a more rapid method of performing the assay using a high-throughput 96 well plate reader format.

- ***The NIR method may be useful for rapid determination of the Somers' spectral measures.***

- ***Modifications to the Somers' assays were validated and will enable higher throughput with the reference method.***

6.1.3.2 Wine quality grading

The ability to accurately assess wine quality is an important part of the wine making process, particularly when allocating batches of wines to styles determined by consumer requirements. Also, grape pricing is often determined by the quality category of the resulting wine – so called "end use" payment. Wine quality, in terms of sensory characteristics, is normally a subjective measure, performed by experienced winemakers, wine competition judges or winetasting panellists. By nature, such assessments can be biased by individual preferences and may be subject to day-to-day variation. An objective quality grading method would therefore be of great assistance. Flavour compounds are often present in concentrations below the detection limit of near infrared spectroscopy but the more abundant organic compounds offer potential for objective quality grading by this technique.

The correlations between NIR spectra and sensory data obtained using (Adelaide) wine show samples were less significant in general. The difficulty in obtaining good spectral correlations with wine score may have been due to excessive sample matrix variation — with most classes in the show, the samples may span vintages, growing areas and winemaking styles, even though they may be made from only one grape variety. For dry red wines, the best calibrations were obtained with a class of Pinot Noir — a variety that tends to be produced in limited areas in Australia and would represent the least matrix variation. Again, the loadings relied predominantly on anthocyanins.

With samples from commercial winery wine quality allocation tastings, the best correlations between NIR spectra and tasting data were obtained with young red wines (current vintage) with minimal secondary winemaking processing (e.g before oak treatment), from a warm climate area. PLS calibrations used factor loadings in the wavelengths related to anthocyanins, ethanol and possibly phenolic compounds. Only a small number of factors were required and the strongest loadings were in the anthocyanin spectral region (400-700nm). The project has data from five vintages (1999, 2000, 2001, 2003 and 2004), demonstrating some success in calibrating for the prediction of red wine quality grading from Vis-NIR spectral data.

For dry white wines, calibrations were less significant and appeared to be more dependent on the ethanol related regions of the spectrum (1700–2400nm), implying that the quality parameters that could be predicted by NIRS correlated with fruit maturity.

- ***NIR can classify quality grades of young, warm climate red wines.***

- ***This work was the first report of the use of NIR for prediction of wine sensory quality and was presented at an ASVO workshop, the 11th Australian Wine Industry Technical Conference (2001), at the 9th International Near Infrared Spectroscopy Conference (1999) and at a CRCV workshop***

In collaboration with Industry Development and Services, the Tannin Group and Wynn's Coonawarra Winery as a side study from a large tasting held by Wynn's, a set of samples representing 50 vintages of bottled Wynn's Coonawarra Cabernet (1954 to 2004) were scanned over the Vis-NIR range. Despite the fact that these were separate wines, rather than following a particular wine over 50 years and with the added complication of possible bottle variation, a distinct progressive pattern of change could be observed with PCA profiles, using the Vis-NIR spectra (Figure 4). Some older wines that anecdotally were of higher quality early in their life appeared further up the profile than expected and *vice-versa* for some younger wines. Thus it appeared that a spectral fingerprint of the original wine quality remained for decades. This method of analysis illustrates the power of spectral information in assessing wine quality and may be able to highlight, for example, abnormal maturation due to bottle variation or poor sample storage.

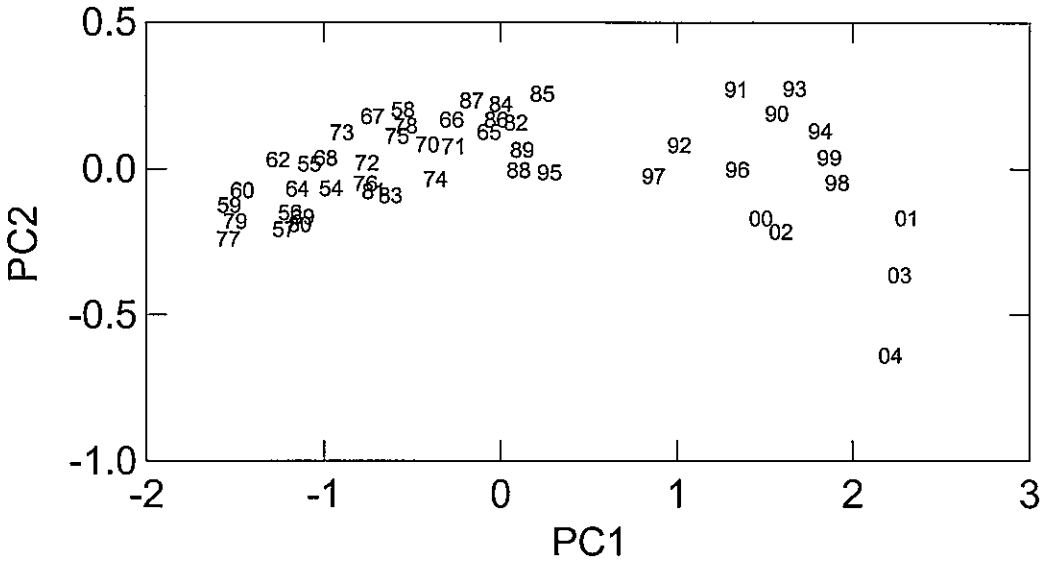

Figure 4. Spectral monitoring of wine maturation: principal component analysis of Vis-NIR spectra collected from 50 vintages of Wynns Coonawarra Cabernet. The first 2 principal components shown here explain 99% of the variation between samples. The wines show a sequential maturation profile but some wines with exceptional storage qualities appear out of sequence.

6.1.3.3 Wine authentication and discrimination

The authentication or identification of food is emerging as one of the most important areas in order to comply with food industry regulations and standards and to address consumers' concerns. Since the early 1980's the development of multivariate statistical techniques, personal computers, and new instrumentation have resulted in the development of IR methods that offer the possibility to analyse more samples than ever before and acquire large amounts of data. Spectroscopic techniques such as NIR or Fourier transform infrared (FT-IR) have proved to be a suitable tool for authentication with demonstrated applications including discrimination between coffee varieties, honey adulteration, and beef contamination and adulteration.

The project team has conducted work using NIR spectroscopy to discriminate between wine varieties and blends. Preliminary investigations have been undertaken into the discrimination of commercial samples of two white wine varieties (Chardonnay and Riesling). Principal component analysis (PCA) of the NIR spectra was used to successfully show discrimination between the two varieties. Further work is continuing in order to understand the influence of the wine matrix on the classification specificity. It is proposed that red wines will also be investigated using this technique.

- ***NIR shows promise as a technique that may be able to classify by variety samples of commercial bottled Riesling and Chardonnay wines.***

A project for a Master's degree student (Liang Liu) from the University of Adelaide (Supervisor: Dr Chris Colby, Department of Chemical Engineering, University of Adelaide) was established to assess the potential use of Vis, NIR and MS-E-Nose to determine the origin and region of wines. Experiments were carried out in order to evaluate the effect of temperature and sample presentation on the measurement of wine compositional characteristics using Vis-NIR spectroscopy. Additionally experiments were carried out in order to evaluate the effect of temperature, salt addition and time on the analysis of wines by MS-E-Nose. The samples used were bottled Tempranillo wines from Spain and Australia, and Riesling wines from New Zealand, France and Australia. Preliminary results showed a great potential for the ability of Vis and NIR spectroscopy to discriminate the geographical origin of the wines.

- ***NIR shows promise as a technique that may be able to discriminate the geographical origin of wines.***

6.1.3.4 Fermentation monitoring

In collaboration with CRCV project 1.2 (the Tannin project), samples from red wine fermentations were scanned using the FOSS *NIRSystems6500* spectrophotometer. Promising correlations were developed for malvidin-3-glucoside, tannin, and pigmented polymers, although there are high coefficients of cross correlation between the constituents. The data set contained two varieties (Cabernet Sauvignon and Shiraz) from three vintages (2001–2003). This promising work has set the foundations for the possibility of on-line monitoring of these constituents in red wine fermentations. Additionally, the potential of using NIRS to rapidly predict such constituents, could provide significant savings in analytical resources required for research fermentation trials and/or increase the potential scope of such research.

- ***NIR has shown promise for monitoring the evolution of colour in fermentations, suggesting potential for on-line implementation in the future. This application also offers significant savings in analytical cost for fermentation research experiments. This finding was presented to the* InVino Analytica *conference (*2003).**

6.1.3.5 Wine sensory attributes

6.1.3.5.1 Panel sensory scores

In collaboration with a CRCV PhD student, Heather Smyth, and the Sensory Team at AWRI the opportunity was taken to include NIR scans from a set of Chardonnay and Riesling wines and relate them to sensory properties measured by descriptive sensory analysis and also attempt varietal classification. With this defined sample set it was shown that the varieties could be discriminated, albeit with some degree of overlap. Additionally good correlations were obtained between aroma properties and NIR spectroscopy.

- ***NIR showed good correlation for aroma attribute scores in a set of commercially available unwooded Chardonnay and Riesling wines — this innovative work has been published in a peer-reviewed journal (Analytica Chimica Acta).***

6.1.3.5.2 Wine spoilage

The project team investigated the most appropriate method to monitor *Brettanomyces* yeast spoilage in red wines. The use of UV, Vis, NIR, MIR and MS-E-Nose as sensors was evaluated with encouraging results. The MS-E-Nose showed most promise, but with little or no predictive capability using the FT-MIR. There is some indication that the NIR may show success but this still needs to be confirmed. Evaluation of UV spectra of aqueous solutions of 4EP indicated that the range had higher sensitivity relative to NIR spectra. However, there were some problems with spectral overloading when addition of 4EP was made to wine and this is being further investigated. Although these preliminary UV calibrations for 4EP were not highly accurate (possibly due to the spectral overloading), samples with 4EP well below the sensory threshold could be discriminated from samples just below and over the sensory threshold. This may be due to detection of other changes in UV absorbing compounds so should be further evaluated.

- ***The prediction of Brettanomyces spoilage by rapid instrumental techniques appears feasible with the MS-based Electronic nose and NIR techniques showing the most promise.***

6.1.4 Other applications

6.1.4.1 Yeast strain identification

In collaboration with AWRI's Molecular Biology team, the potential of NIR and MIR spectroscopy to discriminate and identify yeast strains was examined. By combining NIR spectroscopy and multivariate analysis, it was possible to identify different ecological yeast strains of Saccharomyces cerevisiae selected from the EUROSCARF data base including wild and deletion strains. Visible (VIS) and NIR spectra (400 - 2500 nm) were acquired with a FOSS *NIRSystems6500* spectrophotometer in transmittance mode using samples from controlled fermentations of each strain. Principal component analysis (PCA) and linear discriminant analysis (LDA) were used in order to visualise graphically the relative distribution of the different yeast strains. The results showed that all the wild strains were correctly classified, indicating that the NIR might distinguish

between the ecological types of yeasts. However, there was lower discriminatory power between deletion strains with similar metabolic profiles (pathways) meaning that other analytical techniques might be required to refine classifications. Whilst NIR shows promise as a preliminary screening technique, further studies are necessary to determine the effect of sample presentation and fermentation conditions on the classification models developed.

- ***NIR spectroscopy might be used as a rapid preliminary screening of yeast strains, and a more precise method such as GC/MS will probably be required for more detailed analysis of the strains, particularly very closely genetically related strains.***

6.1.4.2 Grapevine sap composition

A preliminary investigation in collaboration with Serve-AG Research in Tasmania of the potential use of NIR and FT-MIR to predict mineral composition in grapevine sap samples was initiated during 2003. Serv-Ag Research is the coordinator of a GWRDC-funded RITA project investigating the production of Pinot Noir at several sites across Tasmania. Preliminary calibration tests showed promise however there was insufficient sample numbers to confidently assess the application. This application merits further investigation.

- ***NIR shows promise as a rapid method for grapevine sap analysis and further work is recommended.***

6.1.5 Protocols

With the current and expected implementation of NIR technology, there is a need for wineries and growers to understand its workings. It is clear that there will be a need for education and training in the procedures and protocols that must be followed in order to have reliable results. Aside from the NIR methods, inter-laboratory differences with the reference laboratory analysis remain an issue. It also became apparent that there was an urgent need for the team to develop stable reference standards for validation and instrument matching as are already available for grains and fodder industries. This work was given high priority in the last three years of the project, as it is an essential requirement if calibration transfer was to be successful. To support these needs, the project had a focus on standardising the reference methods, developing reference standards and establishing an industry inter-laboratory proficiency-testing programme.

6.1.5.1 Sample presentation

The project has investigated the effect of sample presentation in NIR analysis, comparing homogenised with whole red grapes. Initial work by the project team on the prediction of quality parameters in red grapes using NIR began with the scanning of homogenised grape samples using a research grade laboratory NIR spectrometer, the *FOSS NIRSystems 6500*. However, the later availability of faster scanning Vis/NIR spectrometers with alternative optical configurations provided the possibility of presenting the grape samples to the instrument without the need for homogenisation. Simplifying the sample presentation for NIR prediction of colour, TSS and pH could present an opportunity for laboratories to dramatically increase sample throughput. Furthermore, this mode of presentation might even offer the potential of scanning whole, single berries.

We have investigated of the feasibility of scanning intact whole berries for prediction of colour, TSS and pH using the Perten *DA7000*, Zeiss *CORONA* and FOSS *NIRSystem6500* spectrophotometers. The Perten *DA7000* instrument showed considerable promise, however, was withdrawn from the market by Perten because of a dispute over intellectual property with a third party. Apparently, Perten are developing an alternative to replace this instrument. The Zeiss *Corona,* also shows considerable promise for scanning intact fruit and is rapid, compact, robust and relatively inexpensive (~A$30,000). BRL Hardy has been using this instrument since 2002 for scheduling the harvest and streaming of incoming fruit—a less accurate calibration is acceptable for this application since the results are not used for payment purposes. The project team is now investigating in detail the capability of the instrument to scan grapes in various conditions (i.e. whole, machine harvested, homogenised) for reproducible and reliable prediction of composition. Scanning intact berries relies heavily on the integrity of the samples since juicing of berries may have a profound influence of the reflectance values recorded by the spectrophotometer. In general, the accuracy of prediction appears to be significantly less than for homogenates. Results so far on whole berries suggest only "indicator" level accuracies perhaps more suited to streaming the product rather than for analytical or payment purposes.

- ***Alternative commercially available instruments were evaluated for their potential to scan intact fruit and appear to offer some promise although with reduced accuracy of prediction.***

Preliminary investigations conducted in collaboration with a major winery for whole grape berry presentation were promising, indicating that NIR may have potential for use in the streaming of fruit on receival at the weighbridge or for in-field analysis. These studies were performed using several compact, fast, diode-array spectrometers including the Zeiss CORONA, and included investigation of the feasibility of scanning samples directly in plastic jars that are commonly used when collecting grape samples. Trials using a rotating turntable fitted to the Zeiss CORONA were successfully conducted at the collaborating winery as part of their 2004 vintage testing program. An evaluation of the practice of using a stab blender directly in the plastic sample jars used to collect grape samples was carried out during the 2004 vintage — trials with scanning the homogenate produced by the stab blender directly in these jars indicated improved accuracy over that obtained from non-homogenised weighbridge samples

Calibration accuracy for various sample presentation methods and different instrument types for the measurement of red grape composition are shown in Table 1.

Table 1. Standard error in cross validation for NIR determination of the concentration of total anthocyanins and total soluble solids in red grape homogenates using various instrument types and sample presentation modes.

		Standard error in cross validation	
Instrument type	**Sample presentation**	**Total anthocyanins (mg g^{-1})**	**Total soluble solids (%)**
Diode array	Mechanically harvested fruit scanned on turntable	0.06	1.26
Diode array	Mechanically harvested fruit scanned in jar	0.14	-
Diode array	Homogenates of mechanically harvested fruit scanned in jar	0.17	1.0
Monochromator	Whole grapes scanned in cuvette	0.18	1.70

- ***It has been demonstrated that different sample presentation or processing status will give rise to different NIR calibration accuracy.***

A further innovative approach involved the assessment of the potential for the use of NIR for the non-destructive analysis of bottled wine *in situ*. As well as being able to measure, for example the relative degree of wine oxidation, as previously demonstrated by the AWRI Wine and Oxygen team, it was possible to perform some more routine analyses, such as for example alcohol content, for on-line monitoring of packaging for quality control purposes. This revelation led to the preparation and subsequent successful application for a patent as discussed later. Some trials were carried out at commercial wineries in order to investigate the feasibility of using the Zeiss CORONA spectrophotometer to monitor the degree of oxidation in white wines within sealed bottles. Several hardware set-up options for the Zeiss spectrometer were tested, together with a computer software program that the team developed for routine on-line analysis.

- ***It has been demonstrated that it is possible for NIR to determine the chemical composition and relative degree of oxidation in bottled wine non-destructively*** **in-situ.**

- ***An Australian innovation patent has been granted for this application.***

6.1.5.2 Standardising reference methods

For many Australian wineries, storage of grape samples before analysis is often necessary because of the high throughput requirements (e.g. sometimes greater than 500 samples per day) and often freezing is required. Sample storage and processing are important issues that require consideration when performing analysis on grape samples especially when the results may be used to affect or modulate grape payments. The effects of freezing, homogeniser type and storage time on the accuracy and recovery of the laboratory determination and NIR prediction of colour, TSS and pH were evaluated to provide the basis for developing recommended standard protocols for the analysis of red grapes by both reference and NIR methods.

- ***Standard operating procedures for both the NIR and reference laboratory methods for colour have been developed and published***

6.1.5.2.1 Reference laboratory method

Feedback from industry about the testing procedures for the determination of the concentration of total anthocyanins as described in scientific publications alerted the team to the need to investigate the effects of laboratory handling procedures (including storage and homogenisation) which can differ between laboratories. Therefore, a comprehensive study was planned and undertaken to investigate these effects.

Neither the homogeniser type, nor the sample state (fresh or overnight freezing) had significant effects on the determination of total anthocyanins and TSS by the laboratory methods. However, we observed an effect of overnight freezing on total anthocyanins in samples of high concentration. The strongest effects of both homogeniser type and the state of the sample were observed with pH measurement, however, these statistically significant differences might not be very important from the practical point of view. Phenolic extraction was also affected by the type of homogeniser used, but not by overnight freezing. Longer than three months frozen storage affected all the measured quality parameters.

- ***Neither freezing of samples prior to homogenisation, nor the type of homogeniser had any significant effect on the accuracy of laboratory determinations of colour and TSS of red grapes.***

- ***Storage of grapes in a frozen state longer than three months did have a significant effect on the laboratory determinations of colour, TSS and pH of red grapes.***

This study brought to light evidence for the need and benefits of a strict, detailed laboratory protocol outlining the conditions of sample processing and storage for grape compositional analysis. As a result, the team prepared a technical note outlining the laboratory procedure for determination of the concentration of total anthocyanins in red grapes that is now available to industry and has now become the industry's 'standard' method through endorsement by the Winemakers Federation of Australia's legal metrology group.

- ***Standard operating procedures for the reference laboratory methods for the determination of red grape colour have been developed by the project team, endorsed by the industry and published.***

6.1.5.2.2 NIR method

Samples from the same study assessing the effects of sample handling procedures on the reference laboratory method were also scanned by Vis-NIR. This allowed the effects on Vis-NIR calibrations to be evaluated in a similar manner.

Although samples can be analysed either fresh or after storage in a frozen state, the effect of freezing and frozen storage on the Vis-NIR spectra has not been reported before. Calibration equations for colour were developed using either fresh or frozen samples alone, respectively. The results showed that the standard error of prediction (SEP) for total anthocyanins in all samples was slightly increased by freezing and storage when compared with calibrations developed on fresh or frozen samples alone. These results suggest that it might be possible to use NIR calibrations developed on fresh or frozen samples alone to measure the concentration of total anthocyanins in either fresh or frozen samples after appropriate slope and bias correction. Furthermore, in a collaboration with a PhD student from James Cook University, these data were further investigated using alternative multivariate analysis techniques to evaluate the effect of freezing and frozen storage on the NIR spectra.

It was observed that the type of homogeniser and overnight freezing of red grapes had a slight effect on the Vis-NIR spectra of the homogenates; however these did not affect the performance of the resulting calibrations for total anthocyanins, TSS and pH (Table 2). However, longer frozen storage period (greater than one month) did have an effect on the predictive ability of calibrations. These results suggest that it might be possible to use Vis-NIR calibrations developed on fresh or short-term frozen samples alone to measure the concentration of total anthocyanins, TSS and pH in either fresh or short-term frozen samples after appropriate slope and bias correction.

Table 2. Statistics for the performance of NIR prediction of the concentration of total anthocyanins, TSS and pH in red grape homogenates using various combinations of validation and calibration samples sets.

	n	SEP	R	RPD
Fresh predicted with overnight frozen	143			
Total anthocyanins mg g^{-1}		0.14	0.95	3.1
Total soluble solids		0.68	0.97	4.3
pH		0.05	0.90	2.8
Fresh predicted with 1 month frozen	143			
Total anthocyanins mg g^{-1}		0.17	0.91	2.5
Total soluble solids		1.60	0.85	2.0
pH		0.10	0.87	1.4
Overnight frozen predicted with fresh	176			
Total anthocyanins mg g^{-1}		0.11	0.93	3.6
Total soluble solids		0.69	0.97	4.2
pH		0.05	0.89	2.5

Notes: n: the number of samples in the validation set; SEP: standard error of prediction, R: correlation coefficient, RPD = SD/SEP where SD is the standard deviation of the sample set.

- ***The type of homogenizer and overnight freezing of red grapes had some effect on their Vis-NIR spectra, but did not influence the performance of the resulting calibrations for total anthocyanins, TSS and pH.***

- ***The biggest effect in the spectra was observed in samples stored frozen for longer than one month and had a large effect on the Vis-NIR calibration statistics***

- ***It appears possible to use Vis-NIR calibrations developed on fresh or short-term frozen samples alone to measure the concentration of total anthocyanins, TSS and pH in either fresh or short-term frozen samples after appropriate slope and bias correction.***

- ***Standard operating procedures for the NIR laboratory methods for determination of red grape colour have been developed by the project team, endorsed by the industry and published.***

The project team was involved in the testing and assisting in the development of an automated grape sample preparation system 'Bioprep5 Robotics', which was released commercially by ARRM, a local Adelaide company. The unit was designed primarily to process grape samples for colour analysis, whether by NIR or by the reference laboratory method, but it can be put to other uses. The benchtop-sized unit is fully automated, carrying out grape sample homogenisation, sub-sampling, weighing, addition of an extraction solvent, mixing during extraction, and finally centrifugation, and is multitasking. Following extensive evaluation of the commercial system, the unit has been found to perform well in all tests, including those with sets of grape berry samples with challenging physical properties. In recent testing 156 grape samples were processed and analysed. The data from the automated system compared well to the results obtained from the manual method in terms of accuracy and precision. No serious practical deficiencies were observed in the testing, with aspects such as ease of access to the parts of the unit, ease of cleaning and control of the processing being satisfactory. The unit could be configured to act as an automated homogeniser, with no further processing, to allow NIR scanning of the samples only (i.e. with no reference analyses), in which case the system could process approximately 112 samples/8 hour day. For full processing (including extraction and clarification) with one operator carrying out other analytical tasks on the samples, 48 samples would be the likely maximum that could be processed in an 8-hour period.

- ***A commercially available robotic sample preparation device for preparing red grape homogenates for colour analysis was evaluated and shown to perform well.***

6.1.5.3 Developing reference standards

The combination of spectroscopy and chemometrics offers opportunities for rapid analysis of agricultural products. However, the accuracy of the technique is very dependant on instrument calibration and validation. The analysis of "check" samples to monitor day-to-day performance is usually required under most laboratory quality management systems, but this presents problems in the case of unstable, high moisture products such as grapes.

We have investigated the use of commercial plastic polymer material as reference tiles for use in validation of instrument and calibration performance. Dupont Corian® is produced from poly-methyl-methacrylate (PMMA), with inorganic fillers to introduce opacity and dyes to introduce visible colour. Corian® therefore has a signal that spans the visible to near infrared (Vis-NIR) range, has the desired reflectance properties, and the spectra vary with formulation so that a variety of "standards" are available at low cost from the local hardware store. We have examined three Corian® products as potential standards for use with a FOSS *NIRSystems 6500*. First derivative treatment of the spectra removes baseline effects and although they are by no means a perfect match with red grape homogenates, they offer a high degree of spectral matching and would be suitable as check samples.

An innovation patent relating to spectrophotometer reference tiles has been granted and the first batch of tiles has been produced for distribution with the Integrated Spectronics instrument that is being manufactured for the CRCV.

- ***There was a need to develop stable reference standards for calibration validation, daily instrument checks and instrument matching***

- ***Information on a stable, cost-effective reference tile for Vis-NIR reflectance spectrophotometers was presented at the 11th International Near Infrared Spectroscopy conference (2003)***

- ***An innovation patent for Vis-NIR reference tiles has been granted – this will protect the intellectual property for the Australian wine industry***

6.1.5.4 Industry inter-laboratory proficiency-testing

Grape colour testing, even by the wet chemistry method, is relatively new in commercial winery laboratories. To assist in validation and bench-marking, an inter-laboratory proficiency study was performed during the 2004 vintage with AWRI and a small group of laboratories that routinely conduct the total anthocyanin assay. Results were excellent, with low inter-laboratory standard errors. This also been performed for subsequent vintages (2005 and 2006). Participants include the AWRI Analytical Service, the project team, CSIRO and several wine industry laboratories that routinely use this method.

- ***The project team has facilitated inter-laboratory proficiency studies to assist in validation of the Australian wine industry's grape colour testing procedures.***

6.1.5.5 IR spectroscopy users group

The project team proposed in 2003 that an 'IR spectroscopy users group' be established bringing together the CRCV project team, and other researchers and practitioners involved in the development and application of IR spectroscopy in the research and industrial communities. This was an important step in further strengthening the links between researchers and industry in the application of the IR technology in the Australian wine industry. Personnel from wineries in Australia and New Zealand have attended meetings. The potential of the group to be used as a unified user's lobby group with manufacturers has been discussed, as were IP issues resulting from the desire of members for open collaboration. The issue of IP remains unresolved and will be a challenge for the future.

- ***An Infrared spectroscopy users group had been established and may be a useful forum to distribute industry standard calibrations once IP issues have been finalised.***

6.2 Practical application of NIR

This project has the aim of developing NIR methods that are suitable for practical use in the wine industry. The project has always involved close collaboration with industry, and as a result, has been used extensively by one winery for determination of quality variables for subsequent payment purposes. This clearly demonstrates the availability of the technology for broad industry adoption, however, there remains a need to find the most appropriate mechanism by which the technology can be easily implemented by the broader industry. Therefore, a major objective of this project is to be able to scan grapes with minimal sample preparation, for example, whole bunches or grapes in the

vineyard or at the weighbridge, or directly within a bin of machine harvested fruit with acceptable accuracy and reliability in order to reduce costs and analysis turnaround time. The potential uses for such rapid measurement are many from streaming of fruit for scheduling harvest date to precision agriculture.

The project considered that the highest priority was the development of a stable and robust calibration for red grape colour prediction through investigation over successive years of harvest. The next most important issue was the identification of suitable hardware, and the consequent need to be able to transfer the laboratory calibration to the 'working' instruments.

6.2.1 Commercialisation

Significant effort was expended by project staff in the clarification of matters arising in respect of intellectual property (IP) and of the potential commercialisation of the technology. Extensive professional advice was sought from both legal and commercial viewpoints and several important aspects were documented. These documents included a technology audit, a chronology of the project, market research and estimates, collation of all existing contracts, establishment of confidentiality agreements, discussion on commercialisation options, software licensing contracts, instrument sales agreements, and transfer of IP agreements. Preliminary discussions with several international instrument companies that had approached AWRI indicated that commercialisation issues needed to be resolved as the potential value of the IP would diminish rapidly if delayed. Unfortunately, differing views of the value of the IP and the prerogatives of the AWRI and CRCV slowed progress. CRCV Technologies assumes full responsibility for all activities of commercialisation of the technology arising from this project. It was seen that transfer of the technology to the industry might best be achieved through direct commercialisation activities, and this is being carried out under the responsibility of the Cooperative Research Centre for Viticulture's dedicated commercialisation company, CRCV Technologies Ltd, who continued to negotiate with several instrument manufacturers to this end. Whilst there were some potential hurdles to the chosen path of commercialisation, none of these were insurmountable and did not prevent the intended activities.

The model of commercialisation that was adopted by the project to achieve the aim of implementing the technology to the industry included:

- development of calibrations and/or suitable instruments; and
- provision of training and education programs about the technology.

- ***The commercialisation of the CRCV's intellectual property in NIR technology was implemented by CRCV Technologies.***

A considerable effort was made to clarify the commercial and practical impacts arising from the existence of several patents related to this technology. A 'watch' has been placed upon one of these, and background material relating to prior art for another has been prepared in the event of a future challenge being required.

6.2.1.1 Calibrations and instruments

Much work focussed on the development of robust calibrations, which for some parameters was achieved. The red grape spectral and composition database, which over the lifetime of the project has been accumulated using more than 3500 samples from all across Australia, is in itself an extremely valuable property. Therefore, the potential for commercialisation is large and was investigated by CRCV Technologies. Two main approaches to commercialisation of the IP were explored. Firstly, CRCV Technologies attempted marketing of the calibrations to potential customers, mainly the larger NIR instrument manufacturers, however, no commercialisation agreements were secured. Secondly, collaboration with a commercial instrument manufacturer led to the development and trial of custom made instruments capable of using the CRCV calibration database. The instruments were designed and intended to be used for the measurement of colour, pH and TSS in red grape homogenates for use as a screening tool. More detailed information on this is given below.

- ***Commercialisation of the calibration database has occurred through partnership with a commercial instrument manufacturer.***

6.2.1.2 Provision of training and education programs about NIR technology

The team maintained a strong commitment to the training and teaching of the principles and use of spectroscopy and multivariate analysis techniques (chemometrics) to other team members as well as for industrial and external research partners. In-house workshops and training commenced in March 2004 and were extended to industry through workshops conducted as part of the 12th Australian Wine Industry Technical Conference held in Melbourne in July 2004. Draft training materials had already previously been trialled at one workshop at the 11th Australian Wine Industry Technical Conference (AWITC) in 2001, and at the 12th AWITC in 2004. Further planning is now underway for more workshops at the 13th AWITC in 2007. The team developed materials for a workshop for winemakers, viticulturist and laboratory staff that were trialled at several wineries in South Australia during 2004 to 2006.

- ***Workshops and handout materials have been developed on the use of spectroscopy and multivariate techniques and presented to the Australian wine industry.***

6.2.2 Instrumentation

Whilst it is clearly not within the scope of the project nor feasible to evaluate all available instruments and develop calibrations for them, several different types and brands of spectrophotometers have been investigated. These have ranged from expensive (>$120,000) research-grade scanning monochromators (e.g. FOSS *NIRSystems6500)* to cheaper (~$30,000) silicon diode array instruments (e.g. Zeiss *CORONA*).

6.2.2.1 Custom made NIR instruments

Five custom-made, relatively low cost, prototype field-type instruments (NIRTech *Dionys*) were trialled by the collaborating partners from industry. Preliminary results indicated capability to analyse grape homogenates with an acceptable level of accuracy, and other investigations to assess the suitability of these instruments for direct measurement in machine harvest bins showed some merit. Therefore CRCV Technologies directed a trial 'limited commercial release' of these instruments during the 2002 vintage. However, this was met with limited success possibly due to:

- poor spectral matching of new prototypes with the master instrument with the resultant difficulties in transferring calibrations;
- hardware failures with the second phase prototypes; and
- large differences between 2002 vintage samples and the previous vintages used to develop the calibration that was used.

It is apparent that this model represents quite a high-risk approach in attempting to achieve the outcomes of the project.

- ***A preliminary trial commercial release with low cost custom-made instruments in 2002 was limited by hardware capability and was unsatisfactory.***

Following the successful bid for supplementary funds in 2003, a new approach was taken in sourcing instrumentation that would be relevant and useful for the industry. In a several step approach, CRCV Technologies commissioned various surveys of the industry to determine their needs and assess the likely demand for NIR instrumentation. Following that, discussions were held with commercial instrument suppliers and eventually a selection was made for a partner that was most likely to be able to deliver the outcomes demanded by industry. As part of that process, team staff assisted CRCV Technologies in assessing responses from companies interested in becoming partners in the commercialisation activities.

An agreement was reached between an Australian-based spectroscopic instrument manufacturer and the CRCV which made possible the development of a relatively cheap and robust prototype instrument for the industry to measure grape composition for screening purposes. This instrument utilised the CRCV calibration database that was transferred using novel techniques also developed by the project team. In collaboration with wine industry partners, the prototype instrument and the calibration transfer routine was evaluated during 2005 and 2006 vintages. In addition, it was necessary to develop a practical and affordable customised sample container for use with the instrument, which was design and developed using readily available plastic components, and a large number prepared for future use, by the CRCV's Technology Applications Manager. The prototypes were field-tested in some winery laboratories, with a view to getting feedback on the general operation and obtain suggestions for modifications. The software in particular still requires some development, but the general operation of the instrument appears to be sound. Preliminary

feedback from trial sites was generally positive. Figure 5 shows the correlation between values for colour predicted by one of the prototype instruments using the CRCV calibration and those obtained by the reference laboratory method in red grape homogenates.

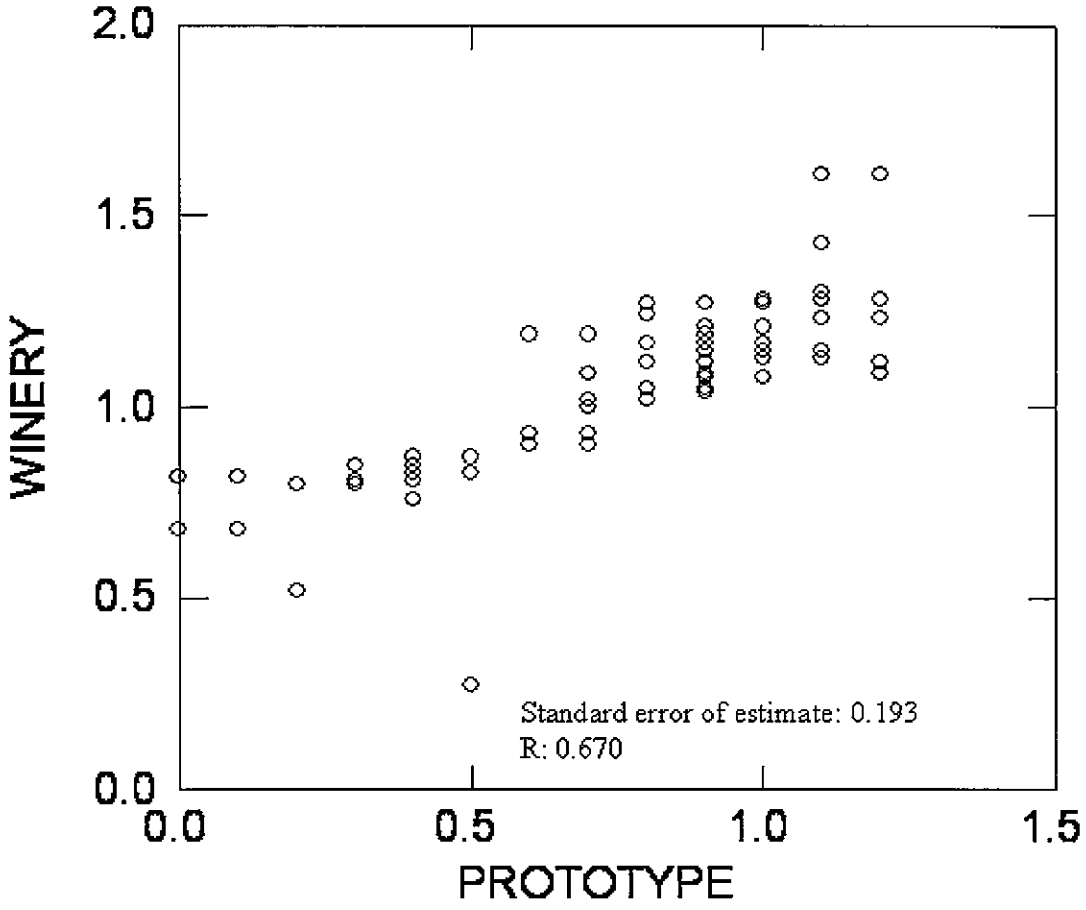

Figure 5. Relationship between the concentration of total anthocyanins (colour, mg/g) in red grape homogenates predicted by the prototype instruments using the transferred CRCV calibration ('PROTOTYPE') and the winery laboratory by the reference method ('WINERY').

- ***Commercial trials with a commercial partner, conducted following additional funding through the 'supplementary bid', have proved successful in delivering a relatively cheap, robust NIR instrument for determining red grape colour, pH and TSS for quality screening purposes.***

6.2.2.2 Generally commercially available NIR instruments

We have investigated of the feasibility of a range of commercially spectrophotometers including the Perten *DA7000*, Ocean Optics, NIRTech *Dionys,* FOSS *InfraXact*, Integrated Spectronics, Zeiss *CORONA* and FOSS *NIRSystem6500*. All appear to have merit for various applications and specific sample presentation modes. The intended use of the results will have a bearing on the system chosen for any particular application.

6.2.3 Calibration transfer

Until recently, it was a commonly held belief that calibrations for NIR instruments were highly specific and therefore each user had to generate their own calibrations. This was seen as an expensive exercise and an impediment to implementation of the technology by many industries, and, in response to this need, techniques were developed for transferring calibrations from one instrument to another. Another view emerged that this was limited to transfer between instruments of the same make that also had to be carefully matched for the spectral performance and response.

Calibration transfer between instruments is a critical issue that affects the ease of uptake across the broader industry. From our investigations, the direct calibration of a single instrument has proven to be relatively straightforward, but the simultaneous standardisation of a population of several or many instruments to the one calibration is a more complex issue. Initial work with five custom-made prototype, relatively low cost, field-type instruments (NIRTech *Dionys*) showed promise in being able to transfer calibrations using a very simple approach, but met with limited success in industry trials conducted by CRCV Technologies during the 2002 vintage probably for the same reasons as described previously (section *Instrumentation*).

Commercially available software (*WinISI*) that is designed for calibration transfer and standardisation of instruments, such as the more expensive research grade *FossNIRSystems6500* spectrometer, was also investigated in a small trial conducted in the 2002 vintage by CRCV Technologies. This approach had some success and also highlighted the need for 'refreshing' the LOCAL and global calibration database with current vintage samples. Another trial was conducted in the 2005 vintage, of transfer between two FOSS instruments *viz. NIRSystems6500* and the *InfraXact* with similar results.

More recent work has shown that it may also be possible, although difficult, to transfer calibrations between distinctly different types of instruments (i.e. FOSS to Zeiss) although this still requires validation now that standard reference materials for the standardisation procedure (see section *Protocols* below) are available. This type of calibration transfer used some new chemometric techniques that are available in the *WinISI* software package.

- ***Calibration transfer both within- and between- instrument types appears feasible but requires validation***

Prediction accuracy is of paramount importance so that new chemometric techniques such as artificial neural networks (ANN) are being investigated. A preliminary trial has shown some promise for the use of ANN chemometrics for transferring calibrations between comparable NIR instruments in Australia and overseas.

- ***The use of artificial neural network algorithms for more accurate calibration may offer some potential to improve calibration transfer and this approach requires further validation.***

6.2.4 Process applications — On- or at-line (harvester, weighbridge, crusher, packaging line)

The development of faster NIR instrumentation has created intense interest in the area of on-line applications, for example for real time analysis on harvesters. Assistance was given to two industry partners using 400-1100 nm (Visible) and 400-1700nm (Visible and NIR) Zeiss diode array instruments to very rapidly measure the colour on weighbridge and vineyard samples for screening and harvest scheduling purposes. The project team purchased such an instrument and the potential applications were explored. Its capability for scanning intact and homogenised fruit has been compared as well as the next logical step of scanning machine harvested fruit directly without any further processing. This proved feasible, and therefore it was proposed to conduct several trials in conjunction with other organisations, being (i) a winery for on-line measurement of colour after the crusher, and (ii) CRCV project 1.1.1 for on-harvester measurement of colour.

- ***Direct scanning of samples from bins of machine harvested fruit showed some promise for on-line applications. Further work is required to validate this application, for example on-harvester.***

A further innovative approach involved the assessment of the potential for the use of NIR for the non-destructive analysis of bottled wine *in situ*. As well as being able to measure, for example the relative degree of wine oxidation, as previously demonstrated by the AWRI Oxygen and Wine team, it was possible to perform some more routine analyses, such as for example alcohol content, for on-line monitoring of packaging for quality control purposes. This revelation led to the preparation and subsequent successful application for a patent as discussed later. Some trials were carried out at commercial wineries in order to investigate the feasibility of using the Zeiss CORONA spectrophotometer to monitor the degree of oxidation in white wines within sealed bottles. Several hardware set-up options for the Zeiss spectrometer were tested, together with a computer software program that the team developed for routine on-line analysis.

- ***It has been demonstrated that it is possible for NIR to determine the chemical composition and relative degree of oxidation in bottled wine non-destructively*** **in-situ.**

6.3 Other rapid analytical technologies

6.3.1 Fourier transform (FT) mid-infrared spectroscopy (MIR)

Feasibility trials have been conducted using mid-infrared (MIR) Fourier Transform Infrared (FT-IR) spectroscopy. Advantages in using FT-IR are that MIR peaks are due to the relatively intense and sharp fundamental vibrational modes of molecules and thus better defined and more easily identified compared with NIR spectra which involve weaker, broader and complex overtones and combinations of the fundamental vibrations. MIR is also sensitive to molecular groups with C, N and O atoms whereas in the Near IR range only high-order vibrations involving the H atom and some weak electronic transitions are seen. Using a novel sample presentation device (Attenuated total reflectance, ATR) to obtain scans has allowed grape homogenates, juices, wines and extracts to be scanned directly in an internal reflectance mode. The principals in ATR are similar to those in evanescent wave spectroscopy using remote fibre optics sensors. Sample presentation for the ATR method is greatly simplified compared to the usual liquid cuvette resulting in easy application, cleaning (just rinse and wipe), and no clogging due to sample sediments — ideal for grape homogenates. It also avoids the common problem of scanning through transmission cuvettes where extreme absorption of the infrared signal by water causes complete absorbance of the spectrum at some frequency regions, a major problem with some dedicated FTIR spectrometers in current use for routine wine and grape juice analysis. Comparative calibration statistics for the two techniques suggest that MIR may have superior predictive capability for pH and TSS, but is less accurate than NIR for colour. However, the best prediction for colour was achieved using a combination of MIR and Vis-NIR, which may offer opportunities for improved prediction for some materials.

- ***The combination of NIR with MIR appears to have some advantages in terms of accuracy of prediction of colour of red grape homogenates.***

Using a relatively affordable mid-infrared FT-IR instrument (Perkin Elmer *Spectrum-One*), the project team has continued with investigations on the application of the FT-IR technique, using the ATR. While extreme spectral absorbance from transmission cells can be reduced by using very thin cells, such short path-length cells are susceptible to blockages from suspended particles (i.e. grape homogenates). With the ATR method, the liquid sample of grape homogenate, wine or juice is simply spread onto the ATR crystal surface and scanned, thus avoiding the possibility of blockages. Prediction calibrations developed for total anthocyanins (colour), pH, total soluble solids (TSS) and electric conductivity (EC) in red grape homogenates, and ammonia, α-amino and yeast assimilable nitrogen (YAN) in white grape juice, were compared with those using a FOSS *NIRSystems6500* spectrometer. While better calibrations were obtained with MIR ATR for pH, TSS and EC than for NIR, prediction of colour (2003 vintage grape homogenate) gave a prediction error that was greater than for NIR (SECV of 0.25 mg/g compared to 0.17 mg/g for the *NIRSystems6500*). In contrast, the predictions of ammonia, α-amino and YAN nitrogen in white juice were slightly better than for NIR. It was concluded that MIR-ATR is worthy of further investigation as a method for grape and juice analysis. It can be used in the same manner for both white juice and red grape homogenates, as well as for other grape-derived products, with very little sample preparation thus providing several advantages as a simple cost-effective technique.

- ***The simple ATR reflection sample presentation method for FT-IR gave better predictive ability for grape parameters other then grape colour.***

The MIR ATR technique was tested for the study of grape phenolic and tannin compounds in alcoholic extracts. The ATR technique allows the scanning of very small amounts of sample as liquids or thin films, with particular advantages in studying the composition of the very low concentrations of non-volatile residual material in thin films arising from the evaporation of alcoholic extracts onto the surface of special reflecting ATR crystals. Preliminary tests gave spectra that clearly showed dramatic enhancement of the MIR ATR spectra, with characteristic frequencies of phenolic compounds clearly observable without the strong interference and dilution of alcohol and water normally seen when using the usual transmission cells. A set of 45 commercially available tannin preparations, was scanned by this in order to build calibrations and a reference library of spectra for diagnostic and interpretation purposes. As a further test of the efficacy of the MIR ATR technique, grape extracts and wines (n = 245) from the 2002-2004 Clare Valley trial were analysed to assess the possibility of calibrating for the determination of a range of properties including tannin, anthocyanin and other phenolic components, as well as to identify the type of tannin (seed or skin).

HPLC reference data for total phenolics including tannins and individual anthocyanins were used to develop calibrations for total tannin, malvidin-3-glucoside and malvidin-coumaryl-glucoside. In all cases, results were encouraging and the fact that there was no co-linearity between these parameters in this sample set precluded the possibility of the calibrations being simply cross-correlation i.e. the calibrations had different spectral loadings and appeared to be unique.

- ***FT-IR using ATR is promising technique for identification and quantification of grape phenolic compounds.***

- ***Prediction of tannin concentration using the dried film ATR method gave even better predictions***

6.3.2 Chemical sensors—mass spectrometry-based 'Electronic nose' instruments

The project has also investigated the potential of an *Agilent* 'electronic nose' (MS-E-Nose) chemical sensor system for applications related to wine spoilage (TCA) and adulteration. It appears that the chemical sensor does not have sufficient sensitivity for detecting 2,4,6 trichloroanisole in wine or 12% ethanol samples spiked with known amount of TCA (it has a detection limit above 10,000 ng/L, which is clearly far above the aroma threshold of TCA of approximately 2 ng/L). Given that this system responds to volatiles from the headspace above a wine, it is likely that this instrument is better suited for applications where the compounds at highest concentration in the air above a wine are the most important to quality — compounds such as yeast derived fermentation flavour compounds (e.g. fatty acid esters), monoterpenes in floral varieties, and fault or taint compounds present at relatively high concentration, such as ethyl acetate, acetaldehyde or acetic acid.

On the other hand, the instrument showed more promise in classification of wines for blending purposes as it was able to easily distinguish between two unblended wines (oaked 1999 Padthaway Chardonnay and a 1999 Padthaway Riesling) and there was a clear separation of their 25%, 50% and 75% blends. Also the 90% and 10% blends could be differentiated from 100% wines. The concentration of the major fermentation esters appeared to be the components that enabled the separation of the wines. In another trial, sensing of many different Chardonnay and Riesling wines showed there was a good separation of the two varieties with some further grouping among regions.

- ***Chemical sensors (e.g. 'electronic noses') have potential for discriminating between wines***

Further studies with both red and white wines investigated a range of applications for the MS-E-Nose. These studies have been promising and it is recommended that this instrument be further investigated.

- ***MS-E-Nose has shown potential for classification of wines by varietal origin***

- ***MS-E-Nose has shown potential for monitoring wine spoilage, for example Brettanomyces yeast spoilage.***

6.3.3 Combining instrumentation — the 'hybrid' approach

The combination of mass spectrometry (MS) based electronic nose (E-Nose) with visible (VIS) and near infrared spectroscopy (NIR) was explored as an objective tool to measure sensory attributes in commercial Riesling wines grown in Australia. Calibration models were developed between instrumental data and sensory scores using partial least squares (PLS) regression with full cross validation. Good correlations ($r > 0.70$, RMSECV: 0.66) were found for *developed* and *floral*; intermediate ($0.70 > r > 0.60$, RMSECV: 0.84 and 0.63) for *tropical* and low ($r < 0.50$, RMSECV: 0.98) for *green* characters measured by a sensory panel and the combination of both techniques. The results suggested that data from instrumental techniques coupled with chemometrics might be related with sensory scores measured by a trained panel. This study showed that combining data from different analytical instruments can have advantages and might be used to rapidly estimate specific sensory attribute scores in specific types of wine styles, compared to the use of a single technique. The pre-processing of the spectra before the chemometrics analysis was an essential element that enabled the development of the calibration models from such diverse and complex instrumental data. The study was considered a starting point in order to evaluate useful sources of information generated by different instrumental techniques with the objective to select combination of sensors for specific wine quality attributes. As in any stage of development of a new method, further

validation work needs to be conducted in order to assess and extend the application of this method to other wine varieties and types.

- ***Combining sensor types (e.g. Vis-NIR and MS-E-Nose) has been demonstrated as a feasible means of improving calibration accuracy in certain applications, such as prediction of sensory properties in white wines.***

6.4 Multivariate statistical techniques

The team continues to maintain a strong commitment to the training and teaching of the principles and use of multivariate analysis techniques (chemometrics) to other team members as well as for industrial and external research partners. In-house workshops and training commenced in March 2004 and the training program was extended to industry through workshops as part of the 12th Australian Wine Industry Technical Conference held in Melbourne in July 2004. One example of an external collaboration is that with a GWRDC-funded RITA project that was conducted in Tasmania on benchmarking Pinot Noir production, where team members provided advice and assistance in the use of multivariate techniques to analyse large amounts of complex data from the analysis of grapevine sap, vineyard soil and harvested grapes. Another example of an internal collaboration is the team's contribution in the use of chemometrics to relate sensory assessment of wine to visible wavelength spectral data of samples from the AWRI Oxygen and Wine project, which revealed that the critical wavelengths differ from those traditionally used to compare relative oxidation in white wines. These are just a few illustrations of the value of the multivariate data analysis approach to complex problem solving. A CRCV participating partner agreement was established with James Cook University's statistical data analysis group and data relating to the processing of grape homogenates was evaluated using some advance mathematical techniques. A PhD student, David Donald, at JCU evaluated the data under the co-supervision of Yvette Everingham (JCU) and Daniel Cozzolino (AWRI). These confirmed our earlier findings that are described above (Standardising reference methods).

- ***The project has developed skills, experience and know-how in chemometrics and data mining that can be applied to situations well beyond both NIR spectroscopy and grape and wine analysis.***

7 Outcome/Conclusion

This project has demonstrated that spectroscopic techniques in particular NIR and MIR techniques show promise as a useful tool for the industry and as a result several companies have adopted the technology into their production business activities. A detailed article was published in the 2003 Australian and New Zealand Grapegrower and Winemaker Technical Issue and includes descriptions of how several large wine companies have now put the NIR technology into practice. For example, during the 2003 vintage, the total number of red grape samples analysed for colour by NIR (albeit primarily conducted by one large company) represented approximately 300,000 tonnes of fruit, in excess of 20% of the total national harvest of red grapes, which was estimated at around 1.4 million tonnes. Significant benefits have also been demonstrated from the use of NIR in the research environment. Using NIR to predict red grape quality parameters (colour, TSS and pH), researchers at CSIRO (Land & Water's Precision Agriculture group) and the University of Adelaide (Roseworthy campus) have been able to significantly reduce the cost of analysis and the necessity for wet chemistry in relation to their research projects spatially relating grape/wine quality indicators to vineyard properties.

The project has successfully evaluated the feasibility of many applications of the technology for rapid determinations of several constituents of grapes, wines and spirits. In response to the wine industry's need for rapid analytical methods for the determination of objective indicators of grape quality, the project has concentrated its efforts on development of an NIR method for determining colour, total soluble solids (TSS) and pH in red grapes that would be suitable for practical industry use.

Investigations into potential laboratory-based applications of the technology have resulted in the following conclusions:

For spirits:

- The ability of NIR to measure methanol in spirits has been established and a manuscript has been published in a refereed scientific journal.

For grapes, must or juice:

- Calibrations have been developed for the measurement of colour, pH, TSS and phenolic compounds in red grape homogenates for several distinctly different types of NIR instruments.
- The accuracy of the calibrations varied depending on the nature of the dataset used — from general 'pan-Australian' to more accurate vintage, region and variety specific. It appears that matrix effects are important and it is necessary to encompass sources of variation in a developed calibration.
- The use of novel chemometric techniques, such as locally weighted regression (e.g. LOCAL) and ANN modelling, has been found to ameliorate the non-linearity effects observed in the NIR calibration for red grape colour that was developed for a wide range of viticultural regions over seven vintages.
- A paper describing the use of laboratory based NIR methods to quantify colour, pH and total soluble solids in grapes has been published in Australian Grapegrower and Winemaker (2003 Annual Technical Issue). The main points of the paper relate to issues of matrix variation (effects of variety, region and vintage), calibration transfer and calibration strategies.
- An infrared calibration at the required level of prediction accuracy does not appear to be feasible for G-G in grape homogenates, however, it does appear feasible for G-G in white grape juices.
- The use of NIR and MIR to predict yeast assimilable nitrogen (YAN) in grape juice shows considerable some promise, with semi-quantitative accuracy for the NIR and analytical quality for the MIR.
- NIR shows promise in discriminating different levels of fungal infection but requires further validation work

For wines:

- The Vis-NIR method appears useful for rapid determination of the Somers' spectral measures in red wines.
- Preliminary experiments have shown promise that NIR may be able to classify by variety samples of commercial bottles of Riesling and Chardonnay wines. FTIR has been shown to have potential for authentication of commercial wines by spectral discrimination or "fingerprinting".
- NIR has shown promise for monitoring the evolution of colour, tannin and pigmented polymer in red wine fermentations, suggesting potential for possible on-line implementation. This application also offers significant savings in analytical cost for fermentation research experiments.
- NIR has shown potential to predict sensory properties in a set of white wines (Riesling and Chardonnay).
- NIR has shown promise for monitoring *Brettanomyces* yeast contamination in red wines.

Whilst the development of laboratory methods has been a primary aim of the project, it was also recognised that the techniques developed must also be suitable for practical use in the industry. The Cooperative Research Centre for Viticulture is managing the commercialisation of the intellectual property developed by the project through their commercialisation agent, CRCV Technologies. Meanwhile, the project team has continued to investigate areas of scientific challenge related to the technology in order to facilitate the implementation by industry.

Investigations into the practical aspects of implementation of the technology have resulted in the following conclusions:

Instrumentation:

- Commercialisation trials conducted by CRCV Technologies highlighted the significant challenges with both standardisation of the instruments and transfer and robustness of calibrations.
- A limited trial commercial release with low cost custom-made instruments in 2002 was unsatisfactory. However, trials with similar low cost instruments manufactured by an alternative more reliable commercial partner and equipped with the CRCV calibration with screening accuracy during 2005 and 2006 were far more successful.
- The evaluation of an NIR instrument that can rapidly scan whole intact grape berries indicated that the technique was feasible but with the cost of lower accuracy compared to scanning of homogenates of grape berries
- Preliminary trials with scanning of intact whole grape berries, unprocessed samples from bins of machine harvested fruit, and intact wine bottles have shown indications of promise for on-line applications.
- Calibration transfer both within- and between- instrument types appears feasible but requires validation

Protocol and procedures:

- Standard operating procedures for both the NIR and reference laboratory methods for colour have been prepared and published.
- Neither freezing of samples prior to homogenisation, nor the type of homogeniser had any significant effect on the accuracy of laboratory determinations of colour and TSS of red grapes.
- Frozen storage of grape samples for up to month did not affect the accuracy of prediction of grape composition by NIR
- stable reference standards for validation and instrument matching were developed by the project team

Whilst the project focussed mainly on the use of NIR technology, it still maintained considerable effort in investigating the use of other rapid techniques. MIR FT-IR spectroscopy with the FOSS *WineScan* and other similar devices is currently used worldwide for the analysis of grape juice and wine. Alternative sample presentation methods such as ATR may provide more accurate measurement of compounds that are present in relatively low concentration in grapes such as phenolic compounds, tannins, G-G, and amino acids than NIR and preliminary trials have shown some promise. It appears that the combination of NIR with FT-IR may have some advantages in terms of accuracy of prediction of colour of red grape homogenates.

Investigations into the potential of an 'electronic nose' chemical sensor system showed promise for applications related to wine spoilage (e.g. *Brettanomyces*, TCA). Combining this technique with NIR appears to offer great potential, for example in the prediction of sensory attributes of white wine.

The project team has developed skills, experience and know-how in chemometrics and data mining that can be applied to situations well beyond both NIR spectroscopy and grape and wine analysis. This skill and know-how can be used in future collaborative projects for the benefit of the wine industry.

7.1 Performance against planned outputs

Performance against the planned outputs has been discussed in section 4.3. In summary, the project team have achieved all targets and in many cases exceeded them.

7.2 Practical implications for industry

The GWRDC 2002-2007 R & D Plan shows priorities and directions developed after extensive consultation with industry. The industry planning document *Strategy 2025*, launched in 1996, was followed in 2000 by an update in the form of *The Marketing Decade*, and both documents have contributed significantly to the thinking behind the plan.

This project was clearly aligned to *Program 1 Innovation and Technology Adoption* of the GWRDC plan — its basis is innovation through implementation of techniques that not been traditionally been used by the wine industry and have been demonstrably successful in other industries. The project used several of the strategies identified in the GWRDC plan including:

- Reinforce innovation culture — *the application of technologies well established outside the wine industry*
- Foster wine industry specific R & D skills and an internationally competitive institutional capability; Stocktake and articulate significant changes in best practice to enable industry to assimilate new knowledge in grape and wine production — *increase the store of knowledge at the AWRI and the industry as it grew through the collaborative project*
- Encourage increased investment/involvement in R & D from individual producers and suppliers; Facilitate regional networks for R & D adoption — *the project is highly collaborative*
- Develop and package R & D outcomes to make them application ready — *where possible, applications for implementation have been developed*
- Communicate R & D results in user friendly formats with a focus on producer benefits; Disseminate R & D outcomes through a range of demonstration activities — *delivery through the existing infrastructure of the CRCV and the AWRI's Industry Development and Support group.*

In addition, the project as a whole can be seen to meet many of the needs identified in other parts of the GWRDC plans, for example, Programs 2 (*Sustainable production*) and 3 (*Quality and Differentiation*). This project was aimed at improving production efficiency and aligned with several of the key areas of GWRDC's program 3 (*Quality and differentiation*), which is to improve producer capability to manage the quality and flavour dimensions of grapes and wine in order to satisfy market requirements and maximise earnings.

Of the strategies in the GWRDC plan to achieve this objective, those addressed within the project included:

- "Specify and calibrate the standards and formulate the practices to manage the determinants of wine product integrity;
- Determine the impact of different viticultural and production processes on desired wine quality attributes;
- Develop objective measures of quality and of flavour attributes in grapes and wine; and
- Identify and specify techniques to manage and to improve quality."

Of the needs identified in the plan for both grapegrowers and winemakers, the following were addressed by the project:

- Know in greater detail the relationships between factors influencing grape and wine quality and will have the tools to measure quality;
- Have a greater capacity to measure and control quality factors in association with grape producers;
- Be able to optimise characteristics like mouth feel and colour of red wines; and
- Have more options for managing wine sensory properties.

One stream of activity additional to those originally proposed and was subsequently successfully undertaken following the supplementary bid was method standardisation. This underpins one of GWRDC's identified measures of success, which is the provision of 'Quality and flavour measurement technology that are accepted as both objective and accurate.'

In the modern technical business environment, it is generally recognised that commercialisation activities are likely to be the most appropriate mechanism to facilitate rapid uptake of the technology by the industry, and CRCV Technologies Pty Ltd has been responsible for this aspect. This process was managed by the CRCV Technologies Pty Ltd and they were responsible for ensuring that commercial applications of the technology are delivered to target audiences in 'application-ready' state for adoption by industry. The corporate governance requirements of CRCV Technologies Pty Ltd ensured that evaluations of all outcomes, including economic, social and environmental, were conducted. However, there is always some risk that the chosen method of commercialisation activities may not turn out to be the most appropriate means and any perceived shortfalls in this aspect may be attributable to such risk.

The GWRDC conducted a cost-benefit analysis of this project in 2001 that also included an assessment of the potential adoption and likelihood of success. The project returned good economic return with a relatively high chance of success (see also GWRDC Annual report 2001-2002).

- The project had by that time already provided demonstrable benefits to the Australian wine industry. It has been so successful in fact that it is now used commercially by wine processing facilities that represent more than 20% of the total Australian crush. This technology has resulted in direct savings in analysis time for grape colour alone that could represent well over $200,000 per year. An alternative way of looking at this is that the technology has now allowed an additional perhaps 6,000 samples each year to be analysed to assist wineries to more fully understand the quality of the grapes they receive. This is a highly significant outcome in only a relatively short period of investment by the industry through the GWRDC.
- Commercialisation trials managed by CRCV Technologies with relatively cheap robust custom-made NIR instruments conducted with industry in 2005 and 2006 have shown a remarkable degree of success, with excellent feedback received from participating wineries and other participating organisations. This was a marked turnaround from earlier trials during the 2002 vintage that did not proceed as smoothly as was hoped. Some of the fundamental research needs in understanding the best mechanism of transferring calibrations to instruments in practical use were identified during the early trials and these challenges were successfully addressed.

The beneficiaries of the project outputs vary to some extent depending on the specific output. In general terms there are three main beneficiaries:

1. Research—the provision of an ability to predict analytes normally determined by complex techniques within a closed experimental system means that analysis costs of research can be greatly reduced. For example a calibration might be developed on a relatively small proportion of the experimental samples and used to predict the remaining samples from the set. This has clearly been demonstrated in this project. Communication to this audience was through the generally accepted means of dissemination as used by the scientific community, including

conferences and scientific publications. In particular, the CRCV reporting and symposia structure has greatly assisted in effectively reaching other researchers within Australia.

2. Wineries—wineries may be able to use the technology to increase the amount of testing that they can conduct on the grapes and wine they process. This will allow them to generate large amounts of data from which they can gain greater understanding of the specifications for both the feedstock and products. This audience was also reached by similar mechanisms but with an added emphasis on industry or trade-type publications and seminars (e.g. ASVO, WineTech). Use of the Institute's extension activities, such as 'Roadshow seminars' has also been made to provide an extremely effective means of dissemination.

3. Grapegrowers—provision of rapid measurement techniques will mean that growers are able to target their practices and monitor them for effectiveness as they strive to achieve the target specifications given to them by their customers downstream. Growers have also be reached effectively though similar mechanisms, but with a slightly different emphasis—eg. Field days and associated seminars, and viticulture-specific publications.

7.3 Benefits of the research

To enhance further the quality of its products, the wine industry has a clear need for simple, rapid and cheap techniques for objectively evaluating the quality of grapes and wines. The measurement of grape characteristics which impact on product quality is a basic requirement for vineyard improvement and for optimum production of desired wine styles. It is common industry practice for quality assessment to be achieved by total soluble solids (TSS) and acidity measurement (pH), visual assessment, and also by tasting assessment of fruit and of wines following vinification. Acidity and soluble solids measures are insufficient as quality indicators, and it is not possible to adequately assess quality by tasting alone. There is a strong need in the modern wine industry for timely information that can be used for grape berry maturity assessment, identification of vineyard blocks or sections of a vineyard that should be segregated, and load quality assessment. Existing analytical methods for measurement of grape composition are not well suited to the demands of production, where the two factors of rapidity and low cost are of paramount importance. Even simple analyses currently require samples to be sent to a geographically separate laboratory, with inherent delays in achieving results. More complex analyses, such as grape colour measurement, terpene analysis, nitrogen concentration, phenolics analysis and the recently developed glycosyl-glucose (G-G) assay, are not considered viable options by my many industry practitioners because of their high cost and slow turnaround time. Assessment of juice, wine and spirit quality is also restricted at present for similar reasons. However, near infrared spectroscopy (NIR) is a correlative technique that offers the potential to reduce analytical times from hours to less than seconds in a range of measurements. It is a technique that is widely used in other agricultural industries and has the several advantages of:

- short analysis time,
- little or no sample preparation,
- ability to measure several parameters simultaneously, and
- potential in-field or on-site use (i.e. in vineyard, on mechanical harvesters, at the winery weighbridge).

An independent cost-benefit analysis commissioned by the GWRDC in 2001 found that the use of NIR for only two compositional variables (colour in red grapes and methanol in spirits) could return benefits of up to $1.1 million per annum. In addition, the industry would benefit in indirect ways from:

- enhancement of the ability for wineries to better define grape quality specifications through the use of a simple, rapid and cost-effective tool to objectively evaluate fruit characteristics (including colour, pH, total soluble solids); and
- reduction in the cost of objective measurement of grapes thereby allowing many more tests to be conducted throughout the industry.

The same study also found that maximum adoption would be achieved in 5 years with benefits projected from the year following project completion.

In the course of reviewing some preliminary 'commercialisation' trials in the application of the technology during the 2002 harvest, project participants confirmed an opportunity to also apply the technology for direct in-vineyard use. Commercial interest has been shown in this potential application and the present proposal is focussed on developing the technology to achieve this aim. While not replacing weighbridge measurement, in-vineyard measurement of grape quality would have the significant advantage over weighbridge applications by facilitating direct measurement and spatial referencing of grape quality within vineyards. This knowledge would allow growers or

wineries to more accurately segregate and stream fruit to match the required product specifications. If technical difficulties of variable sample presentation can be overcome then GPS-linked mechanical harvester mounted application of the technology may facilitate even greater precision in within-vineyard spatial referencing.

This project is clearly aligned to *Program 1 Innovation and Technology Adoption* of the GWRDC plan — its basis is innovation through implementation of techniques that have not traditionally been used by the wine industry and have been demonstrably successful in other industries. The project uses several of the strategies identified in the GWRDC plan.

In addition, the project can be seen to meet many of the needs identified in other parts of the GWRDC plans, for example, Program 3 (*Quality and Differentiation*).

The GWRDC conducted a cost-benefit analysis of this project in 2001 that also included an assessment of the potential adoption and likelihood of success and found that the project returned good economic return with a relatively high chance of success (see also GWRDC Annual report 2001-2002).

The benefits for the Australian wine industry and broader community are:

- Rapid analytical methods can help prevent quality loss in large volumes of red wine. This, in addition to the reduced analysis costs of such screening techniques, will result in higher profitability for companies leading to a positive economic impact on Australia.
- Successful outcomes will provide the industry with a means for rapid and cost effective monitoring for quality during wine production, thereby minimising potential losses in quality and value of wine produced in Australia. Our industry partners are extremely keen to implement any such methods as soon as possible; therefore our initiative will enhance their contribution to R&D.
- This might result in a **reduction in quality loss** and, using a conservative estimate of 15% of wine production being improved, at a saving in quality value of only $1 per litre, would represent **almost $10 million per year.**
- Commercialisation of this development will most likely be of two types. Firstly, the collaborating wine company will be implementing the techniques as it is being developed, and secondly, the technique can be transferred to the AWRI Analytical Service or other similar entity and thereby made available to the whole industry.

8 Recommendation

This project has generated a large amount of output in a relatively short time and been a driving force behind a significant uptake by the industry of this technology. Notwithstanding this success, it is clear that there are many more potential applications that warrant significant investment in further research work.

The main focus to date has been on the development of practical NIR methods for the rapid prediction of red grape composition for quality indication purposes–the measurement of total anthocyanins (colour) being the most important of these. It is clear from the work so far that calibrations for red grape colour are subject to matrix variation that may be influenced by season and therefore ongoing further work over successive seasons to examine the robustness is clearly necessary. Since such measurements appear likely to be used for trade purposes (i.e. grape payment), and the project has provided the means for strict standardisation, improved accuracy and validation of the methods of determination being used by the industry, whether by NIR or standard laboratory techniques, through the defining of standard laboratory protocols and development of standard reference materials. Now that these compositional measures have been examined, it is necessary to move on to the next level and search for and validate other compositional parameters that have an influence on wine quality.

The project has conducted some preliminary investigations into some qualitative prediction applications and demonstrated the potential of NIR for use in quality grading of wines for blending or allocation purposes, as well as classification of white wine samples by variety. Clearly such applications might offer broad potential use by industry and therefore must be examined further. In addition, it has been demonstrated that NIR can discriminate between different yeast strains and again this may have potential applications in both the research and industrial sectors of the wine industry. This observation requires further validation and development.

Another area of extreme interest is the application of NIR for prediction of wine sensory scores that are normally collected by the expensive and time-consuming method of human sensory panels. We have demonstrated some potential for savings in this area and it is clearly worthy of further investigation.

NIR technology inherently offers itself as a potential tool for on-line measurement applications because it can be non-destructive, non-contact and extremely fast. One such example already identified is the potential for monitoring of red wine fermentation for the evolution of colour and phenolics. Another is the demonstrated feasibility for measuring wine composition directly in intact bottled wine. A future potential also exists for the determination of grape composition directly as it is harvested or in the harvest bin, or even in-line after tipping or crushing. The challenges for this application of NIR will mainly be in relation to sample presentation, but also significant software and engineering issues will need to be considered and is clearly an area for future research.

Significant players in the Australian wine industry have readily embraced the uptake of NIR technology predominantly in one area of application (viz. the measurement of red grape colour). To obtain the maximum benefit that the technology can offer it is necessary for the industry to begin increasing its own skills base and understanding of the technique. Therefore, there is clear need for further 'development' or 'extension' activities emanating from the research and the expertise that was encouraged through the activities of the CRCV. Such activities include the provision of information through publications and advisory services, but also through development and provision of specialised targeted training programs that are of direct relevance to the industry, and some pilot trials of these types have already taken place. A significant challenge in this respect will be the identification of appropriate infrastructure or organisation capable of such delivery and the management and coordination of such proposed activities on a national scale.

Whilst it is clear that NIR has much to offer the wine industry, both for researchers and for industry, there are many other alternative rapid techniques that are worthy of investigation and are currently being assessed. Spectroscopic techniques such as ultra-violet, visible, MIR or Raman may offer other niche applications of potential benefit and it is efficient to utilise the expertise gained from this project to expand and investigate these. Since FT-MIR is currently used worldwide as a routine analytical tool and there are opportunities to expand the capabilities of the FT-MIR to include new analytes and new research directions. There may be other new techniques also with benefits to offer and the investigative capabilities developed by the team as a result of the current research are likely to provide efficiency in furthering such investigations. Such techniques include the range of chemical sensors, such as mass spectrometry-based electronic nose or tongue instruments — investigations conducted in this project indicate great potential for such instruments.

This project which began with investigation of NIR spectroscopy has tantalisingly opened the door to a new approach and application of the combined disciplines of analytical chemistry, physics, mathematics, biochemistry and engineering to the production of wine. Along the way the project has provided a glimpse of many opportunities for the application of a range of rapid analytical techniques and multivariate statistical techniques for the wine industry. Further work in the applications of these disciplines may provide the stepping-stone to a further maturing of the Australian wine industry with a commensurate increase in competitive advantage.

- ***Further investigations of other rapid analytical technologies are required to enhance the suite of tools available to grapegrowers and winemakers for monitoring and assessing the quality of their products***

- ***Highly targeted extension and training activities are required to assist industry technical staff to uptake and implement the technologies of benefit to their businesses.***

- ***Delivery of the abundant outputs from this project into outcomes for the wine industry requires the fostering of a culture of delivery though a range of commercialisation activities. A significant challenge in this respect will be the identification of appropriate infrastructure capability of such delivery on a national scale.***

9 Appendix 1: Communication

9.1 Public relations and communication — Media reports (radio, newspapers, television

1. Gishen, M. Technology moves into vineyard. GrapeGrowers newspaper, February 1999; p. 14.
2. Dambergs, R.G. Colour helps define quality. National GrapeGrowers, January 2000; p. 16.
3. Gishen, M. Limits on colour quality. Riverland and Northern Mallee Countryside Quarterly, March 2000; p. 5.
4. Dambergs, R.G. Red grape colour testing with near infrared spectroscopy. ABC Radio 5MV Rural Report (Cathy Parker), 7 August 2000.
5. Dambergs, R.G. Red grape colour testing with near infrared spectroscopy. Win TV News. 7 August 2000.
6. Gishen, M. Red grape quality testing. ABC radio, SA, 5CK: Country Hour (Andrew Maddern), 8 August 2000, 12.25 pm
7. Dambergs, R.G. Red grape colour testing with near infrared spectroscopy. ABC Television Lateline (Neil Cross/Tony Jones). 8 August 2000.
8. Gishen, M. Red grape quality testing. ABC radio, QLD/NT, Tropical FM: Drive (Anne O'Keefe), 9 August 2000, 16.46 pm
9. Gishen, M. Near infrared spectroscopy. ABC Radio National, Interview with Natasha Mitchell, Science Unit, Australian Broadcasting Corporation (ABC), 12 March, 2001
10. Viticulture: Technology to benefit grapes. The Grower April 2002; pp 12-13.
11. Press interview with Mark Gishen: Kerry Lush, ABC Riverland: Explanation following release of AWRI standard method on red grape colour measurement on CRCV website. Aired Wednesday 29 March 2006 - Rural Report 5MV
12.

9.2 Publications and written technical communications

9.2.1 Scientific publications (refereed)

1. Dambergs, R.G.; Kambouris, A.; Francis, I.L.; Gishen, M. (2002) Rapid analysis of methanol in grape derived distillation products using near infrared transmission spectroscopy. *J. Ag. Food Chem.* 50: 3079–3084.
2. Dambergs, R.G.; Esler, M.B.; Gishen, M. (2004). Near Infrared Spectroscopy in Agriculture: applications in analysis of beverages and brewing products. In: Near Infrared Spectroscopy in Agriculture; Roberts, C.A., Workman, J. and J.B. Reeves (Eds.). Agronomy Monograph 44; ASA, CSSA, and SSSA; Madison, WI, USA. pp. 465-486.
3. Cozzolino, D.; Smyth, H.; Gishen, M. (2003) Feasibility study on the use of visible and near infrared spectroscopy together with chemometrics to discriminate between commercial white wines of different varietal origins. *J. Agric. Food Chem.* 51: 7703-7708.
4. Cozzolino, D.; Kwiatkowski, M.; Parker, M.; Gishen, M.; Dambergs, R.G.; Cynkar, W.U.; Herderich, M.J. (2003) Prediction of phenolic compounds in red wine by near infrared spectroscopy. *Analytica Chimica Acta* 513: 73 - 81.
5. Cozzolino, D.; Esler, M.B.; Dambergs, R.G.; Cynkar, W.U.; Boehm, D.R.; Francis, I.L.; Gishen, M. (2004) Prediction of colour and pH in grapes using a diode array spectrophotometer (400 - 1100 nm). *J. Near Infrared Spectroscopy* 12: 105 – 111..
6. Cynkar, W.; Cozzolino, D.; Dambergs, R.G.; Janik, L.; Gishen, M. (2004). The effects of homogenisation method and freezing on the determination of quality parameters in red grape berries of *Vitis vinifera*. *Australian Journal of Grape and Wine Research* 10:3, 236-242.
7. Gishen, M.; Cozzolino, D.; Dambergs, R.G. Grape and wine analysis in the Australian wine industry — enhancing the power of spectroscopy with chemometrics (2005). *Australian Journal of Grape and Wine Research* 11: 296 - 306.
8. Cozzolino, D.; Smyth, H.; Lattey, A.; Cynkar, W.U.; Janik, L.; Dambergs, R.G.; Francis, I.L.; Gishen, M. (2005) Relationship between sensory analysis and near infrared spectroscopy in Australian Riesling and Chardonnay wines. *Analytica Chimica Acta* 539: 341-348.
9. Cozzolino, D.; Smyth, H.; Cynkar, W.U.; Dambergs, R.G.; Gishen, M. (2005) Usefulness of chemometrics and mass spectrometry based electronic nose to classify Australian white wines by their varietal origin. *Talanta* 68: 382 - 387.
10. Cozzolino, D.; Dambergs, R.G.; Cynkar, W.U.; Janik, L.J.; Gishen, M. Effect of both homogenization and storage on the spectra of red grapes, and on the measurement of total anthocyanins, total soluble solids and pH by Vis-NIR spectroscopy (2005). *J. Near Infrared Spectroscopy* 13: 213 - 223.
11. Dambergs, R.G.; Cozzolino, D.; Cynkar, W.U.; Janik, L.J.; Gishen, M. (2006). The determination of red grape quality parameters using the LOCAL algorithm. *J. Near Infrared Spectroscopy* 14: 71 - 79.

12. Cozzolino, D.; Smyth, H.; Lattey, A.; Cynkar, W.U.; Janik, L.; Dambergs, R.G.; Francis, I.L.; Gishen, M. (2006) Combining mass spectrometry based electronic nose, visible–near infrared spectroscopy and chemometrics to assess the sensory properties of Australian Riesling wines. *Analytica Chimica Acta*. 563: 319 - 324.
13. Sarneckis, C.J.; Dambergs, R.G.; Jones, P.; Mercurio, M.; Herderich. M.J.; Smith, P.A. (2006) Quantification of condensed tannins by precipitation with methyl cellulose: development and validation of an optimised tool for grape and wine analysis. *Australian J. Grape and Wine Res.* 12: 39-49.
14. Donald; D.; Coomans D; Everingham Y; Cozzolino D; Gishen M; Hancock, T. (2006). Adaptive wavelet modelling of a nested 3 factor experimental design in NIR chemometrics. *Chemometrics and Intelligent Laboratory Systems*. 82: 122-129.
15. Howell, K.S., Cozzolino, D., Bartowsky, E.J., G. Fleet, Henschke, P.A. (2006). Metabolic profiling as a tool for revealing Saccharomyces interactions during wine fermentation. *FEMS Yeast Research*. 6: 91 - 101.

9.2.2 Technical reports and industry journal articles (non-refereed)

1. Francis, L. Cynkar, W. Investigation of an 'electronic nose'. Aust. NZ Wine Ind. J. 15(6): 54-56.
2. Gishen, M.; Francis, I.L. (2001) NIR spectroscopy and grape berry colour measurement: an update. *Technical Review* No. 134 October 2001. The Australian Wine Research Institute; Urrbrae, SA.
3. Anon. (2002) Near infrared spectroscopy shows promise as a useful tool: CRCV update. Aust. NZ Wine Ind. J. 17(1) 41–42.
4. Dambergs, R.G.; Cozzolino, D.; Cynkar, W.U.; Kambouris, A.; Francis, I.L.; Høj, P.B.; Gishen, M. The use of near infrared spectroscopy for grape quality measurement. The Australian & New Zealand Grapegrower and Winemaker. Annual Technical Issue (No. 476a), 69 – 76, (2003).
5. Gishen, M. Dambergs, R. Near infrared spectroscopy at the Australian Wine Research Institute (Part 1). *NIR. News* 13 (6): 9, 15; 2002.
6. Gishen, M. Dambergs, R. Near infrared spectroscopy at the Australian Wine Research Institute (Part 2). *NIR News* 14 (2): 26–27; 2002.
7. Cozzolino, D.; Cynkar, W.U.; Janik, L.; Francis, I.L.; Gishen, M.; Dambergs, R.G. Advances in infrared (IR) spectroscopy at AWRI: novel applications for grape and wine analysis. *Technical Review* No. 145, July 2003. The Australian Wine Research Institute; Urrbrae, SA.
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9. Cynkar, W.; Cozzolino, D.; Dambergs, R. The effects of homogeniser-type and frozen storage time on the determination of total anthocyanins (colour), total soluble solids and pH in red grape berries. Technical Review (151): 20-24; 2004. Adelaide, SA The Australian Wine Research Institute.
10. Cynkar, W.; Cozzolino, D.; Dambergs, R.; Gishen, M. A recommended industry 'standard' method for determination of the concentration of total anthocyanins (colour) in red grapes. Technical Review (151): 19; 2004. Adelaide, SA The Australian Wine Research Institute.
11. Pollinitz, A., Eglinton, J., Siebert, T., Smyth, H., Henschke, P., Parker, M., Francis, L., Cozzolino, D. Herderich, M. (2004). Research links vineyards, vintage, aroma, flavour to bottled wine. Aust. Vig. 4 (2): 17-21.
12. Gishen, M.; Dambergs, R.G.; Kambouris, A.; Kwiatkowski, M.; Cynkar, W.U.; Høj, P.B.; Francis, I.L. Application of near infrared spectroscopy (NIRS) for quality assessment of grapes, wine and spirits. Davies, A.M.C.; Giangiacomo, R., eds. Near Infrared Spectroscopy: proceedings of the 9th International conference; Chichester, UK. NIR Publications; 2000: 917–920.
13. Esler, M.B.; Gishen, M.; Francis, I.L.; Dambergs, R.G.; Kambouris, A.; Cynkar, W.U.; Boehm, D.R. Effects of variety and region on near infrared reflectance spectroscopic analysis of quality parameters in red wine grapes. Davies, A.M.C.; Cho, R.K. Near infrared spectroscopy: proceedings of the 10th international conference; Chichester, UK. NIR Publications. 249-253. 2002.
14. Dambergs, R.G. Kambouris, A. Schumacher, N. Francis, I.L. Esler, M.B. Gishen, M. Wine quality grading by near infrared spectroscopy. Davies, A.M.C.; Cho, R.K. Near infrared spectroscopy: proceedings of the 10th international conference; Chichester, UK. NIR Publications. 187-189. 2002.
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17. Gishen, M. Opportunities for NIRS and chemometrics in viticulture. In: OIV proceedings, Adelaide 11-12 October 2001.
18. Dambergs, R.G.; Cozzolino, D.; Cynkar, W.U.; Kambouris, A.; Francis, I.L.; Høj, P.B.; Gishen, M. The use of near infrared spectroscopy for grape quality measurement. The Australian Grapegrower and Winemaker; Annual Technical Issue (No. 476a) 69 – 76; 2003.
19. Gishen, M. Dambergs, B. Near infrared spectroscopy at the Australian Wine Research Institute (Part 1). *NIR News* 13 (6): 9, 15; 2002.
20. Gishen, M. Dambergs, B. Near infrared spectroscopy at the Australian Wine Research Institute (Part 2). *NIR News* 14 (2): 26–27; 2002.
21. Cozzolino, D.; Cynkar, W.U.; Janik, L.; Francis, I.L.; Gishen, M.; Dambergs, R.G. Advances in infrared (IR) spectroscopy at AWRI: novel applications for grape and wine analysis. *Technical Review* No. 145, July 2003. The Australian Wine Research Institute; Urrbrae, SA.
22. Pollinitz, A., Eglinton, J., Siebert, T., Smyth, H., Henschke, P., Parker, M., Francis, L., Cozzolino, D. Herderich, M. (2004). Research links vineyards, vintage, aroma, flavour to bottled wine. Australian Vigneron 4: 17-21.

9.2.3 Draft papers in preparation (as at 26/05/2006)

1. Dambergs, R.G.; Kambouris, A.; Bevin, C.; Lim, A; Gishen, M. Wine quality grading with visible and near infrared spectroscopy. *Food Quality and Preference.*
2. Janik, L.J., Cozzolino, D., Dambergs, R., Cynkar, W. and Gishen, M. The prediction of total anthocyanin concentration in red-grape homogenates using near-infrared spectroscopy and artificial neural networks. *Analytica Chimica Acta.*
3. Janik, L.J., Cozzolino, D., Cynkar, W.U., Dambergs, R. and Gishen, M. Mid-infrared attenuated total reflectance (ATR) spectroscopy for the analysis of grape composition. *Analytica Chimica Acta.*

9.3 Presentations

9.3.1 Conference and meeting addresses

1. Assessment of fruit and product quality in the wine industry using near infrared spectroscopy. Dambergs R.G.;:, Gishen, M. 8th Australian Near Infrared Spectroscopy Group Conference, Palm Cove, 21-23 August 1998 (Bob Dambergs).
2. Fruit and product quality evaluation using NIRS. BRL Hardy Technical Conference, Hobart, 7-10 June, 1999 (Bob Dambergs)
3. Measuring fruit quality. In: "Grape quality for our future", BRL Hardy grower roadshow seminars, Loxton, Berri, Barmera, Renmark, Mildura, 6-10 September, 1999 (Bob Dambergs).
4. Near infrared spectroscopy: potential applications for viticulture and oenology. Charles Sturt University, 18 August 1999 (Bob Dambergs)
5. NIRS in the new millennium: applications for the wine industry. Winery Engineer's Association conference, Griffith, 9 September, 1999 (Mark Gishen).
6. The GG assay and NIRS: what are they and what are the applications in the wine industry? Orange Region Vignerons' Association Annual Field Day, Orange, NSW, 5th February 2000 (Mark Gishen).
7. NIRS – rapid analysis and quality assessment for agriculture. Renmark Agricultural Bureau, Renmark, 3 April 2000 (Bob Dambergs).
8. Gishen, M.; Clancy, P.J.; Francis, I.L.; Dambergs, R.G.; Johnstone, R.S. Development of a robust portable scanning NIRS instrument for quality assessment of grapes in the wine industry. 9th Australian Near Infrared Spectroscopy Group Conference, Victorian Institute of Dryland Agriculture, Horsham, 5-6 April 2000 (Mark Gishen).
9. NIRS assessment of fruit and wine quality. BRL Hardy Technical Conference, Queenstown, N.Z., 5-8 June 2000 (Bob Dambergs).
10. Measuring grape quality with a spectrophotometer. Interwinery Analysis Group Annual Technical Meeting. Tanunda, 7 July 2000 (Bob Dambergs).
11. Berri Ag bureau 24 July 2000(Bob Dambergs)
12. Grape colour as a quality indicator. Wine Grapes Marketing Board workshop, Griffith, NSW, 10 August 2000 (Bob Dambergs).
13. Grape colour as a quality indicator. Australian Vintage growers meeting, Loxton, 25 September, 2000 (Bob Dambergs)
14. Application of near infrared spectroscopy (NIRS) for quality assessment of grapes, wine and spirits in the Australian wine industry, AWRI staff seminar series, AWRI: Adelaide, 27 September 2000 (Mark Gishen).

15. NIRS assessment of grape quality. GWRDC Board meeting. Banrock Station, 26 October 2000 (Bob Dambergs).
16. An update on the use of Near Infrared Spectroscopy for measurement of grape quality parameters, Outlook Seminar, Department of Horticulture Viticulture and Oenology, University of Adelaide, November 2000 (Peter Høj).
17. The use of NIR for the measurement of grape, juice and wine components; The GG assay: an objective measure of fruit quality? — the latest developments and results from the CRCV national survey; The Australian Wine Research Institute, 'Roadshow' seminar series, 9 November to 6 December 2000; Adelaide Hills, Barossa valley, Clare Valley, Riverland, Sunraysia (Mark Gishen.)
18. New Techniques/Technologies: NIR and FTIR Spectroscopy, Interwinery Analysis Group, Technical seminar, 24 November 2000 (Michael Esler).
19. NIRS applications in the wine industry. Riverland grape quality focus group, Berri, 13 December 2000 (Bob Dambergs)
20. Near infrared for rapid grape analysis "Grape expectations" University of California Davis Cooperative Extension symposium. Davis, California, April 12 2001 (Leigh Francis)
21. Near infrared spectroscopy- a rapid analytical tool for the wine industry. AWRI staff seminar series, AWRI: Adelaide, 13 June 2001 (Bob Dambergs)
22. Opportunities for NIRS and chemometrics in viticulture. In: OIV proceedings, Adelaide 11-12 October 2001 (Mark Gishen).
23. The use of NIR to measure the composition of grapes, juice and wine, The Australian Wine Research Institute, 'Roadshow' seminar series, 19–21 November 2001; Toowoomba, Queensland (Mark Gishen.)
24. Vintage colour measurements: sampling and testing methods. Interwinery Analysis Group Technical Seminar, Clare, 30 November, 2001 (Bob Dambergs).
25. Chemometrics in general — Qualitative and quantitative chemometric algorithms: PCA and PLS. Workshop #19 Introduction to chemometrics: NIR spectroscopy of grapes and wine; The eleventh Australian wine industry technical conference; 7–10 October 2001. Adelaide, SA (Michael Esler)
26. Practical application of PCA to multivariable wine data: qualitative analysis of non-spectroscopic data. Workshop #19 Introduction to chemometrics: NIR spectroscopy of grapes and wine; The eleventh Australian wine industry technical conference; 7–10 October 2001. Adelaide, SA. (Leigh Francis)
27. Chemometrics applied to the NIR spectroscopic analysis of grape fortifying spirit. Workshop #19 Introduction to chemometrics: NIR spectroscopy of grapes and wine; The eleventh Australian wine industry technical conference; 7–10 October 2001. Adelaide, SA. (Bob Dambergs)
28. Opportunities for NIRS and chemometrics in the wine industry. Workshop #19 Introduction to chemometrics: NIR spectroscopy of grapes and wine; The eleventh Australian wine industry technical conference; 7–10 October 2001. Adelaide, SA. (Mark Gishen)
29. Introduction to NIR spectroscopy and calibration. Workshop #79 Objective measures of grape and wine quality; The eleventh Australian wine industry technical conference; 7–10 October 2001. Adelaide, SA (Mark Gishen)
30. Practical grape assessment using NIR methods. Workshop #79 Objective measures of grape and wine quality; The eleventh Australian wine industry technical conference; 7–10 October 2001. Adelaide, SA (Michael Esler).
31. Potential applications of NIR. Workshop #79 Objective measures of grape and wine quality; The eleventh Australian wine industry technical conference; 7–10 October 2001. Adelaide, SA. (Bob Dambergs).
32. Gishen, M.; Iland, P.G.; Dambergs, R.G.; Esler, M.B.; Francis, I.L.; Kambouris, A.; Johnstone, R.S.; Høj, P.B. Objective measures of grape and wine quality. The eleventh Australian wine industry technical conference; 7–10 October 2001. Adelaide, SA. (Mark Gishen)
33. Opportunities for NIRS and chemometrics in viticulture. In: OIV proceedings, Adelaide 11-12 October 2001. (Mark Gishen)
34. The Australian Wine Research Institute, 'Roadshow' seminar series, 19–21 November 2001; Toowoomba, Queensland. Seminar talks: The use of NIR to measure the composition of grapes, juice and wine (Mark Gishen).
35. Vintage colour measurements Interwinery Analysis Group annual review workshop, Friday 30 November 2001, Clare, SA. (Bob Dambergs)
36. Dambergs, R.G.; Esler, M.B.; Cynkar, W.U.; Kambouris, A.; Janik, L.J.; Boehm, D.A. Francis, I.L; Høj, P.B.; Gishen, M. Non-linearity and matrix effects in NIRS calibrations for measurement of grape quality variables - or "straightening the PLS banana." The tenth Australian Near Infrared Spectroscopy Group conference, 6-7 May 2002. Coffs Harbour, NSW. (Bob Dambergs)
37. Gishen, M.; Dambergs, R.G.; Janik, L.J.; Cynkar, W.U.; Boehm, D.A.; Francis, I.L.; Høj, P.B.; Esler, M.B. Spectroscopic analytical techniques in the wine industry (NIRS and FT-IR). Beer and

wine users group meeting, FOSS Directions 2002 conference, 8-9 May 2002. Coffs Harbour, NSW. (Mark Gishen)
38. Science and technology, their importance in the production of quality wines for the consumer. Keynote Address, 3rd Foro Mundial Del Vino (3rd WorldWine Forum), May 2002, Logrono (Rioja), Spain (Peter Godden).
39. Gishen, M.; Dambergs, R.G.; Janik, L.J.; Cynkar, W.U.; Boehm, D.A.; Francis, I.L.; Høj, P.B. Near Infrared Spectroscopy - Future directions. Presentation to CRCV 2002 Symposium, Mildura Victoria, 17-18 June 2002. (Mark Gishen)
40. Applications of NIRS in the wine industry; presented by Bob Dambergs to CSIRO Plant Industry, Adelaide, 9 August, 2002. (Bob Dambergs)
41. NIRS in the wine industry; Wirra Wirra Growers Day, 27 August 2002. (Bob Dambergs
42. NIRS in the wine industry; The Australian Wine Research Institute, 'Roadshow' seminar series presented to: Yarra Valley and Mornington Peninsula growers and winemakers, 16-18 September 2002; Swan Valley, Great Southern and Margaret River growers and winemakers, 18-22 August, 2002. (Bob Dambergs).
43. Defining and Measuring Grape and Wine Quality, La Trobe University, (Wodonga Campus), 23 January 2003 (Peter Godden).
44. NIR in the wine industry; Gishen, M.; Dambergs, R.G.; Janik, L.; Cynkar, W.U.; Boehm, D.; Francis, I.L; Cozzolino, D.; Høj, P.B. FOSS Directions 2003 conference, Taupo, N.Z.; 28–29 May 2003.
45. Legal metrology in the wine industry; Gishen, M.; Harvey, G. FOSS Directions 2003 conference, Taupo, N.Z.; 28–29 May 2003. (Mark Gishen)
46. FOSS 'WineScan'—Calibration development for wine analysis; Janik, L.; Buick, D.R.; Holdstock, M.G.; Massis, A.; Catalano, A.; Henry, M.; Gishen, M. FOSS Directions 2003 conference, Taupo, N.Z.; 28–29 May 2003 (Les Janik)
47. Measuring grape colour; CRCV field seminar, Mildura, 28 May 2003. (Bob Dambergs)
48. Measuring grape colour; CRCV "Talking Technology" workshops, Adelaide, 10 and 11 July, 2003 (Bob Dambergs).
49. Spectral analysis of commercially graded red wines. Riverlink Cabernet quality workshop. CSIRO, Merbein , 29 July 2003 (Bob Dambergs).
50. Esler, M.B.; Gishen, M.; I.L. Francis; Dambergs, R.G.; A. Kambouris; Cynkar, W.U.; D.R. Boehm. Effects of variety and region on near infrared reflectance spectroscopic analysis of quality parameters in red wine grapes. In: Proc. 10th International NIR Conference. A.M.C. Davies and R.K. Cho (Eds). NIR Publications, UK; 2002: 249 – 253.
51. Dambergs, R.G.; Kambouris, A.; Schumacher, N.; Francis, I.L.; Esler, M.B.; Gishen, M. Wine quality grading by near infrared spectroscopy. In: Proc. 10th International NIR Conference. A.M.C. Davies and R.K. Cho (Eds). NIR Publications, UK; 2002: 187 – 190.
52. Cozzolino, D.; Kwiatkowski, M.J.; Parker, M.; Cynkar, W.U.; Dambergs, R.G.; Gishen, M.; Herderich, M.J. Prediction of phenolic compounds in red wine fermentations by near infrared spectroscopy. 3rd Symposium *InVino Analytica Scientia*, Aveiro, Portugal, 10–12 July, 2003.
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54. Cozzolino, D.; Dambergs, R.G.; Cynkar, W.U.; Janik, L.J.; Gishen, M.; Høj, P.B. An overview of the use of near infrared spectroscopy in the Australian grape and wine industry. Presented at the11th Australian Near Infrared Spectroscopy Group conference 19–21 April 2004, Fremantle, WA.
55. Smyth, H.; Cozzolino, D.; Herderich, M.; Sefton, M.; Francis, L. Identification of key aroma compounds in Australian Riesling and unwooded Chardonnay wines. Hofman, T.; Rothe, M.; Schieberle, P., eds. State-of-the-art in flavour chemistry and biology: [proceedings of the 7th Wartburg Symposium on flavour chemistry and biology; Eisenach, Germany 21–23 April 2004]. Garching, Germany: Deutsche Forschungsanstalt für Lebensmittelchemie; 2004: 508-512.
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57. Smyth, H.E.; Cozzolino, D.; Francis, I.L. Identification of key aroma compounds in Australian Riesling wines. Proceedings of the Australian Society of Viticulture and Oenology: Viticulture and Oenology Seminars. Tanunda, SA, 10-11 July 56 - 58. 2003
58. Janik, L.J.; Gishen, M. FOSS 'WineScan' — Calibration and protocols for wine analysis. Wine Industry User's Group, FOSS Directions 2004, Swan Valley, 22-23 April 2004.
59. Henschke, P.; Bellon, J.; Capone, D.; Coulter, A.; Cowey, G.; Cozzolino, D.; Curtin, Ch.; Field, J.; Gishen, M.; Graves, P.; Lattey, K.; Robinson, E.; Francis, I.L.; de Barros Lopes, M.; Godden, P. (2004). Incidence and control of Brettanomyces: The Australian Perspective. In. Proc. 55th

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60. Cozzolino, D.; Kwiatkowski, M.; Parker, M.; Dambergs, R.G.; Cynkar, W.; Gishen, M. and Herderich, M. (2004). Determination of several phenolic compounds in red wine by near infrared transmittance spectroscopy. In. Near infrared spectroscopy: Proc. 11th International conference. Davies, A.M.C.; Garrido-Varo (eds.). West Sussex. UK. NIR Publications, 573-576.
61. Dambergs, R.G.; Cozzolino, D.; Cynkar, W.; Esler, M.B.; Janik, L.; Francis, I.L. and Gishen, M. (2004). Strategies to minimise matrix-related error with near infrared analysis of wine grape quality parameters. In. Near infrared spectroscopy: Proc. 11th International conference. Davies, A.M.C.; Garrido-Varo (eds.). West Sussex. UK. NIR Publications, 183-186.
62. Cozzolino, D.; Gishen, M.; Dambergs, R.G.; Cynkar, W.U.; Janik, L.; Francis, I.L. and P.B. Hoj (2004). Calibration transfer between different instruments for determination of colour in red grapes. In. Near infrared spectroscopy: Proc. 11th International conference. Davies, A.M.C.; Garrido-Varo (eds.). West Sussex. UK. NIR Publications, 273-275.
63. Cozzolino, D.; Esler, M.B.; Gishen, M.; Dambergs, R.G.; Cynkar, W.; Boehm, D.R.; Francis, I.L. and P.B. Hoj (2004). Prediction of colour and pH in grapes using a diode array spectrophotometer. In. Near infrared spectroscopy: Proc. 11th International conference. Davies, A.M.C.; Garrido-Varo (eds.). West Sussex. UK. NIR Publications, 393-398.
64. Daniel Cozzolino, Multivariate analysis (chemometrics): a novel approach in modern interdisciplinary sciences. 24-29 July 2004 Presented at 12th AWITC, Melbourne, VIC,.
65. Daniel Cozzolino, Relationship between sensory analysis and near infrared spectroscopy in Australian Riesling wines. 11-16 April 2005. Presented at the 12th International NIR Conference, Auckland, NZ.
66. Bob Dambergs, Five years of NIR research at The Australian Wine Research Institute. 8-10 June 2005. FOSS Directions, Surfers Paradise.
67. Daniel Cozzolino., Heather E. Smyth, Kate A. Lattey, Wies Cynkar, Les Janik, Robert G. Dambergs, I. Leigh Francis, Mark Gishen. Combining mass spectrometry based electronic nose, visible-near infrared spectroscopy and chemometrics to assess the sensory properties of Australian Riesling wines. InVino Analytica Scientia, 7–9 July 2005, Montpellier France
68. CRCV Symposium, Objective measurement of grape quality, Adelaide, 19th July 2005. Commercial wine quality grading - correlations with spectral properties Bob Dambergs, Wies Cynkar, Les Janik, Daniel Cozzolino and Mark Gishen.
69. CRCV Industry Reference Group presentation, Adelaide, 12th August 2005. Rapid instrumental methods – past success and future potential, Daniel Cozzolino, Wies Cynkar, Bob Dambergs, Mark Gishen and Les Janik.
70. Bob Dambergs. The relationship of grape and wine colour with quality, 3 October 2005, Growing Great Grapes, King Valley Vignerons seminar, King Valley.
71. D. Cozzolino, R.G. Dambergs, W.U. Cynkar, and M. Gishen. Rapid instrumental methods and multivariate data analysis of wine. Accepted as Oral contribution, OIV, June 2006, Spain.
72. D. Cozzolino, G. Cowey, K.A. Lattey, P. Godden, R.G. Dambergs, W.U. Cynkar, L. Janik and M. Gishen. Challenging the NIR spectra - prediction of sensory panel response. Accepted as Oral contribution ANISG Conference, May 2006, Queensland.

9.3.2 Conference posters

1. NIRS analysis of grapes — matrix effects. Dambergs, R.G.; Kambouris, A.; Schumacher, N.; Gishen, M.; Francis. I.L.; 9th Australian Near Infrared Spectroscopy Group Conference, Victorian Institute of Dryland Agriculture, Horsham, 5-6 April 2000.
2. Effects of variety, region and season on near infrared reflectance spectroscopic analysis of quality parameters in red wine grapes. Esler, M.B.; Gishen, M.; Francis, I.L.; Dambergs, R.G.; Kambouris, A.; Cynkar, W.U.; 10th International Conference on Near Infrared Spectroscopy, Kyongju, Korea, 10-15 June 2001
3. Wine quality grading by near infrared spectroscopy. Dambergs, R.G.; Kambouris, A.; Schumacher, N.; Francis, I.L.; Esler, M.B.; Gishen, m.; 10th International Conference on Near Infrared Spectroscopy, Kyongju, Korea, 10-15 June 2001
4. Effects of variety, region and season on near infrared reflectance spectroscopic analysis of quality parameters in red wine grapes. Esler, M.B.; Gishen, M.; Francis, I.L.; Dambergs, R.G.; Kambouris, A.; Cynkar, W.U.; 11th Australian wine industry technical conference 7–10 October 2001, Adelaide, South Australia.

5. Wine quality grading by near infrared spectroscopy. Dambergs, R.G.; Kambouris, A.; Schumacher, N.; Francis, I.L.; Esler, M.B.; Gishen, M.; 11th Australian wine industry technical conference 7–10 October 2001, Adelaide, South Australia.
6. High throughput malic acid analysis using a microplate reader. Blake, P.; M. Frankel, M.; Dambergs, R.G.; 11th Australian wine industry technical conference 7–10 October 2001, Adelaide, South Australia.
7. Strategies to minimise matrix-related error with NIRS analysis of wine grape quality parameters. Dambergs. R.G.; Cozzolino, D.; Cynkar, W.U.; Esler, M.; Janik, L.; I.L. Francis, I.L; Gishen, M. 11th International NIR Conference, Cordoba, Spain. April 6 - 11, 2003.
8. Determination of colour and pH of red grapes using a diode array instrument. Cozzolino, D.; Gishen, M; Dambergs, R.G; Cynkar, W.U.; 11th International NIR Conference, Cordoba, Spain. April 6 - 11, 2003
9. Measurement of phenolic compounds during red wine fermentation by near infrared transmission (NIT) spectroscopy. Cozzolino, D.; Kwiatkowski, M.J.; Parker, M.; Gishen, M; Dambergs, R.G.; Cynkar, W.U.; Herderich, M. 11th International NIR Conference, Cordoba, Spain. April 6 - 11, 2003
10. Calibration transfer between different NIR instruments for determination of colour in red grapes. Cozzolino, D.; Gishen, M.; Dambergs, R.G.; Cynkar, W.U.; Francis, I.L.; 11th International NIR Conference. Cordoba, Spain. April 6 - 11, 2003
11. R.G. Dambergs, Stummer, B.E., Zanker, T., Cozzolino, D, Gishen, M., Scott, E.S. (2004) Detection of powdery mildew in grapes by near infrared spectroscopy. 11th Australian Near Infrared Spectroscopy Group conference 19–21 April 2004, Fremantle, WA.
12. Daniel Cozzolino, Mariola Kwiatkowski, George Skouroumounis, Elizabeth Waters, Wies Cynkar, Bob Dambergs, Les Janik and Mark Gishen Preliminary study on the use of near infrared spectroscopy to assess wine composition in a bottle 24-29 July 2004 12th AWITC, Melbourne, VIC,.
13. Daniel Cozzolino, Les Janik, Chris Bevin, Audrey Lim, Wies Cynkar, Bob Dambergs and Mark Gishen. Calibration of a fast diode array spectrophotometer for the measurement of red grape composition: total anthocyanins, total soluble solids and pH 24-29 July 2004 12th AWITC, Melbourne, VIC,.
14. Daniel Cozzolino, Heather Smyth, Kate Lattey, Wies Cynkar, Leigh Francis, Bob Dambergs and Mark Gishen. White wine varietal discrimination using near infrared reflectance spectroscopy 24-29 July 2004 12th AWITC, Melbourne, VIC,.
15. Daniel Cozzolino, Simon Dillon, Eveline Bartowsky, Paul Henschke, Wies Cynkar, Les Janik, Bob Dambergs and Mark Gishen. Monitoring fermentation of red wine using near infrared spectroscopy 24-29 July 2004 12th AWITC, Melbourne, VIC,.
16. Daniel Cozzolino, Wies Cynkar, Les Janik, Bob Dambergs, Leigh Francis and Mark Gishen. Effect of freezing and frozen sample storage on visible and near infrared calibration for determination of total anthocyanins in red grapes. 24-29 July 2004. 12th AWITC, Melbourne, VIC,.
17. Daniel Cozzolino, Mariola Kwiatkowski, George Skouroumounis, Elizabeth Waters, Wies Cynkar, Bob Dambergs, Les Janik and Mark Gishen. The use of visible and near infrared spectroscopy to measure the relative degree of oxidation in white wine. 24-29 July 2004. 12th AWITC, Melbourne, VIC,.
18. Daniel Cozzolino, Mariola Kwiatkowski, Mango Parker, Bob Dambergs, Wies Cynkar, Mark Gishen and Markus Herderich. Quantitative analysis of phenolic compounds in red wine fermentation using near infrared spectroscopy. 24-29 July 2004. 12th AWITC, Melbourne, VIC,.
19. Daniel Cozzolino, Heather Smyth, Kate Lattey, Wies Cynkar, Leigh Francis, Bob Dambergs and Mark Gishen. A preliminary investigation of the potential of near infrared spectroscopy to predict sensory properties in white wines. 24-29 July 2004. 12th AWITC, Melbourne, VIC,.
20. Daniel Cozzolino, Wies Cynkar, Les Janik, Bob Dambergs, Leigh Francis and Mark Gishen. Prediction of total anthocyanins in individual grape berries using visible and near infrared spectroscopy. 24-29 July 2004. 12th AWITC, Melbourne, VIC,.
21. Daniel Cozzolino, Wies Cynkar, Les Janik, Bob Dambergs, Leigh Francis and Mark Gishen. Measurement of colour, total soluble solids and pH in whole red grapes using visible and near infrared spectroscopy. 24-29 July 2004. 12th AWITC, Melbourne, VIC,
22. Wies Cynkar, Daniel Cozzolino, Bob Dambergs, Les Janik, Leigh Francis and Mark Gishen. Effect of sample preparation and storage on the determination of quality parameters in red grape berries of Vitis Vinifera. 24-29 July 2004. 12th AWITC, Melbourne, VIC,.
23. Wies Cynkar, Daniel Cozzolino, Les Janik, Bob Dambergs, Leigh Francis and Mark Gishen. Prediction of glycosylated compounds in Chardonnay and Riesling grape juice by near infrared and mid-infrared spectroscopy. 24-29 July 2004. 12th AWITC, Melbourne, VIC,.

24. Les Janik, Wies Cynkar, Daniel Cozzolino, Mark Gishen and Bob Dambergs. The potential of attenuated total reflectance mid-infrared spectroscopy for grape compositional analysis. 24-29 July 2004. 12th AWITC, Melbourne, VIC,.
25. Tom Scherer, F. Peacock, S. Stanley, Rachel Walker, Ben Leditschke, Mark Gishen and Daniel Cozzolino. Tasmanian pinot noir vineyard monitoring project. 24-29 July 2004. 12th AWITC, Melbourne, VIC,.
26. Bob Dambergs, Belinda Stummer, Tim Zanker, Daniel Cozzolino, Mark Gishen and Eileen Scott. Near infrared spectroscopy as a tool for detection of powdery mildew in homogenised grapes. 24-29 July 2004. 12th AWITC, Melbourne, VIC,.
27. Bob Dambergs, Daniel Cozzolino, Wies Cynkar, Les Janik, Leigh Francis and Mark Gishen. An examination of the effects of vintage, region and variety on analysis of wine grape quality parameters by near infrared spectroscopy. 24-29 July 2004. 12th AWITC, Melbourne, VIC,.
28. George Skouroumounis, Mariola Kwiatkowski, Mark Sefton, Elizabeth Waters and Bob Dambergs. The use of chemometrics to select optimum wavelengths for the spectrophotometric determination of browning in white wines. 24-29 July 2004. 12th AWITC, Melbourne, VIC,.
29. Bob Dambergs, Bruce Kambouris, Wies Cynkar, Les Janik, Daniel Cozzolino, Paul Henschke and Mark Gishen. A comparison of near infrared and mid-infrared spectroscopy for the analysis of yeast assimilable nitrogen in grape juice. 24-29 July 2004. 12th AWITC, Melbourne, VIC,.
30. Daniel Cozzolino, Mariola Kwiatkowski, George Skouroumounis, Elizabeth Waters, Wies Cynkar, Bob Dambergs, Les Janik and Mark Gishen. Preliminary study on the use of near infrared spectroscopy to assess bottled wine composition in situ. 11-16 April 2005. 12th International NIR Conference, Auckland, NZ
31. Daniel Cozzolino, Mariola Kwiatkowski, George Skouroumounis, Elizabeth Waters, Wies Cynkar, Bob Dambergs, Les Janik and Mark Gishen. Measurement of elements in wine by visible and near infrared spectroscopy. 11-16 April 2005. 12th International NIR Conference, Auckland, NZ
32. Daniel Cozzolino, Wies Cynkar, Bob Dambergs, Les Janik, Leigh Francis and Mark Gishen. Effect of freezing and frozen storage on the visible and near infrared spectra of red grapes from Vitis Vinifera. 11-16 April 2005. 12th International NIR Conference, Auckland, NZ
33. Daniel Cozzolino, Lorelei Flood, Miguel De Barros Lopes, Jenny Bellon, Bob Dambergs and Mark Gishen. Classification of yeast mutants strains by means of visible and near infrared spectroscopy. 11-16 April 2005. 12th International NIR Conference, Auckland, NZ.
34. Bob Dambergs, Daniel Cozzolino, Wies Cynkar, Les Janik, Leigh Francis and Mark Gishen. Applications of Vis-NIR spectroscopy in the wine industry. 11-16 April 2005. 12th International NIR Conference, Auckland, NZ
35. Bob Dambergs, Chris Bevin, Daniel Cozzolino, Wies Cynkar, Les Janik, and Mark Gishen. "Kitchen spectroscopy" - the use of commercial benchtop polymers as Vis-NIR reflectance reference materials. 11-16 April 2005. 12th International NIR Conference, Auckland, NZ
36. Mariola Kwiatkowski, Daniel Cozzolino, George Skouroumounis, Andrew Kleinig, Mark Sefton, Mark Gishen and Elizabeth Waters. The use of visible and near infrared spectroscopy to predict CIELab colour parameters, and sensory properties of red and white wines. 11-16 April 2005. 12th International NIR Conference, Auckland, NZ
37. Daniel Cozzolino, Mango Parker, Robert G. Dambergs, Mark Gishen and Herderich, M. Qualitative monitoring red wine fermentation: combining chemometric and near infrared spectroscopy. In Vino Analytica Scientia, 7–9 July 2005, Montpellier France.
38. W.U. Cynkar, R.G. Dambergs, D. Cozzolino, L. Janik and M. Gishen. Effect of frozen sample storage on Vis and NIR spectra of red grape homogenates. Poster. ANISG, May 2006, Queensland.
39. L. Liu, D. Cozzolino, M. Gishen, C. Colby, B. O'Neill, and D. Abbot. Effect of temperature on wine spectra using near infrared spectroscopy. Poster. ANISG, May 2006, Queensland.

9.3.3 Seminar and workshop presentations

1. Fruit and product quality evaluation using NIRS. BRL Hardy Technical Conference, Hobart, 7-10 June, 1999 (Bob Dambergs)
2. Measuring fruit quality. In: "Grape quality for our future", BRL Hardy grower roadshow seminars, Loxton, Berri, Barmera, Renmark, Mildura, 6-10 September, 1999 (Bob Dambergs).
3. Near infrared spectroscopy: potential applications for viticulture and oenology. Charles Sturt University, 18 August 1999 (Bob Dambergs)
4. The GG assay and NIRS: what are they and what are the applications in the wine industry? Orange Region Vignerons' Association Annual Field Day, Orange, NSW, 5 February 2000 (Mark Gishen).
5. NIRS – rapid analysis and quality assessment for agriculture. Renmark Agricultural Bureau, Renmark, 3 April 2000 (Bob Dambergs).

6. Measuring grape quality with a spectrophotometer. Interwinery Analysis Group Annual Technical Meeting. Tanunda, 7 July 2000 (Bob Dambergs).
7. Berri Ag bureau 24 July 2000(Bob Dambergs)
8. Grape colour as a quality indicator. Wine Grapes Marketing Board workshop, Griffith, NSW, 10 August 2000 (Bob Dambergs).
9. Grape colour as a quality indicator. Australian Vintage growers meeting, Loxton, 25 September, 2000 (Bob Dambergs)
10. Application of near infrared spectroscopy (NIRS) for quality assessment of grapes, wine and spirits in the Australian wine industry, AWRI staff seminar series, AWRI: Adelaide, 27 September 2000 (Mark Gishen).
11. NIRS assessment of grape quality. GWRDC Board meeting. Banrock Station, 26 October 2000 (Bob Dambergs).
12. An update on the use of Near Infrared Spectroscopy for measurement of grape quality parameters, Outlook Seminar, Department of Horticulture Viticulture and Oenology, University of Adelaide, November 2000 (Peter Høj).
13. New Techniques/Technologies: NIR and FTIR Spectroscopy, Interwinery Analysis Group, Technical seminar, 24 November 2000 (Michael Esler).
14. The use of NIR for the measurement of grape, juice and wine components; The GG assay: an objective measure of fruit quality? — the latest developments and results from the CRCV national survey; The Australian Wine Research Institute, 'Roadshow' seminar series, 9 November to 6 December 2000; Adelaide Hills, Barossa Valley, Clare Valley, Riverland, Sunraysia (Mark Gishen.)
15. NIRS applications in the wine industry. Riverland grape quality focus group, Berri, 13 December 2000 (Bob Dambergs)
16. Near infrared for rapid grape analysis "Grape expectations" University of California Davis Cooperative Extension symposium. Davis, California, 12 April 2001 (Leigh Francis)
17. Near infrared spectroscopy- a rapid analytical tool for the wine industry. AWRI staff seminar series, AWRI: Adelaide, 13 June 2001 (Bob Dambergs)
18. The use of NIR to measure the composition of grapes, juice and wine, The Australian Wine Research Institute, 'Roadshow' seminar series, 19–21 November 2001; Toowoomba, Queensland (Mark Gishen.)
19. Vintage colour measurements: sampling and testing methods. Interwinery Analysis Group Technical Seminar, Clare, 30 November, 2001 (Bob Dambergs).
20. The Australian Wine Research Institute, 'Roadshow' seminar series, 19–21 November 2001; Toowoomba, Queensland. Seminar talks: The use of NIR to measure the composition of grapes, juice and wine (Mark Gishen).
21. Vintage colour measurements Interwinery Analysis Group annual review workshop, Friday 30 November 2001, Clare, SA. (Bob Dambergs)
22. Gishen, M.; Dambergs, R.G.; Janik, L.J.; Cynkar, W.U.; Boehm, D.A.; Francis, I.L.; Høj, P.B. ear Infrared Spectroscopy - Future directions. Presentation to CRCV 2002 Symposium, Mildura Victoria, 17-18 June 2002. (Mark Gishen)
23. Applications of NIRS in the wine industry; presented by Bob Dambergs to CSIRO Plant Industry, Adelaide, 9 August, 2002. (Bob Dambergs)
24. NIRS in the wine industry; Wirra Wirra Growers Day, 27 August 2002. (Bob Dambergs
25. NIRS in the wine industry; The Australian Wine Research Institute, 'Roadshow' seminar series presented to: Yarra Valley and Mornington Peninsula growers and winemakers, 16-18 September 2002; Swan Valley, Great Southern and Margaret River growers and winemakers, 18-22 August, 2002. (Bob Dambergs).
26. Defining and Measuring Grape and Wine Quality, La Trobe University, (Wodonga Campus), 23 January 2003 (Peter Godden).
27. Measuring grape colour; CRCV field seminar, Mildura, 28 May 2003. (Bob Dambergs)
28. Measuring grape colour; CRCV "Talking Technology" workshops, Adelaide, 10 and 11 July, 2003 (Bob Dambergs).
29. Spectral analysis of commercially graded red wines. Riverlink Cabernet quality workshop. CSIRO, Merbein , 29 July 2003 (Bob Dambergs).
30. Vis-NIR discrimination of fungal infection in grapes. CRCV Project 1.5.2 Group Meeting, 27 May 2004. (Bob Dambergs)
31. Outline of the rapid instrumental methods project. Team Leader presentation, AWRI internal seminar, 27 August 2003. (Mark Gishen)
32. NIRS in the wine industry. Renmark Agricultural Bureau Annual General Meeting, 24 November 2003. (Bob Dambergs)
33. The use of NIR for the measurement of grape, juice and wine components, including colour. Presented by Mark Gishen to AWRI Roadshows and Workshops: in NSW, 20–24 October 2003 (Hunter Valley, Griffith, Mudgee) and in ACT, 1–2 December 2003. (Mark Gishen)

34. Beyond (and before) NIR. AWRI internal seminar, 8 June 2004. (Bob Dambergs)
35. Spectral analysis of commercially graded red wines. Riverlink Cabernet Quality Workshop, Merbein Vic, 29 July 2003. (Bob Dambergs)
36. Chemometrics and multivariate data analysis. AWRI internal workshop. 5 March 2004. (Daniel Cozzolino and Leigh Francis)
37. Advanced practical chemometrics. AWRI tannin team, AWRI internal workshop. 15 March 2004. (Daniel Cozzolino)
38. Commercial wine quality grading – correlations with spectral properties. AWRI Roadshow in Toowoomba, Qld. 30 November 2004 (Bob Dambergs)
39. Defining and measuring grape quality CRCV Symposium Mildura 6-8 June 2005 (Mark Gishen)
40. Mark Gishen, Grape and wine analysis: chemometrics. AWRI 50th Anniversary special seminars, Adelaide SA. 14 June 2005
41. Daniel Cozzolino. Hands on in chemometrics (W04). 24-29 July 2004. 12th AWITC, Melbourne, VIC
42. Daniel Cozzolino. Introduction to IR spectroscopy (W14). 24-29 July 2004. 12th AWITC, Melbourne, VIC
43. Daniel Cozzolino. Applications of IR spectroscopy: from laboratory to industry (W34). 24-29 July 2004. 12th AWITC, Melbourne, VIC
44. Bob Dambergs and Mark Gishen. Objective measures of grape and wine quality (W71). 24-29 July 2004. 12th AWITC, Melbourne, VIC
45. Wies Cynkar, Bob Dambergs. Colour measurement of red grapes - how to get started (W49). 24-29 July 2004. 12th AWITC, Melbourne, VIC
46. Bob Dambergs. Managing wine instabilities, identifying wine faults and quantifying grape colour and tannin. 1-2 December 2004. AWRI Roadshow Workshop in Toowoomba
47. Bob Dambergs. Trouble free winemaking: the identification, management and avoidance of wine instabilities. 8-9 February 2005. AWRI Roadshow, Cowra
48. Bob Dambergs, Wies Cynkar, Les Janik, Daniel Cozzolino and Mark Gishen. Commercial wine quality grading - correlations with spectral properties. CRCV Symposium, Objective measurement of grape quality, Adelaide, 19th July 2005.
49. Bob Dambergs, Wies Cynkar, Les Janik, Daniel Cozzolino and Mark Gishen. Defining and measuring grape quality CRCV Symposium. Mildura, 6 -7th June 2005.
50. Daniel Cozzolino, Wies Cynkar, Bob Dambergs, Mark Gishen and Les Janik. Rapid instrumental methods – past success and future potential. CRCV Industry Reference Group presentation, Adelaide, 12th August 2005.
51. Bob Dambergs. The relationship of grape and wine colour with quality, 3 October 2005, Growing Great Grapes, King Valley Vignerons seminar, King Valley.
52. Bob Dambergs, Commercial wine quality grading – correlations with spectral properties, AWRI Industry Development and Support seminars. Griffith, NSW, 8 November, 2005.
53. Bob Dambergs, Commercial wine quality grading – correlations with spectral properties, AWRI Industry Development and Support seminars. Irymple, Vic, 9 November, 2005.
54. Bob Dambergs, Development and Application of a Simple and Robust Assay for Quantitation of Tannins in Grape and Wine Samples, AWRI Industry Development and Support seminars. Griffith, NSW, Irymple, Vic, 9 November, 2005.
55. Bob Dambergs, Commercial wine quality grading – correlations with spectral properties, AWRI Industry Development and Support seminars. Renmark, SA, 10 November, 2005.
56. Bob Dambergs, Development and Application of a Simple and Robust Assay for Quantitation of Tannins in Grape and Wine Samples, AWRI Industry Development and Support seminars. Renmark, SA, 10 November, 2005.
57. Daniel Cozzolino, Bob Dambergs, Wies Cynkar, Les Janik and Mark Gishen. Workshop on Chemometrics and Seminar on Advances in spectroscopy related to grape and wine analysis was presented to Orlando Wyndham Group winemakers and viticulturist. 19th August 2005
58. Daniel Cozzolino, Bob Dambergs, Wies Cynkar, Les Janik and Mark Gishen. CRCV — Integrated Spectronics prototype instrument demonstration – Berri SA. March 2006.
59. Daniel Cozzolino, Bob Dambergs, Wies Cynkar, Les Janik and Mark Gishen. An internal workshop for AWRI staff on "Introduction to Chemometrics"20 participants AWRI staff. 28th September 2005.

9.3.4 Public Presentations

Event	Date
Interwinery Analysis Group Annual Technical Meeting. Tanunda SA	7 July 2000

AWRI Roadshow seminars Adelaide Hills Barossa Valley Riverland Sunraysia	 9 November 2000 13 November 2000 4 December 2000 6 December 2000
Outlook Seminar Department of Horticulture Viticulture and Oenology, University of Adelaide	November 2000
Interwinery Analysis Group, Technical seminar, Berri	24 November 2000
"Grape expectations" University of California Davis Cooperative Extension symposium. Davis, California, USA	12 April 2001
Workshop 19 at 11th AWITC, 2001 Introduction to chemometrics: NIR spectroscopy of grapes and wine (convenor: Dr Michael Esler)	7 Sun Oct 2001
Workshop 79 at 11th AWITC, 2001 Objective measures of grape and wine quality. (co-convenors: Mark Gishen, and Dr Leigh Francis)	Wed 11 Oct 2001
AWRI Roadshow seminars - Toowoomba, QLD	19–21 November 2001
CRCV 2002 Symposium, Mildura Victoria	17-18 June 2002
AWRI Roadshow seminars Mornington Peninsula, Yarra Valley Tasmania Swan Valley, Great Southern and Margaret River	 16–18 September 2002 14 August 2002 18-22 August, 2002
Mildura field days 2003	28 May 2003
CRC Talking Technology Workshops, Adelaide	10 and 11 July 2003
Riverlink Cabernet quality workshop. CSIRO, Merbein	29 July 2003
Renmark Agricultural Bureau Annual General Meeting	24 November 2003
AWRI Roadshows and Workshops Hunter Valley, Griffith, Mudgee ACT	 20–24 October 2003 1–2 December 2003
AWRI Roadshow in Toowoomba, Qld	30 November 2004
AWRI Roadshow Workshop in Toowoomba, Qld	1-2 December 2004
AWRI Roadshow, Cowra	8-9 February 2005
AWRI 50th Anniversary special seminars: Adelaide SA. Griffith, NSW Pokolbin, Hunter Valley, NSW Canberra, ACT Cowra, NSW Coonawarra, SA	 14 June 2005 8 August 2005 12 September 2005 13 September 2005 14 September 2005 11 October 2005
King Valley Vignerons seminar, King Valley, Victoria	3 October 2005
AWRI Industry Development and Support seminars Hobart and Launceston	 17-19 January, 2006

9.3.5 Other communications

1. University of Western Australia students, 13 July 2001, AWRI conference room: Quality management advisory services at the AWRI; update on the NIR project. (Mark Gishen)
2. SA Farmers Federation — winegrapes section AGM, 10 Aug 2001, Langhorne Creek: Quality and colour: the science and the usefulness of NIR spectroscopy. (Mark Gishen)
3. Lectures presented to LaTrobe University students in viticulture and oenology at Wodonga campus, 25 January 2002. Topics included: The use of NIR for the measurement of grape, juice and wine components (Mark Gishen).
4. Presentation to visiting group of grapegrowers from SA organised by Elders, Tuesday 21 May 2002. NIR spectroscopy to measure the composition of grapes, juice and wine (Mark Gishen).
5. Lecture presented to Adelaide University, Department of Horticulture Viticulture and Oenology, Graduate diploma students — research seminars, Thursday 6 June 2002. Objective measures of grape and wine quality and the role of NIR and FT-IR spectroscopy (Mark Gishen).
6. Lecture to the University of South Australia, School of Pharmacy and Medical Sciences, Dep. Food Science. 16th September 2005. Rapid methods of food analysis —spectroscopy and chemometrics. (Daniel Cozzolino)

7. Lecture to the University of Adelaide, School of Agriculture and Wine, October 2005. New analytical methods in wine science — spectroscopy and chemometrics. (Bob Dambergs)
8. Bob Dambergs. The relationship of grape and wine colour with quality, 3 October 2005, Growing Great Grapes, King Valley Vignerons seminar, King Valley, Victoria.
9. Bob Dambergs appeared before the Senate Rural and Regional Affairs Committee inquiry regarding evidence for an inquiry into the Australian Wine Industry regarding quality measures, supplier contracts and industry viability.

10 Appendix 2: Intellectual Property

The project has generated significant IP building from a base of background IP contributed by participants and industry collaborators. The IP comprises patents, a database of grape samples and relevant analyses; skills, experience and know-how to transform database information into robust prediction models for various NIR instruments.

10.1 Patents

Several innovations and inventions have been developed during the course of the project. Consequently, several patent applications have been successful.

1 NIR reference tiles: An innovation patent relating to spectrophotometer reference tiles has been granted (Australian Innovation Patent AU 20051002982) and the batches of tiles were produced for distribution with Integrated Spectronics' prototype instrument that was manufactured for the CRCV.

2 Non-destructive analysis by VIS-NIR spectroscopy of fluid(s) in its original container: An innovation patent has been lodged in relation to a discovery about the application of NIR spectroscopy to indicate wine quality and value non-destructively in-the-bottle (Australian Innovation Patent AU 2005100565).

3 Hyperspectral imaging of contaminants in products and processes of agriculture: A provisional full patent for chemical imaging of mould and other contaminants in grapes had been lodged, but must be followed up with more information within twelve months. The provisional patent on chemical imaging of fungus and other grape contaminants has been accepted, acknowledging that it represents innovation and putting a disclosure date on the IP – this allows us to now discuss it in a public forum, but it must be followed up within 12 months with the full application if we are to retain the IP. Samples of fungus infected grapes were collected from the 2006 vintage and will be used for preliminary imaging work later in the year, once links have been established with third party imaging laboratories.

4 Improving the Analysis of grape quality using Artificial Neural Networks: A provisional patent for alternative calibration algorithms (PLS-ANN combination) to predict grape chemistry, including total anthocyanins (colour) from NIR spectra was filed on Tuesday 21 March 2006.

10.2 Calibration database

A database has been collected from which the project team has developed Vis-NIR calibrations for the prediction of certain grape constituents. The Vis-NIR calibrations consist of a database and set of equations protected by confidentiality. This intellectual property (IP) is a mixture of Background IP and Centre IP.

The wineries who owned part of the Background IP have assigned their proportion to CRCV Technologies Pty Ltd in return for a license to any improvements. The AWRI as owner of the balance of the Background IP has licensed it to CRCV Technologies Pty Ltd for an initial term expiring 8th August 2007 with an option to renew.

Integrated Spectronics have been granted a non-transferable, royalty-bearing licence to use the IP (Calibration Technology) within the Territory (Australia only) during the term (initially until 8th August 2007) for the purpose of commercialising the Vis-NIR Calibration Package in accordance with the agreement reached with CRCV Technologies

10.3 Know-how

The project has expanded on the background know-how that was contributed by the participants. This comprises skills, experience and know-how in NIR spectroscopy and chemometrics as well as reference analysis techniques and data interpretation. This know-how is required to transform database information into robust prediction models suitable for use on NIR instruments. As this has been for a range of instruments, an important skill that has been developed is the ability to move and transfer data between various software packages in order to conduct the necessary analyses — no single software package is able to conduct all required tasks in an optimum manner. In addition, the skills gain in understanding the use of multivariate statistics has provided a strong grounding of know-how that can be applied to situations well beyond NIR spectroscopy and beyond grape analysis. Commercialisation of this type of know-how is sometimes delivered through consultancy service provision, however, this has not been explored during this project.

11 Appendix 3: References

Somers, T.C. Evans, M.E. Wine quality: correlations with colour density and anthocyanin equilibria in a group of young red wines Journal of the Science of Food and Agriculture J. Sci. Food Agric. 25 : 1369-1379; 1974

GWRDC, Economic evaluation of GWRDC portfolio, eSYS Development &BDA Group, Grape and Wine Research and Development Corporation, March 2001.

Dambergs, R. G. Cozzolino, D. Esler, M. B. Cynkar, W. U. Kambouris, A. Francis, I. L. Hoj, P. B. Gishen, M. The use of near infrared spectroscopy for grape quality measurement. Australian & New Zealand Grapegrower & Winemaker No. 473a : 69-74, 76; 2003.

12 Appendix 4: Staff

CRCV Program Manager: Dr Elizabeth Waters: 1999–2006

CRCV Technical Applications Manager: Mr Ian Atkinson: 2003–2006

1999–2000

AWRI	Dr Leigh Francis, Mr Mark Gishen, Dr Michael Esler, Dr Wies Cynkar, Prof Peter Høj, Ms Sally-Jean Bell, Mr Peter Godden, Mr Nick Yap
Adelaide University	Dr Graham Jones, Mr Patrick Iland,
BRL Hardy	Dr Bob Dambergs, Dr Bruce Kambouris, Mr Nathan Schumacher
Orlando Wyndham	Mr Russell Johnstone, Ms Inca Lee, Mr Ben Zander,
Southcorp Wines	Dr Andrew Kleinig
Rosemount Wines	Mr Eric Wilkes

2000–2001

AWRI	Mr Mark Gishen, Dr Michael Esler, Dr Bob Dambergs, Dr Wies Cynkar, , Mr David Boehm, Dr Leigh Francis, Prof Peter Høj, Ms Sally-Jean Bell, Mr Peter Godden
Adelaide University	Dr Graham Jones, Mr Patrick Iland
BRL Hardy	Dr Bruce Kambouris
Orlando Wyndham	Mr Russell Johnstone, Ms Inca Lee, Mr Ben Zander
Southcorp Wines	Dr Andrew Kleinig, Dr Mark Smith
Rosemount Wines	Dr Eric Wilkes, Mr Peter Hayes
Mildara Blass	Mr Russell Johnson
Simeon Wines	Mr Peter Stephens

2001–2002

AWRI	Mr Mark Gishen, Dr Michael Esler, Dr Bob Dambergs, Dr Wies Cynkar, Mr Les Janik, Mr David Boehm, Dr Leigh Francis, Prof Peter Høj
Adelaide University	Dr Graham Jones, Mr Patrick Iland
BRL Hardy	Dr Bruce Kambouris
Orlando Wyndham	Mr Russell Johnstone, Ms Inca Lee, Mr Ben Zander
Southcorp/Rosemount	Dr Andrew Kleinig, Dr Mark Smith, Mr Peter Hayes
Beringer Blass	Mr Russell Johnson, Dr Eric Wilkes
Simeon Wines	Mr Peter Stephens

2002–2003

AWRI:	Mr Mark Gishen, Dr Daniel Cozzolino, Dr Bob Dambergs, Dr Wies Cynkar, Mr Les Janik, Dr Leigh Francis, Prof Peter Høj
Adelaide University	Dr Graham Jones, Mr Patrick Iland
BRL Hardy	Mr Chris Bevin, Ms Audrey Lim
Orlando Wyndham	Mr Russell Johnstone, Ms Inca Lee, Mr Ben Zander
Southcorp/Rosemount	Dr Andrew Kleinig, Dr Mark Smith, Mr Peter Hayes
Beringer Blass	Mr Russell Johnson, Dr Eric Wilkes
Simeon Wines	Mr Peter Stephens, Dr Bruce Kambouris

2003–2004

AWRI	Mr Mark Gishen, Dr Daniel Cozzolino, Dr Bob Dambergs, Dr Wies Cynkar, Mr Les Janik, Dr Leigh Francis, Prof Peter Høj
Adelaide University	Dr Graham Jones
BRL Hardy	Mr Chris Bevin, Ms Audrey Lim
Orlando Wyndham	Mr Russell Johnstone, Ms Inca Lee
Southcorp/Rosemount	Dr Andrew Kleinig, Dr Mark Smith, Mr Peter Hayes
Beringer Blass	Mr Russell Johnson, Dr Eric Wilkes
Simeon Wines	Mr Peter Stephens, Dr Bruce Kambouris

2004–2005

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Beringer Blass	Mr Russell Johnson, Dr Eric Wilkes
Simeon Wines	Mr Peter Stephens, Dr Bruce Kambouris

2005–2006

AWRI	Mr Mark Gishen, Dr Daniel Cozzolino, Dr Bob Dambergs, Dr Wies Cynkar, Mr Les Janik, Dr Leigh Francis, Prof Peter Høj
Adelaide University	Dr Graham Jones
BRL Hardy	Mr Chris Bevin, Ms Audrey Lim
Orlando Wyndham	Mr Russell Johnstone, Ms Inca Pearce
Fosters Wine Estates	Mr Russell Johnson, Dr Eric Wilkes
Simeon Wines	Mr Peter Stephens, Dr Bruce Kambouris

13 Appendix 5: Data

All raw data has been archived at the AWRI according to the policies and practices in place at the time. Retrieval can be arranged on request.

14 Appendix 6: Budget reconciliation

(Gaye Jones from CRCV will include information)

Please use Form B, downloadable from the GWRDC website at www.gwrdc.com.au

Grape and Wine Research and Development Corporation

FINAL REPORT

CRV 98/2AWNS

(CRCV 1.4 & S1.4.1)

Appendix 6

TOTAL FUNDING $799,612