The Australian Wine Research Institute **Annual Report 2001**

Council Members

Mr D.J. McWilliam, BSc, MSc
Chairman—Elected a member under Clause 6(e) of the Articles of Association

Mr R.E. Day, BAgSc, BAppSc
Elected a member under Clause 6(e) of the Articles of Association

Mr R.L. Gibson, BAppSc, BAppSc
Elected a member under Clause 6(e) of the Articles of Association

Mr P.F. Hayes, BSc, BAppSc, MSc, DipEd
Elected a member under Clause 6(e) of the Articles of Association

Professor P.B. Høj, MSc, PhD
Ex officio under Clause 6(d) of the Articles of Association as Director of the Institute

Mr T.W.B. James, AssDip(WineProd)
Elected a member under Clause 6(e) of the Articles of Association

Mr G.R. Linton, BAppSc(AppChem), GradDip(SysAnal)
Elected a member under Clause 6(e) of the Articles of Association

Professor G.R. Scollary, MSc, PhD, BEd, BAppSc (Wine Science), FRACI
Charles Sturt University Representative under Clause 6(c) of the Articles of Association

Professor M. Sedgley, PhD
The University of Adelaide Representative under Clause 6(b) of the Articles of Association

Dr R.R. Walker, BAgSc(Hons) PhD
CSIRO Representative under Clause 6(a) of the Articles of Association

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The Company

The Australian Wine Research Institute was incorporated under the South Australian Companies Act on 27 April 1955. It is a company limited by guarantee, it does not have a share capital and it has been permitted, under licence, to omit the word 'limited' from its registered name.

The Memorandum of Association of The Australian Wine Research Institute sets out in broad terms the aims of the Institute and the Report of the Committee of Review for the Institute published in March 1977 identified the following specific aims:

1. To carry out applied research in the field of oenology.
2. To service the extension needs of the winemakers of Australia.
3 To be involved in the teaching of oenology at both undergraduate and postgraduate levels.
4. To assume responsibility for the co-ordination of oenological activities, and the collection, collation and dissemination of information on oenological and viticultural research to the benefit of the Australian wine industry.

The Institute's laboratories and offices are located on the Waite Campus of The University of Adelaide at Urrbrae in the Adelaide foothills, on land leased from the University. The original lease is for a term of 99 years, with a right of renewal clause for a further 99 years. The Institute formally affiliated with The University of Adelaide in 1990. The first buildings were erected and opened in 1957 and alterations and extensions were completed in 1976. The buildings have been extensively modified and refurbished since that time with major extensions being undertaken in 1994 and 1999.

The Institute is adjacent to the Faculty of Agricultural and Natural Resource Sciences of The University of Adelaide, three Divisions of the CSIRO and the South Australian Research and Development Institute.

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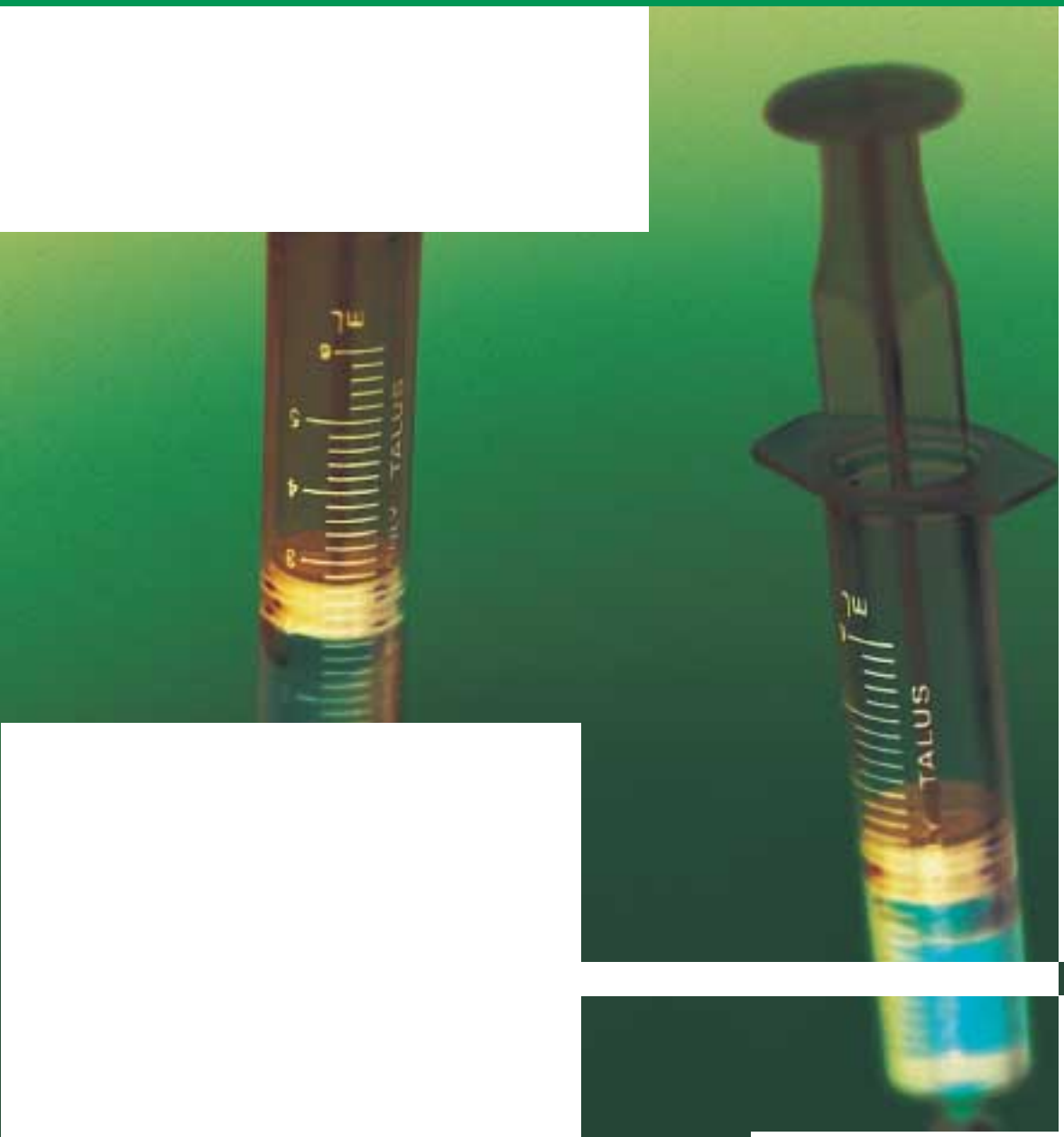

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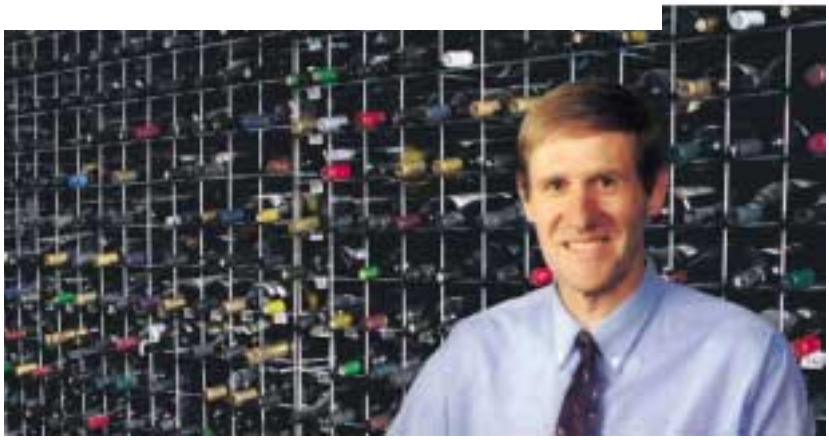
46th Annual Report
Presented to the
Australian Wine Industry
30th June 2001

Chairman's Report



Doug McWilliam, Chairman of Council

It is my pleasure to again present my Chairman's Report for the past year's activities of The Australian Wine Research Institute. This past year has been full of activity and outstanding progress with results now issuing from such projects as the cork closure trial, the potential enhancement of oak flavour during bottle ageing and the ability of NIR to rapidly measure berry colour and other quality parameters. This last project has generated enormous industry interest and has not only seen generous industry support from some of our major companies but has now progressed far enough to be trialled with confidence at the winery weighbridge. The NIR project started as a collaborative project between the Institute and BRL Hardy but has now been incorporated in the new Cooperative Research Centre for Viticulture's programs and along with the direct industry investment has led to a strong industry desire to progress the development of affordable instrumentation that can be readily taken up by industry. The commercialisation of the IP associated with this technique needs to be rapidly implemented and it is hoped to obtain prompt and satisfactory agreement with all vested interests.

The closure trial, now more than 18 months old, has revealed significant differences in the performance of different closures as outlined in the publication which was due to appear in the July issue of the *Australian Journal of Grape and Wine Research*. What is most important to recognise is that the testing protocol and the individual results have to be evaluated from each winemaker's perspective of what performance factors they deem important for their individual situation. The Institute trial was a massive undertaking involving considerable cost and resources and only provides a 'snapshot in time' of the rapidly changing and advancing technology of closure design.

One of our aims has been to strengthen the chemistry side of our activities and we have been fortunate to have Dr Markus Herderich join our Institute staff. Markus undertook post doctoral studies at our Institute in 1994-95 and has strengths in flavour and phenolic chemistry. Other major staff changes are the retirement, after 14 years service, of our Secretary, Janet Currie. She not only kept a tight reign on the financial affairs of our Institute but was a counsel and confidant to all of the staff. We now have a most suitable replacement and welcome Hans Muhlack to fill the vacancy created by Janet's retirement.

The new extensions to the Institute are fully occupied and Analytical Services has continued to expand their range of services offered to the industry during the last year. The Institute urgently needs to address the renovation and extensions of the old chemistry lab. With a stronger emphasis on flavour and phenolic chemistry, these facilities are inadequate for the future needs of both equipment and staff.

It is pleasing to see that the Grape and Wine Research and Development Corporation is proposing to move to a 'rolling' funding cycle which will remove some of the uncertainty of the current annual funding. It has been of mutual benefit to have their representative attend our Council meetings and I thank them for their advice, interest and involvement.

I also thank all members of Council for their diligence and efforts with a special thank you to Professor Margaret Sedgley from The University of Adelaide who retires in favour of Professor Steve Tyerman, the newly appointed Professor of Viticulture. Thank you to all the staff who carry the Institute's messages to industry so well via the forums of roadshows, seminars, workshops, lectures and our publication, *Technical Review*. Congratulations to our Communication and Publicity Manager and the Library staff for the implementation of the on-line searchable database of the John Fornachon Memorial Library.

Finally, I offer my thanks to the Director, Peter Høj, and all of his staff for a successful year. It has indeed been a pleasure for me to have been associated with the Institute and its employees in my capacity as Council member and Chair. I urge all members of our industry to not only continue, but increase, their interest and support for this wonderful asset we all share.

Doug McWilliam
Chairman of Council

Council notes

Chairman

At the Council Meeting held on 30 October 2000, Mr D.J. McWilliam was re-elected Chairman of Council.

Members of the Executive Committee

Mr D.J. McWilliam
Mr R.L. Gibson
Mr P.F. Hayes
Professor P.B. Høj
Mr T.W.B. James
Professor M. Sedgley

Deputy Members of Council

Mr N.P. Blieschke
Mr L.P. Deans
Dr P.R. Dry
Mr J. Northey
Dr N.S. Scott
Dr A.M. Smith
Mr P.J. Wall

Meetings

Ordinary General Meeting

The 46th Ordinary (Annual) General Meeting was held on 30 October 2000.

Council

The Council of the Institute met on the following dates: 25 July 2000, 30 October 2000, 6 March 2001, 1 May 2001. Executive members of Council met on 5 December 2000.

Funding

The Council of the Institute acknowledges the continuing financial support of the Grape and Wine Research and Development Corporation.

Appreciation

The Institute acknowledges the assistance and cooperation of the following organizations throughout the year:

Adelaide University
Australian Wine and Brandy Corporation
Australian Wine Foundation
Charles Sturt University
Commonwealth Scientific and Industrial Research Organization (CSIRO)
Cooperative Research Centre for Viticulture
Department of Agriculture, Forestry and Fisheries Australia
South Australian Wine and Brandy Industry Association
State Departments of Agriculture
Winegrape Growers' Council of Australia, Inc.
Winemakers' Federation of Australia Incorporated

Left to right:

Peter Hayes, Robin Day, Geoff Linton, Doug McWilliam, Margaret Sedgley, Richard Gibson, Steve Tyerman, Tim James, Peter Høj.

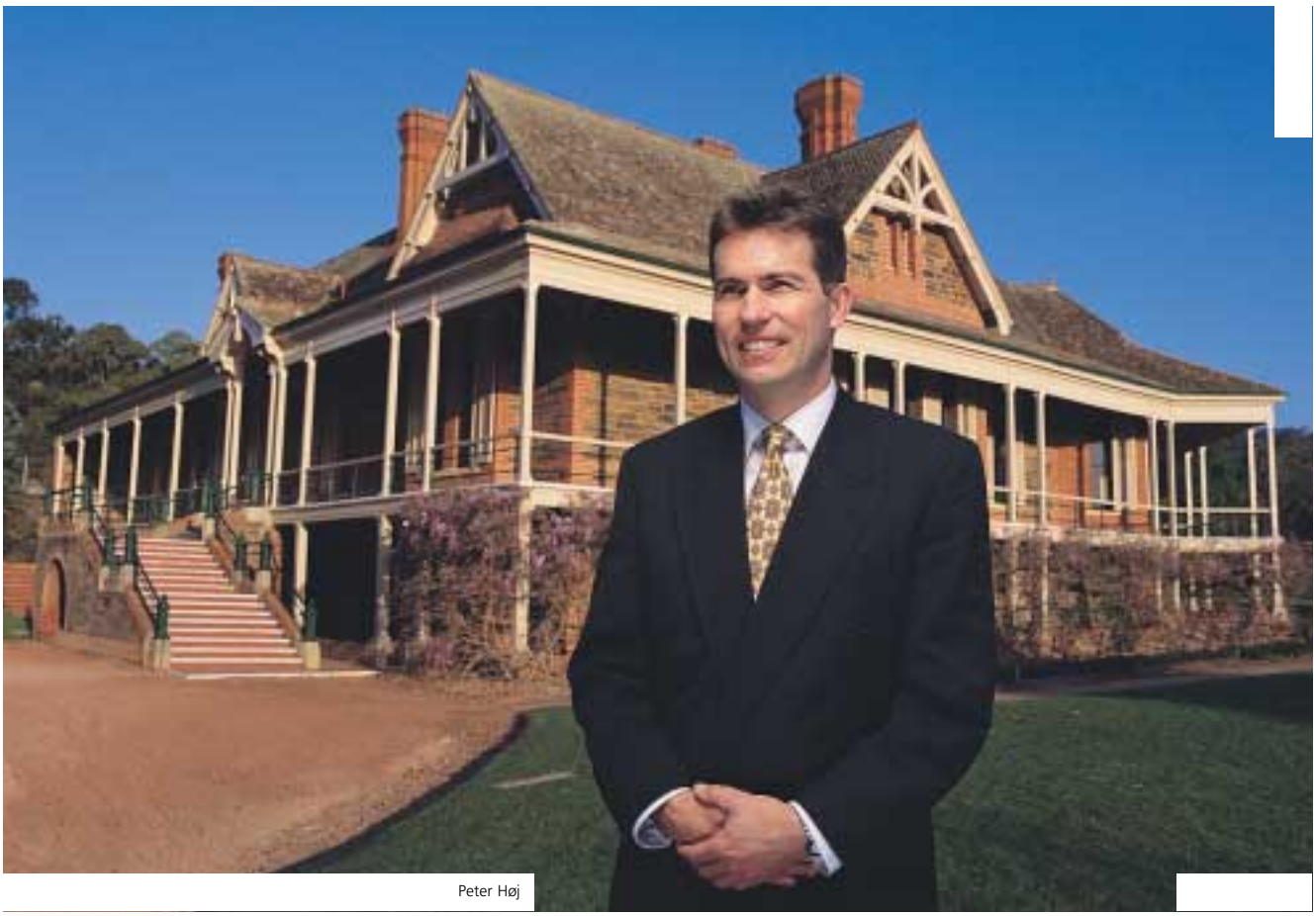
Director's Report

The continued growth of the Australian wine industry and the increase in research levies, with effect from the 1999 vintage, has allowed for an expansion in the total research portfolio managed by the Grape and Wine Research and Development Corporation (GWRDC) and , in turn, for an expansion of funding to a wide range of different research organisations. The funds allocated directly and indirectly to the Institute have also increased in dollar-terms and now constitute about 35% of the total matched levy funds administered by the GWRDC and an estimated 16-18% of public expenditure on grape and wine R&D in Australia. We are appreciative of this funding as it has widened the scope of our activities and further reduced our already very low per capita running costs to service the needs of industry better. These activities will, in turn, help underpin sustained growth of wine industry activities with consequential flow-on benefits to local communities.

Tangible outcomes, about which much is written in the remainder of this Annual Report, include the conduct, data collection, analyses, writing up and acceptance for publication in the *Australian Journal of Grape and Wine Research* of the Institute's seminal closure trial, ably led by our Manager of Industry Services (Peter Godden) with strong support from our sensory science specialist (Leigh Francis) and the dedicated members of our trained sensory panel. This event precipitated unprecedented national and international media interest. Throughout this process, relevant Institute staff conducted themselves with a high degree of professionalism in an area which, due to its commercial significance, had to be navigated with a great deal of discipline and thought. Another highlight relates to the much awaited installation of six computer-controlled Potter fermentors and six rotofermentors in the recently built Hickinbotham-Roseworthy Wine Science Laboratory at the Waite Campus of Adelaide University. This facility and its eventual completion is a prime example of how relentless belief in a cause and distinct contributions by a range of determined individuals can lead to long term gains where others might have given up due to lack of support. The Institute is most appreciative of the University of Adelaide's decision to build this facility and indeed helped start the ball rolling by pledging a $250,000 contribution over five years starting in July 1997; a commitment we now have met fully due to the strong performance of our own Analytical Service and its fourteen staff. In return for this significant contribution, we have been granted access to the Facility for 25 years. The first such substantial access occurred through a collaborative effort in our tannin project where eight tonnes of fruit were fermented in triplicate with different hardware and different yeasts. The wines are now subjected to detailed sensory analyses and outcomes will be communicated to industry upon its completion and presented to industry in workshops conducted at the 11th Australian Wine Industry Technical Conference in October 2001.

The 11th AWITC will be the largest and probably most diverse ever conducted. The Conference Planning Committee is Chaired by the Institute Director, the Program Planning Committee by Southcorp's National Viticulturist (Peter Hayes) while the detailed management is undertaken in a highly professional manner by the Institute's Rae Blair and her Secretariat Staff. This triennial event has grown to become a major Institute activity in the area of knowledge dissemination and adoption. It involves detailed planning, coordination and management of nearly 1700 delegates, the staging of 72 workshops (coordinated by our Industry Services Personnel) and arranging some 10,000 m^2 of trade exhibition comprising 178 individual exhibitors.

The selected examples of Institute activities cited above illustrate the continued commitment of our staff to the wine industry which, in many cases, extends beyond what can reasonably be expected and sustained in the longer term without



Peter Høj

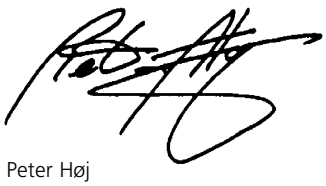
increased funding to employ more staff and upgrade infrastructure significantly. The Institute is working actively to help itself in this regard. One such example relates to the large extension of Institute facilities we undertook in 1998. That $1.1 million extension was facilitated by an injection of $375,000 over three years from the GWRDC and otherwise funded by savings as well as a large commercial loan, the latter on track to be paid back during the financial year 2001/2002.

Notwithstanding our past Institute expansions and the construction of the Hickinbotham Roseworthy Wine Science Laboratory by Adelaide University, more paint is needed to complete the picture, chiefly due to the need to accommodate staff and an increasing number of overseas visitors as well as acquiring new equipment for grape and wine research. The Institute, with very strong support from CSIRO Plant Industry, therefore, acted as a proponent agent in what later turned out to be a successful bid to the Federal Government for funding of $4.5 million under the new and once off 'Major National Research Facility Schemes' (MNRF). This will lead to a $7,000,000 upgrade of facilities at both the Waite Campus of the University of Adelaide and, to a lesser extent, at the Ron Potter Centre of Charles Sturt University. Throughout the bid process, we enjoyed strong support from the South Australian Government (including a $700,000 cash contribution) and from Council members who endorsed the Director to commit to a further commercial loan in order to be in the race. A special mention should go to Mr Robin Day who, on several occasions, gave up his own time to contribute so strongly to our bid as an industry representative.
The conduct and pursuit of the MNRF bid was a heartening exercise where institutions cooperated with good-will and a minimum of fuss to generate an outcome not possible to achieve by any agent alone. Clearly, we have a challenge to put in place a reasonable MNRF management structure which is effective and user-friendly, but we are confident this will happen using structures which have been proven over the long term. In parallel with our quest to secure a better funding base for our research, we must also identify costs (and those potentially hidden) associated with various models for its distribution, management and execution. Throughout Australian research management, there appears to be a case for identifying excellence, fund it appropriately and then let the individuals get on with the job within a framework defined robustly by major stakeholders, in our case the GWRDC, the Australian wine industry and the communities benefiting from its success.

On a more positive note, the Institute, in addition to the outcomes cited above, again looks back on a year with achievements in a diverse range of areas including our trendsetting NIRS work, the protein based fingerprinting of grape varieties and the application of our unique tannin mouthfeel wheel to the desciption of the mouthfeel properties of chemically defined tannins. Only time will tell if any of these developments rate against the impressive work of past illustrious Institute employees such as Fornachon.

Present and past achievements would not have been possible without the strong support of dedicated wine industry individuals, staff and collaborators. The Institute is most appreciative of this much needed support and is looking forward to a long partnership in our quest to serve the Australian wine industry's future increased R&D needs. A special thanks must go to Janet Currie, our long-serving Company Secretary who, over a period of 14 years, steered the financial activities of the Institute without a flinch and allowed us to expand the Institute in a sustainable fashion through several phases. We wish Janet all the best in her retirement.

We must never forget that our endeavor to remain innovative is dependent on our capacity to recruit and further develop talented individuals within all aspects of the wine industry. The Institute is committed to partake in this undertaking with the wine industry as our partner and joint success as the outcome.



Peter Høj
Director

Staff

Peter Bordier Høj, MSc, PhD, Copenhagen, Director

Research team members

Paul Anthony Henschke, BSc(Hons) PhD *UAdel.*, Principal Research Microbiologist
Mark Aidan Sefton, BSc(Hons) PhD *UWA*, Principal Research Chemist
Elizabeth Joy Waters, BSc PhD *UAdel.*, Principal Research Biochemist
Ian Leigh Francis, BSc(Hons) Monash, PhD *UAdel.*, Senior Research Chemist
Robert George Dambergs, BSc(Hons) *UAdel.*, PhD *UQld*, Senior Research Chemist (commenced 6 February 2001)
Markus Johannes Herderich, PhD *UWuertzberg*, Senior Research Chemist (commenced 9/4/01)
Yoji Hayasaka, DipEng(IndChem) *Tokyo I.T.*, MPharm *Vic. Col. Pharm.*, CertIntBusMgt *Monash*, Manager—Mass Spectrometry Facility
Miguel Antonio de Barros Lopes, BSc *Oregon*, PhD *UC (Santa Barbara)*, Molecular Biologist
Eveline Jutta Charlotte Bartowsky, BSc(Hons) PhD *UAdel.*, Research Microbiologist
Zhong Kui Peng, BSc MSc, *Zhejiang*, PhD *UAdel.*, Research Chemist
Alan Percy Pollnitz, BSc(Hons), PhD *UAdel.*, Research Chemist
Gordon Michael Elsey, BSc(Hons), PhD, *Flinders*, Postdoctoral Research Fellow
Michael Brian Esler, BSc(Hons) *USydney*, PhD *UWollongong*, Postdoctoral Research Fellow
George Kyriakos Skouroumounis, BSc(Hons), *Flinders*, PhD *UAdel.*, Postdoctoral Research Fellow
Stéphane Vidal, Eng Dip. (Biochem), Nat. Instit. App. Sc. *Lyon*, MBiochem, *UClaude Bernard Lyon*, PhD *UJoseph Fourier Grenoble*, Postdoctoral Research Fellow
Kenneth Frank Pocock, BAppSc *UAdel.*, FAIFST, Senior Chemist
Michael Raunkjaer, MSc *Copenhagen*, Visiting Research Chemist (concluded 1/12/00)
Anders Håkansson, MSc *Copenhagen*, Visiting Research Chemist (commenced
Dr Encarnacion Gomez Plaza, Chem, PhD *Murcia* Visiting Research Chemist (two month stay, commenced 23/10/00)
Tracey Ellen Siebert, BSc *UAdel.*, Chemist (commenced 14/3/01)
Peter James Costello, BSc (Hons) MSc *UNSW*, PhD *UAdel.*, Microbiologist
Holger Gockowiak, BSc(Hons) *UAdel.*, Microbiologist (concluded 13/11/00) Part time Laboratory Manager (commenced 16/4/01)
Jeffrey Mark Eglinton, BSc(Hons) *UAdel.*, Microbiologist/Senior Computer Systems Officer
Dimitra Capone, AssDip(Chem) *USA*, Laboratory Technician
Maria Jolanta Kwiatkowski, MSc *Gliwice*, Laboratory Technician
Simon Dillon, BSc(Hons) *Flinders*, Research Assistant (commenced 11/9/00)
Bianca van Wegen, BEnvSc(Hons) *Flinders*, Part-time Research Assistant (commenced 21/11/00)
Wieslawa Cynkar, BSc PhD *Wroclaw*, Technical Officer
Gayle Ann Baldock, BSc(Hons) *Guelph*, Technical Officer/Casual Analyst
Jennifer Bellon, Part-time Laboratory Assistant
Jane Melissa McCarthy, AdCertMedLabSc *USA*, CertVetNurs, CertAnimHand *TAFE*, Technical Assistant (commenced 21 November 2000).
Shauna Liam Brown, BBiotech(Hons) *Flinders*, Postgraduate Student
Anthony John Heinrich, BBiotech(Hons) *Flinders*, Postgraduate Student
Anita Oberholster, BSc(Hons), *Stellenbosch*, Postgraduate Student (19/4/01)
Kate Susan Howell, BSc(Hons) *UNSW*, Postgraduate Student
Kerry Leigh Wilkinson, BSc(Hons) *Flinders*, Postgraduate Student
Carolyn Jane Puglisi, BSc *Flinders*, BSc(Hons) *UAdel.*, Postgraduate Student
Agnieszka Janusz, BSc(Hons) *Flinders*, Postgraduate Student (commenced 26/3/01)
Heather Eunice Smyth (nee Pain), BSc(Hons) *UAdel.*, Postgraduate Student (commenced 2/4/01)
Nadia D'Incecco, BAgSc(Food Tech)(Hons) *Padova*, Visiting Italian Postgraduate Student (concluded 6/2/01)
Paolo Sivilotti, BAgSc *Udine*, Visiting Italian Postgraduate Student (commenced 8/11/00, concluded 31/5/01)
Teresa Girbau Solà, BPharm, DipOphtham *Barcelona*, Visiting Spanish Postgraduate Student (commenced 14/2/01, concluded 3/8/01)
Sandra Hoffman, Visiting German Postgraduate Student (commenced 18/6/01)

Industry Services team members*

Peter William Godden, BAppSc (Wine Science) *UAdel.*, Winemaker/Manager Industry Services
Mark Gishen, BE(Chem)(Hons) MEngSc(Chem) *UMelb.*, Quality Liaison Manager
Sally-Jean Bell, BSc(Hons) *UWA*, GradDip(Wine) *Roseworthy*, PhD *UWA*, Viticulturist
Adrian Dermott Coulter, BSc *Flinders*, GradDipOenol, *UAdel.*, Oenologist
Peter Valente, BSc(Hons) *Flinders*, Chemist
Ella Margaret Clare Robinson, BA, BSc(Hons) *UAdel.*, Chemist
Raelene Joan Blair, CertAppMgt(Marketing) *AIM*, Communication and Publicity Manager/Personal Assistant to the Director
Creina Standish Stockley, BSc(Hons) *UAdel.*, MSc *Flinders*, MBA *USA*, Health and Regulatory Information Manager
Catherine Grace Daniel, BA *ANU*, GradDip(Lib) RMIT, Librarian
Ingrid Betty-Maud Oats, DipLibInfo *Adel. Tafe*, Library Technician
Jodi-Ann Slade, Library Assistant (concluded 20/2/01)
Anna Capone, Library Assistant (commenced 20/2/01)
*Several members of the Industry Services Team lead or take part in a number of research projects.

Analytical Service

Donald Robert Buick, BSc *UAdel.*, Grad Dip(BusAdmin) *USA*, AIFST, Manager – Analytical Service
John Benjamin Hughes, DipWineMrktg *UAdel.*, Analytical Service Supervisor – Administration
Matthew Grant Holdstock, BSc *Flinders*, Analytical Service Supervisor – Laboratory
Gregory Andrew Ruediger, BAppSc *SAIT*, GradDipOenol, *UAdel.*, Trace Analysis Laboratory Supervisor
Kevin Herbert Pardon, AssDip(AppChem) *SAIT*, Analyst
Andrea Dale Kemp, AssDip(Farm Mgmt), *Roseworthy*, BA, *UAdel.*, Casual Analyst
Randell Leith Taylor, BSc(Hons) *UAdel.*, Casual Analyst
David Rolfe Boehm, BSc *UAdel.*, Casual Analyst
Amanda Louise Cook, AdvCert (Lab Tech) *Mackay*, Casual Laboratory Technician
Radka Kolouch, AssDip(Food Tech) *Czech Republic*, Casual Laboratory Technician
Matthew James Cream, Casual Laboratory Technician
Jeremy Crispin Hack, Casual Laboratory Technician
Bao Tran, AssocDipChemTech *USA*, Casual Laboratory Technician
Sandra Lloyd-Davies, BA *Flinders*, Casual Laboratory Administration Assistant

Administration

Janet Currie Currie, BA *Glasgow*, MAICD, Company Secretary (retired 15/1/01)
Hans Engelbert Muhlack, BEc *Adelaide*, CPA Aust., Company Secretary (commenced 3/10/00)
Sita Soetratma, B.Bus(Mgmt) *USA*, Accountant (concluded 8/12/00)
Rachel Lee Edwards, Accountant (commenced 13/12/00)
Heather Margaret Donnell, Secretary to the Director
Carolyn Debra Grant, AssDip(Acc) *Panorama TAFE*, Secretary to the Company Secretary/Conference Secretariat
Narelle Elizabeth D'Costa, Administration Officer
Emma-Kate Darling, Receptionist
Maria Concettina Mills, Casual Receptionist
Elizabeth Morgan, Administration Support
Branka Zepina, Cleaner (commenced 12/3/01)

Highlights of the year

The outcomes of the Institute's major wine closure trial were analysed 20 months post-bottling and submitted to the *Australian Journal of Grape and Wine Research* for publication with expected publication of the 42 page article in mid July 2001.

Flavour scalping studies showed a very large differential in the rate of disappearance of known wine aroma compounds when stored in 'bag in box' containers.

The terminology developed by our sensory panel and collaborators to characterise the mouthfeel of red wines is gaining acceptance as a 'mouthfeel wheel' by researchers and winemakers worldwide.

Gram quantities of nine different tannin fractions from grape seeds and grape skins for sensory evaluation were isolated and characterised using HPLC and electrospray mass spectrometry followed by sensorial assessment by a tasting panel over an eight week period.

Combined laboratory and industry-based winemaking trials, coupled with chemical and quantitative sensory descriptive analysis, continued to demonstrate the potential of *S. bayanus*, *Candida* and new hybrid yeast as positive modulators of wine sensory characteristics.

A laboratory model system for predicting the effects of fermentation yeast on malolactic bacteria has been developed to enable selection of the most appropriate combination of strains to provide greater reliability of malolactic fermentation.

The Institute secured strong private financial support from Lallemand to boost our research on the effect of wine yeast on red wine phenolic compounds.

The Institute paid its final instalment of its $250,000 donation to the Hickinbotham Roseworthy Wine Science Laboratory and conducted a series of triplicated ferments on a commercial scale to study the effect of winemaking practices and yeast strains on colour, mouthfeel, flavour and aroma of both white and red wines.

Successful organic synthesis of a high-yielding precursor to the ubiquitous wine flavor compund ß-damascenone was achieved as a first step to understand its formation from non odoriferous grape precursors.

Organic synthesis disproved the structural assignment of an oak lactone precursor which has been assumed for more than 20 years.

Our Analytical Service increased its turnover and recorded an increase in contract research as well as a very large demand for analyses for 4-ethylphenol, an indicator of *Brettanomyces* activity which may be more widespread than initially assumed.

A double modification of a laboratory yeast by means of molecular biology gives a remarkable demonstration of the power of the technology and it is demonstrated that the genomes of commercial wine strains are quite different to those of laboratory strains.

Formal 'Roadshow' visits were made to SA and Vic in November and December 2000 (see Appendix 1). Senior Institute staff presented seven full-day seminars, each seminar consisting of a minimum of twelve presentations focussing on current areas of Institute research, or topical issues in the wine industry.

Institute staff authored/co-authored 37 publications; gave 35 seminars/talks (excluding the Roadshow seminars above); presented 11 workshops; presented 3 posters; gave 38 lectures and supervised 36 students during the year.

Institute staff hosted more than 113 international visitors to the Institute during the year including five visiting scientists on extended stay.

The new website of The Australian Wine Research Institute @ www.awri.com.au was launched including an extensive educational/troubleshooting section on wine hazes and deposits.

Requests for information from the John Fornachon Memorial Library staff increased by 108% over requests received in 1999/00.

The 14th Advanced Wine Assessment Course was held in July 2000, giving another 30 participants the opportunity to develop and test their sensory evaluation performance. This was the second Course presented under a four-day format, which includes over 40 hours of activities over the four days and a contribution by more than ten senior Australian wine show judges.

Institute staff responded to more than 4,400 enquiries in relation to wine industry matters.

The human capital of the Institute is further enhanced by qualification of one additional staff member for the PhD degree, by qualification of one additional staff member for the Postgraduate Diploma in Oenology, by recruitment of a distinguished German scientist to lead our tannin project and by the recruitment of a PhD qualified chemist with almost two decades of experience in the wine industry.

Staff Activities

In addition to undertaking research and other projects described in this report, the Institute performs a large number of external activities in support of the Australian wine industry.

Information on seminars, talks and poster papers given to outside organizations, academic lectures delivered, graduate students supervised, and the papers published is tabulated and can be found in Appendices 1–4 of the Annual Report. Activities in addition to those in the Appendices are described below.

Peter Høj is a member of the following:

- Prime Minister's Science, Engineering and Innovation Council
- South Australian Innovation, Science and Technology Council
- International Trade and Technical Advisory Committee (AWBC)
- Compliance and Technical Advisory Committee (AWBC)
- Technical Committee (Winemakers' Federation of Australia)
- Wine Committee (Royal Agricultural and Horticultural Society of South Australia)
- Waite Campus Management Committee
- Committee of Management, Viticultural Publishing, publisher of *Australian Journal of Grape and Wine Research*
- Editorial board of the *Journal International des Sciences de la Vigne et du Vin*
- Conference Planning Committee of the Eleventh Australian Wine Industry Technical Conference (7-11 October 2001, Adelaide) (Chair)
- Australian Organising Committee of the 26th World Congress and 81st General Assembly of the Office International de la Vigne et du Vin (OIV) (11-18 October 2001, Adelaide) and is Section President of the Oenology Sub-Commission for the Australian OIV Congress.

He is also the Institute's representative on the following Adelaide University's Boards/Committees:

- Faculty Board
- Department of Horticulture, Viticulture and Oenology Advisory Committee
- Management Committee of the Department of Horticulture, Viticulture and Oenology

Hans Muhlack is the Public Officer of the Australian Wine Industry Technical Conference Inc.

Rae Blair is a member of the Conference Planning Committee and is the Treasurer and Conference Manager of the Eleventh Australian Wine Industry Technical Conference. She is also a member of the Australian Organising Committee of the 26th World Congress and 81st General Assembly of the OIV.

Creina Stockley is a member of the Australian Wine and Brandy Corporation's Legislative Review Committee, a member of the Technical Committee of the Winemakers' Federation of Australia, a member of the National Reference Committee – Environment Strategy Development (South Australian Wine and Brandy Industry Association) and was elected Vice-President of the Nutrition and Wine Expert Group for the Australian OIV Congress.

Elizabeth Waters is Leader of Program 1 of the Cooperative Research Centre for Viticulture II.

Mark Sefton is on the editorial review board of the *International Journal of Vine and Wine Sciences* and is the project leader of project 1.3 of the Cooperative Research Centre for Viticulture II.

Leigh Francis is an Editorial Board member of the *Journal of the Science of Food and Agriculture*, Editorial Review Board member of the *American Journal of Enology and Viticulture*, Chairman of the Royal Australian Chemical Institute (SA Branch), Medicinal and Agricultural Chemistry Group.

Ken Pocock serves on the Waite Campus Occupational Health and Safety Coordinating Committee.

Paul Henschke serves on the Editorial Review Board of the following journals: *Australian Journal of Grape and Wine Research; American Journal of Enology and Viticulture; and Australia and New Zealand Wine Industry Journal*.

Peter Godden is a member of the Conference Planning and Program sub-Committees and is the Workshop Coordinator of a program of 72 workshops to be held at the Eleventh Australian Wine Industry Technical Conference.

Matthew Holdstock is a member of the Interwinery Analysis Group Inc.

Visitors to the Institute

Australia

- Eric Parnis and Neil Grant, South Australian Department of Industry and Trade (1 August 2000)
- Linda Lee, Hong Kong Trade Development Council, Sydney (2 August 2000)
- Matthew Wingfield, International South Australia (2 August 2000)
- Dr Erika Winter, Agriculture Victoria (3 August 2000 and other occasions)
- Group of 30 Advanced Viticulture students from South Eastern TAFE, accompanied by Denis Vice (3 August 2000)
- Linda Bowes and Brian Smedley, SAWBIA (18 August 2000, and other occasions)
- Senator Judith Troeth, Parliamentary Secretary to the Minister for Agriculture, Fisheries and Forestry, Australia (25 August 2000)
- Michael Alder, Agriculture, Fisheries and Forestry, Australia (25 August 2000)
- David Monck, South Australian Centre for Manufacturing, Marilyn Arnold, Flavour South Australia, The Food Industry Association Inc. (28 August 2000)
- Sandy Grieve, Australian Council of Agricultural Journalists Inc (4 September 2000)
- Jenny Eichler and four Year 11 Chemistry Students from Murrayville College (6 September 2000)
- Members of the Executive Committee of the Australian Academy of Technological Sciences and Engineering (6 September 2000)
- Ross Dobinson and Tim Hoban, Technology Structuring Limited (7 September 2000)
- David Hall and Dr Deann Glenn, Grape and Wine Research and Development Corporation (on several occasions)
- Richard Heinemann, The Business Centre (14 September 2000)
- H-Michael Schwandt, Consul-General, Federal Republic of Germany, Pat Vaughan, Commonwealth visit co-ordinator, Department of the Prime Minister and Cabinet, Nektarios Tsirbas, Policy Officer, Department of Agriculture, Fisheries and Forestry, Thomas Kruckemeyer, Interpreter, SA, Dean Dempsey, Deputy Manager, Protocol, Department of the Premier and Cabinet, Peter Hayes, Rosemount Estates (3 November, 2000)
- Juan Bournas, Trade Commissioner of Chile and Kees van Haasteren, AgTour (15 November 2000)
- Noel Hayes, Amorim (20 November 2000)
- Olga Kostic, International Marketing Manager and Brian Dolling, Managing Director, NuKorc (22 November 2000)
- Peter Flinn, Agriculture Victoria (22 November 2000)
- Tom Angove, Angove's Wines (2 January 2001)
- Andrew McPherson, Andrew Dean, Hamish MacGowan, Leigh Clarnette, Guida Vazzoler, McPherson Wines; Alister Purbrick, Allan George, Neil Larson, Chateau Tahbilk; Geoff Merrill, Scott Heidrich, Joe DiFabio, Geoff Merrill Wines (2 February 2001)
- Tony Royal, Seguin Moreau (12 February 2001)
- Michael Burleigh, Foss Pacific (28 February 2001 and 24 April 2001)
- Cecil Camilleri, Yalumba Wines; Alex Sas, BRL Hardy; Wendy Allan, Prudence Honner, Southcorp Wines; Peter Hayes, Rosemount Estate; Prue Henschke, C.A. Henschke & Co; David Bruer, Temple Bruer; Russell Johnstone, Orlando Wyndham Group; Vic Patrick, Syd Kyloh, Blass Beringer; John Sabine; Andrew Benger; Lou Cremasco; Geoff Hardy,

Pertaringa Vineyards; James Altmann, Fruit Doctors; Nic Fazekas, IAMA; Bill Murray, IHD, Leon Redunz, AvCare, Bayer; Keith Jones, South Australian Wine and Brandy Industry Association; DeAnn Glenn, Grape and Wine Research and Development Corporation; Matt Cahill, Dow AgroSciences; Stephen Strachan, Winemakers' Federation of Australia; David Baker, David Braybrook, Cooperative Research Centre for Viticulture; Robyn van Heeswijck, Anu Kuma, Department of Horticulture, Viticulture and Oenology; Trevor Wicks, South Australian Research and Development Institute; Ary Hoffman, LaTrobe University; Peter Merriman, Alison MacGregor, Rob Sward, Mark Krstic, M. Mollah, Department of Natural Resources and Energy; Rai Kookana, Ray Correll, CSIRO (27 February 2001)
Natasha Mitchell, ABC radio (12 March 2001)
Imre Lele, Bottle Magic and Ken Craig, SA Centre for Manufacturing (13 March 2001)
Duncan MacDonald, Rod Pemberthy, Sanjay Sinha, Burns Philp (14 March 2001)
A/Professor Graham Bell, Director, University of New South Wales, Centre for ChemoSensory Research (15 March 2001)
Rob Lewis, South Australian Research and Development Institute and Nigel Scott CSIRO Division of Plant Industry (26 March 2001)
Dr John Radcliffe, CSIRO (27 May 2001)
Jennifer Gardiner, Adelaide University (27 May 2001)
Libby Boschen, WINETAC (25 May 2001)
B. Sara, C. Belperio, T. Mitani, K. Ringwood, M. Wong, L. Pattison, R. Elfenbein, M. Trewartha, Food Technology students from the Regency Institute of TAFE (31 May 2001)
Robert Druscetta and Anne Patterson, Australian Wine and Brandy Corporation (28 June 2001)
Don Woolman and Paul Clancy, Australian Wine Industry Suppliers' Association (29 June 2001)
Cooperative Research Centre for Viticulture Board members: G. Scollary, D. Gall, D. McWilliam, P. Barnes, T. Martin, J. Dal Bro, P. Kriedemann, P. Mansfield (4 July 2001)
Professor Edwina Cornish, Adelaide University (5 July 2001)

Chile

Agapito Garcia Vidal; Ximena Torti Solar, Agricola San Diego de Puquillay Ltda; Guillermo Achurra Montes, Agricola Tunquelen Ltda; Jorge Luis Ollé Cordero, Agricola y Comercial Crucesillas Ltda; Carlos Valenzuela Olave; Patricio Azocar Monasterio, Cooperativa Agricola Pisquera Elqui Ltda; Carlos Alfredo Terres Silva, Cooperativa Agricola Vitivinicola de Curico Ltda; Diego Garcia de la Huerta, Juan Sutil Servoin; Maria Adriana Cerda Arriagada, Nevada Export S.A.; Roberto Echeverria P. De V; Claudio Salgado Villablanca, Sergio Salgado Segura, Armando Fuenzalida Salgado, Sociedad Agricola Chequen Ltda, Ana Maria Flores Lopez, Sociedad Agricola Ganadera y Forestal Arboleda Ltda; Santiago Achurra Hernandez, Sociedad Agricola Requingua Ltda, Javier Eduardo Ramirez Vodnizza, Sociedad Eduardo Ramirez Letelier y Cia Ltda; Luis Antonio Paredes Nuñes, Sociedad Nuñez y Cia Ltda; Fernando Almeda Ollé, Sociedad Vinicola Miguel Torres S.A.; Gonzalo Bertelsen Diaz, Terramater S.A.; José Antonio Esturillo Castaño, Vinos del Sur S.A.; Dario Polloni Sch, Viña Cantera S.A.; Roberto I. Echeverria Z, Viña Echeverria Ltda; Eugenia del Carmen Diaz Aedo, Viña Morandé S.A.; Teresa Medina Fernández, Viña Santa Mónica Ltda; Max Undurraga, Viña Undurraga S.A.; Jorge Rojas Garcés, Viña ValdiviesoS.A.; Eugenio Maffei Rojo, Viñedos y Bodegas Corpora S.A.; Angel Domper Pons, Viñedos y Bodegas Domper S.A.; Sara Cabrera Gajardo, Fontec; Tonchi Tomic Jakaf; Carmen Sutil Servoin; Manuel Madrid Baeza, Corporación Chilena del Vino; Chile (15 November 2000)

Denmark

Thorvald Akselsen, Foss Electric, Denmark (26 April 2001)
Mai Nygaard, Chr. Hansen, Denmark (17 November 2000)

France

Mounir Saoma, Michel Picard, France (2 February 2001)
Laurent Lebrun, Seguin Moreau, France (12 February 2001)
Marc Sabate, Michel De Tapon, Sabaté Diosos, France (12 February 2001)

Germany

His Excellency, Mr Karl-Heinz Funke, Federal Minister of Food, Agriculture and Forestries, Mr Jürgen Detken, Head of Division, Generlal EU Policy, Mr Hellmut Altpeter, Director, Economic Relations to non-European Industrial Countries, Ms Sigrun Neuwerth, Head of the Press Office, Mr Wolfgang Jaeger, Executive Officer, Minister's Office, Ms Ines Mann, Interpreter, Germany (3 November 2000)

Greece

Yanis Vogiatzis, John Boutari & Son – Wineries S.A., Greece (12 March 2001)

Hong Kong

Antonio Y.L. Koon, Ponti Trading Limited, Kowloon, Hong Kong (2 August 2000)
Johnny Wan, Hong Kong Trade Development Council, Wanchai, Hong Kong (2 August 2000)

India

Dr K.K. Jindal, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan, India (3 August 2000)

Israel

Amos Mizrach, Volcani Institute, Bet Dagan, Israel (23 February 2001)

Italy

Elio Novello, Gianpalo Vaona, Bolla, Italy (2 February 2001)

Japan

Dr Yoshio Kodama, Meiji Seika Ltd, Japan (15 December 2000)

Portugal

Antonio Amorim and Professor Miguel Chabral, Amorim, Portugal (20 November 2000)

United Kingdom

Dr Steven Walker, Campden & Chorley wood Food Association Group, United Kingdom (31 August 2000)
Mr John Gumbley, Senior Producer, Sky News, United Kingdom (14 October 2000)
Eddie Deaville, ADAS Nutritional Sciences Research Unit, United Kingdom (22 November 2000)
Carmel Kilcline, Sainsbury's, United Kingdom (3 April 2001)
Professor Ian R Peterson , Coventry University, United Kingdom (22 May, 2001)

United States of America

Dr Charles Edwards, Washington State University, USA (17 November 2000)
Paul Dolan, Dennis Martin, John White, Bob Blue, Nancy Walker, Heath Dolan, Bill Leair, Cara Hane, Jill Jepson, Fetzer Vineyards, Hopland, CA, USA (2 February 2001)
Rick Boyer, Marc Imbert, Jekel Vineyards, USA (2 February 2001)
Paul Ahvenainen, Korbel, USA (2 February 2001)
Lois Levitan, Cornell University, Environmental Risk Analysis Program Centre for the Environment, USA (27 February 2001)
Members of the University of California Cooperative Extension Service: Rhonda Smith – Sonoma, Larry Bettiga – Monterey, George Leavitt – Madera, Steve Vasquez – Fresno, Christian Butzke – Enologist, UC Davis, Chuck Ingles – Sacramento, Jennifer Hashim – Kern, Ed Weber – Napa, Paul Verdegaal – San Joaquin, USA (12 March 2001)
Paul Heck, Scott Laboratories, USA (27 March 2001)

Other

Thirty participants from the International Federation of Agricultural Journalists' Ninth World Congress (4 September 2000)

Sevenhill Winery: 1900
Photograph courtesy of The State Library of South Australia

Sample changer for autotitrator

Wine grape tannin and colour specification

Staff: Dr Markus Herderich, Dr Elizabeth Waters, Dr Stéphane Vidal, Anita Oberholster, Dr Z Peng, Stella Kassara, Mariola Kwiatkowski, Dr Mark Sefton, Dr Leigh Francis, Yoji Hayasaka, Gayle Baldock, Dr George Skouroumounis, Heather Donnell, Richard Gawel
Collaborators: Adelaide University - Dr James Kennedy, Dr Robert Asenstorfer, David Lee, Stephanie Lambert, Dr Graham Jones, Renate Ristic, Dr Patrick Iland, Stephen Clarke

This project was initiated as collaboration between The Australian Wine Research Institute and The University of Adelaide at the request of industry; an Industry Reference Group composed of twelve senior winemakers advises it. Funding for the tannin project is provided by the GWRDC as well as by the CRCV.

The ambitious objectives of this challenging multi-disciplinary project are:

- To gain an understanding of the types of compounds important to red wine colour and mouthfeel and of conditions favouring their presence in wine.
- To determine whether the interaction of proanthocyanidins (tannins) with other grape and wine compounds modifies their sensory properties.
- To establish viticultural and oenological techniques that enhance wine colour and desirable mouthfeel.

General observations

The new Leader of the Tannin Project, Dr Markus Herderich, commenced at the Institute on 9 April 2001. Dr Jim Kennedy, post-doctoral fellow at the University of Adelaide, has accepted a tenure track position as an Assistant Professor in the department of Food Science and Technology at Oregon State University and left the project in May 2001. Other fluctuations of the involved staff include Robert Asenstorfer, student at the University of Adelaide, who qualified for the award of the degree of Doctor of Philosophy; Anita Oberholster, PhD student at the AWRI, who finished the experimental part of her PhD research; and Stella Kassara, chemist at the AWRI, who left for maternity leave in July 2000 and recently resigned.

Despite these changes good progress has been made and the appointment of Dr Markus Herderich as new leader of the Tannin project has further focussed to our activities. Dr Liz Waters, the preceding project leader, is thanked for her significant past contribution which, in the longer term, was not sustainable due to her demanding task as Manager of Program 1 of the Cooperative Research Centre for Viticulture. Besides other measures of success and progress, it is very pleasing to see that the outcomes from this project are continuously being communicated in peer-reviewed journals. In this reporting period, three papers have been published, four have been submitted for publication, and at least a further four are in draft form. Just as important is the public and international recognition of the achievements obtained by the Tannin project. A fine example is the terminology developed by our sensory panel to characterise the mouthfeel of red wines, which - after publication in 2000 - instantly was accepted and identified as 'mouthfeel wheel' by researchers and winemakers all around the world. The international significance of the outcomes of the Tannin project is also reflected by various invited presentations, for example, the Institute was invited to give a 45 minute plenary presentation entitled 'Red wine colour and mouthfeel: Analysis of phenolic pigments and tannins' at the Annual Meeting of the American Society for Enology and Viticulture, held in San Diego from 27-30 June 2001. This task was admirably performed by the new project leader and appeared to generate much interest and follow-up enquiries from parties interested in establishing collaborations.

A similar reception was given when the tannin project and some of its recent outcomes were presented by Dr Vidal at an international conference in Bordeaux, where much praise was given for our achievements in a difficult area of research. More specific achievements relate to outcomes listed below.

Method development

Methods to quantify the levels of tannins in seed extracts and in wine have been developed, and the identity of polymeric proanthocyanidins (tannins) has been rigorously demonstrated by mass spectrometric analysis.

The results have been published (Z. Peng, Y. Hayasaka, P.G. Iland, M. Sefton, P. Høj and E.J. Waters, J. Agric. Food Chem., 49, 2001, Institute publication #660) and another manuscript was submitted for publication (Z. Peng, P.G. Iland, A. Oberholster, M.A. Sefton and E.J. Waters). The main outcomes are:

- Reversed phase HPLC methods have been developed for quantitative analysis of polymeric procyanidins (tannins) in grape seed extracts and for quantitative analysis of polymeric pigments in wines. The recovery of polymeric pigments with this method was 95%.

- Results obtained with the HPLC method correlated well with spectrophotometric measures of total pigmented polymers and indices of wine age.
- Using this technology, a study of 120 wines has confirmed that the proportion of pigmented material incorporated into polymeric proanthocyanins increases with wine age.
- At present, these analytical protocols are adopted and validated as routine methods for the determination of oligomeric and polymeric polyphenols in grape, seeds, and wine samples from numerous viticultural and winemaking trials. Libraries summarizing UV- and mass spectral data will facilitate the identification of key polyphenols in the future. These initiatives will result in significantly reduced analysis time and secure higher through-put of samples.

Wine pigments

Robert Asenstorfer's PhD thesis was passed.

- The findings from Robert Asenstorfer's thesis have now been prepared for publication by Robert and Dr Graham Jones.

In a collaboration with Robert, Yoji Hayasaka developed and optimised methods for the detection of stable wine pigments by Electrospray ionastion mass spectrometry (ESI-MS/MS). Applying tandem mass spectrometric experiments numerous complex red pigments could be detected in wine samples. With this approach we also identified four novel anthocyanin polyphenol derivatives for the first time as natural products in wine.

A paper outlining this methodology has been submitted for publication.

Analytical methods to identify the existence of new wine compounds is a necessary but not sufficient tool to advise winemakers on how to modulate wine composition to meet set quality specifications. Once identified, the relative importance of such compounds must be established. The next step is thus to synthesize some of the newly discovered compounds in sufficient quantities to describe their chemical and sensorial properties. To this end, David Lee, a PhD student funded by CRCV, and Mr Anders Haakansson, a visiting synthetic chemist from Denmark, has initiated synthesis of these new wine pigments.

Copigmentation

Copigmentation reactions are strongly influenced by pH, with its relative importance being greatest at higher pH values. Ethanol decreases the copigmentation interaction.

Of the common simple phenolics in wine, quercetin has been shown to form the most stable intermolecular complex with malvidin-3-glucose.

Self-association of malvidin-3 glucose also appears to be a major cause of colour enhancement. As model experiments show that the extent of intermolecular copigmentation depends on the concentration of anthocyanins,

more is likely better in young red wines. Conversely, aged products feature significantly reduced concentrations of anthocyanins and the contribution of intermolecular copigmentation to the color of older red wines remains to be established. Stephanie Lambert, an Adelaide University PhD Scholarship holder, presented a poster on her physical chemistry studies at In Vino Analytica Scientia, held in Bordeaux from 14-16 June.

Grape seed and skin proanthocyanidins

As reported previously, adding extra seeds to a red wine ferment produced wines with increased levels of phenolics, including tannins and pigmented polymers, and enhanced colour density.

After ageing in bottle for two years, wines made from must with extra seeds contained more colour and were higher in astringency than wines made conventionally. Importantly, the wines appeared not to be more bitter.

This material is being drafted for publication (Z. Peng, P.G. Iland, I.L. Francis, Y. Hayasaka and E.J. Waters).

A method for characterising the composition of grape and wine tannins using acid hydrolysis in presence of phloroglucinol has been developed.

The analytical protocol has been published (J.A. Kennedy and G.P Jones, J. Agric. Food Chem., 49, 2001, 1740.).

For the first time, nano electrospray ionisation in combination with mass spectrometry allowed us to detect individual tannins on a molecular level. This method has been developed by Yoji Hayasaka and represents a major progress for our studies aiming to understand the chemical properties of tannins.

Application of this methodology enabled us to characterize the composition and purity of several different tannin fractions from grape seeds and grape skins. This is essential when attempting to ascribe specific sensorial properties to individual classes of isolated tannins.

An investigation on structural changes of grape skin tannins and pigmented polymers at different stages of berry development has been conducted. Again, the methods developed proved to be essential for this study on the composition of grape skin proanthocyanins.

The results of the latter experiments have been submitted for publication (J.A. Kennedy, Y. Hayasaka, S. Vidal, E.J. Waters and G.P. Jones – manuscript in review).

Shiraz grapes were collected during the period of berry development from a range of viticultural regions (Riverland, Barossa and Coonawarra). In some cases samples were collected from trials where different irrigation schedules had been applied. These studies showed that levels of catechin, epicatechin and extractable tannins (procyanidins) in seeds increased up until veraison, where they were at their maximum, and then declined. The differences in seed composition of grapes sourced from the range of viticultural regions were less than the differences in skin colour (anthocyanin levels).

When the seed colour of berries from different vineyards within one region are compared at the same stage of ripeness (e.g. 24 °Brix) there is little difference in the degree of seed colour, indicating that once berries have ripened to a particular point, assessment of seed colour is not a discriminating factor between the potential quality of different vineyards.

A viticultural trial to investigate the differences between seed physical characteristics (seed number, size and weight) and seed chemical composition of grapes that develop in shaded canopy conditions versus that of grapes that develop in moderately and well exposed canopy conditions has been established by Pat Iland and Renata Ristic at The University of Adelaide.

The extractable tannin content of seeds was maximal in the three weeks prior to veraison and then decreased as the fruit ripened. The seed content of the flavan-3-ol monomers also decreased after veraison.

Visually seeds were initially green, plump and had pliable seed coats. Beginning from veraison, the seeds browned in colour, became desiccated and the seed coats hardened.

These changes in content and visual aspects can be explained by oxidative events occurring in the seed coat during fruit ripening. This information has been published (J.A. Kennedy, G.J. Troup, J.R. Pilbrow, D.R. Hutton, D. Hewitt, C.R. Hunter, R. Ristic, P.G. Iland and G.P. Jones, Aust. J. Grape Wine Research 6, 2000, 244).

There were no differences in total amounts (monomers and procyanidins), subunit composition (amount of terminal and extension units) or mean degree of polymerisation, of seeds of different canopy treatments.

Linking the astringency and mouthfeel properties of wines to proanthocyanidin and or other polymer fractions in wine

Funded by the CRCV and employed by the Institute, Stéphane Vidal commenced work in Véronique Cheynier's laboratory at INRA-IPV in Montpellier on 6 June 2000, where he prepared tannin fractions from seeds and skins for sensory assessment.

Stéphane has isolated and purified gram quantities of ten different tannin fractions from grape seeds and grape skins.

A panel of tasters has been convened and trained by Richard Gawel over the last few months. In late March these tasters completed a very extensive tasting of the tannin fractions isolated by Stéphane.

This is the first time that such fractions have been tasted and profiled for their astringency sub-qualities. The data will allow us to understand how the chemical properties of tannins relate to their sensory properties and are currently being subjected to extensive statistical analysis.

Stéphane presented this work at In Vino Analytica Scientia, held in Bordeaux from 14-16 June.

Winemaking practices that effect proanthocyanidin levels and sensory properties of wine

To study the effect of winemaking practices on colour and mouthfeel the first commercial scale winemaking trials have been conducted at the Hickinbotham Roseworthy Wine Science Laboratory located on the Waite Campus with support from our Industry Services Manager and continued input by the facility Manager, Mr Stephen Clarke, a winemaker with 20 years commercial experience.

Winemaking was performed according to the guidelines of the industry reference group.

The objectives of these initial 20 fermentations are to analyze the influence of red grape varieties (Coonawarra Shiraz and Adelaide Hills Cabernet Sauvignon), fermentation tanks (rotary tanks versus stationary fermenters), and fermentation temperatures (18°C, 25°C) on red wine phenolic compounds. A complementary trial conducted by our Wine Microbiology team assessed the influence of yeast strains on tannin profiles.

In addition, we aim to study the variation between replicate fermentations and to identify key parameters which contribute to variation.

Reference samples of juice and skins have been collected on a daily basis during fermentations, and are continuously kept on a weekly basis prior to bottling.

Comprehensive chemical analysis of several hundred samples for polyphenol and tannin content has been initiated. It will be complemented with sensory analysis after short term bottle storage, and several of the wines produced will be presented to industry in workshops conducted at the Eleventh Australian Wine Industry Technical Conference.

An analysis of compositional differences between grape marc derived tannin material and tannins extracted from skins during fermentation, indicates differences in the relative proportions of polymeric vs monomeric pigments and the ratio of malvidin-3-glucoside-6' acetates to malvidin-e-glucoside-6'-p-coumarate anthocyanins. For example, the proportion of these natural polymeric pigment in young wines is approximately 15% but can constitute greater than 60% in grape marc extracts. Similarly, the proportion of malvidin-3-glucoside-6'-p-coumarate is higher in marc extracts than in most young wines.

These characteristics can be determined by a single HPLC analysis using the methods developed by Peng.

Further analysis of a liquid grape marc extract indicated that dextrins were not detectable in the preparations analysed. This contrasts with previously published results on the composition of solid grape marc extracts.

Rapid instrumental techniques

Staff: Dr Leigh Francis, Dr Bob Dambergs, Dr Michael Esler, Dr Bruce Kambouris, Dr Wies Cynkar, David Boehm, Professor Peter Høj, Dr Sally-Jean Bell, Peter Godden
Collaborators: Adelaide University - Dr Graham Jones, Dr Patrick Iland Orlando Wyndham Group - Russell Johnstone, Ben Zander, Inca Lee Southcorp Wines - Dr Andrew Kleinig, Eric Wilkes

A comprehensive suite of accurate and precise calibrations has been developed for the measurement of colour, pH, and TSS in red grapes on three distinctly different types of NIR instruments. These calibrations range in specificity from general 'pan-Australian' to more precise region and variety specific. Greater than 2,000 samples have been collected this vintage by collaborating companies and some of these have been delivered to the Institute and analysis has commenced.

As part of our obligations to the Cooperative Research Centre for Viticulture and to the Commonwealth, publication of this method had earlier been held over pending finalisation of intellectual property issues.

Dr Leigh Francis and Dr Bob Dambergs

These matters have now been largely resolved and the potential for commercialisation of the technology has been examined more fully. While it was deemed timely to delay publication earlier to allow the Institute to secure the advantage of being an early entrant into the field by not disclosing information to potential commercial competitors or imitators, it is suggested that this delay is no longer warranted. The publication of the NIR method for colour, pH and total soluble solids, will now be accelerated.

The sample collection program provided more than 2000 samples from the 2001 vintage from grape growing regions in NSW, VIC, WA as well as SA. It is anticipated that by the end of 2001 the chemical and spectroscopic analysis of this data set will be completed and the data used to refine earlier NIR calibrations based on 1999 and 2000 data and develop something approaching a pan-Australian calibration or set of calibrations. The significance of regional, varietal and especially seasonal variation for NIR analysis will be further investigated. At this point in time, it appears that region-specific and variety-specific calibrations will be slightly better than pan-Australian calibrations. Whether the differences have real practical consequences is yet to be resolved (see Figure 1).

Negotiations with the CRCV and the collaborating wine companies have taken place in order to clarify the mode of commercialisation and provide a mandate for the management of such activity.

The transfer of calibrations between well-matched or standardised instruments appears likely to be successful and will be examined in detail with samples from the 2001 vintage. It should be noted however that this is not a trivial exercise and may require significant effort. While

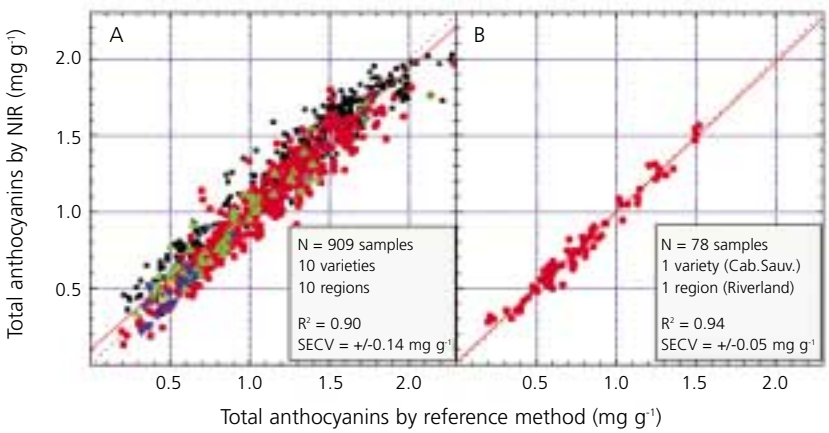


Figure 1: NIR calibrations for total anthocyanin concentration for a population of ~900 red wine grape samples and one of its sub-populations. A. 'Global' calibration; 909 samples across 10 growing regions, 10 grape varieties and 2 seasons, coloured by variety. B. Subpopulation, localised for both a single grape variety, Cabernet Sauvignon, and a single growing region, the Riverland. Observe that NIR measurement precision (SECV) improves by focusing on a particular region and variety.

the direct calibration of a single instrument has proven to be relatively straightforward, the simultaneous standardisation of a population of several or many instruments to the one calibration is a more complex issue. In particular, it appears that not all types of instruments are equally capable of being standardised. On the other hand, there now exists recognised protocols for the standardisation of more expensive research grade instruments, such as the NIRSystems6500 spectrophotometer. Commercially available software that is designed for calibration transfer and instrument standardisation is to be evaluated in standardisation of the Institute's NIRSystems6500 spectrometer with BRL Hardy's based on 2000 and 2001 vintage data.

Investigations with instruments capable of scanning whole fruit are continuing in association with two wine companies, but no data has yet been received. The Perten DA7000 instrument offers the possibility of being able to rapidly analyse samples of intact whole berries. The investigation is proceeding on a large data set already provided by BRL Hardy using samples from the 2001 vintage.

Trials with an existing prototype bin probe have commenced and data is being collected. Initial trials have focussed on off-site evaluation of reproducibility of spectral collection.

Trials with prototype bin probes continued during the 2001 vintage to further examine the feasibility of the concept. Whilst the trials have demonstrated the promise of this approach of in-situ scanning, there is some indication that commercial development of a NIR 'bin-probe' may be ambitious at present given the current state of commercially available NIR technology. Institute efforts may be better focused on addressing the more tractable issues encountered in development of a compact laboratory-based instrument. The latter is likely to be deliverable to industry earlier than the former.

Three of the relatively-inexpensive prototype NIRTech instruments will be tested in parallel at the Institute in order to evaluate instrument effects in the absence of all other experimental variables. The successful standardisation of cheaper diode-array based instruments in particular (such as this prototype), is still a subject of considerable research inquiry internationally.

It was planned to expand the trial of these prototype instruments to a small number of companies for the 2001 vintage. Disappointingly, there have been unforeseen delays and only one instrument is likely to be supplied to industry in 2001.

Considerable effort has been expended in investigating previously identified variation in the glucosyl-glucose (G-G) assay calibrations (particularly for white grapes). Detailed scrutiny of the reference method has led to some refinements of the analytical techniques. In particular, the use of a 'stomacher' for gently macerating white grapes in a reproducible manner that is also more relevant to real winemaking practices, has improved the degree of uncertainty in the measurement.

Further samples of Chardonnay in particular are being collected this season with cooperation from BRL Hardy. These samples will be tracked from juice right through to wine and linked with the subsequent quality rating determined at the in-house allocation tastings. Other wines assessed at the allocation tastings will also be scanned and then discrimination models will be tested.

Studies using commercial tasting data indicate the approach of using NIR for allocation tastings and measurement of G-G in white fruit may have some value and this will be addressed more fully in future work. Samples from the 2001 vintage have now been received at the Institute and work will commence shortly.

In some recent preliminary studies, grape and other tannin fractions of various sizes, chemical composition and degree of polymerisation that have been prepared by Dr Stèphane Vidal as part of project AWR 96/1 (Wine grape tannin and colour specification) have been scanned by NIRS. The spectra appear to show significant differences and suggest that the technique may be useful in distinguishing these tannin fractions either quantitatively, qualitatively, or both.

Yeast flavour and fermentation activity

Staff: Dr Paul Henschke, Dr Eveline Bartowsky, Dimitra Capone, Dr Peter Costello, Nadia D'Incecco, Jeff Eglinton, Dr Leigh Francis, Holger Gockowiak, Kate Howell, Jane McCarthy, Dr Mark Sefton, Tracey Siebert, Professor Peter Høj
Collaborators: Professor Graham Fleet, The University of New South Wales, Stephen Clarke, The University of Adelaide

This GWRDC-funded project has as a primary objective, to investigate microbiological interventions which can be used to generate a wider spectrum of wine sensory properties than currently achieved with conventional use of well-characterised *Saccharomyces cerevisiae* strains and well-known malolactic bacteria. Three current main activities are related to:

- Development of new isotopic dilution assays for quantifying the key impact compounds produced by yeast, as a tool for determining the importance of yeast strain and fermentation conditions,
- Evaluation of the oenological properties of non-conventional yeasts with an emphasis on correlating chemical composition and organoleptic attributes of wines made under winery conditions,
- Laboratory and winery studies concerning the use of mixed yeast strain starter cultures, compared with conventional single strain cultures, as a strategy for simulating 'natural ferments', and

Quantification of yeast key impact aroma compounds—methods development

As stated in the previous annual report, the aim of this collaboration with Drs Mark Sefton and Leigh Francis and their teams, is to develop stable isotope dilution assay methods for quantifying the key impact compounds formed during the yeast alcoholic fermentation. Work has focused on the principal esters produced by yeast which are an important contributor to the fruity/floral/estery character of wine, which is often referred to as the 'fermentation bouquet' of wine. Good progress has again been made with the synthesis of ten isotopically-labelled ester standards being completed. The development of validated assays for the ethyl esters has commenced. Ms Tracey Siebert, who has recently joined the team, will focus on applying the assays to wines produced through our fermentation trials; a series of Chardonnay and Cabernet Sauvignon wines are currently undergoing extensive chemical analysis and sensory evaluation. This analytical approach will allow us to relate the ester profile of wine determined chemically and by sensory techniques and to determine the relative importance of yeast strain and fermentation conditions on the development of the fermentation bouquet of wine. In addition to assays on esters, several other stable isotope dilution assays for important wine aroma and flavour compounds is underway.

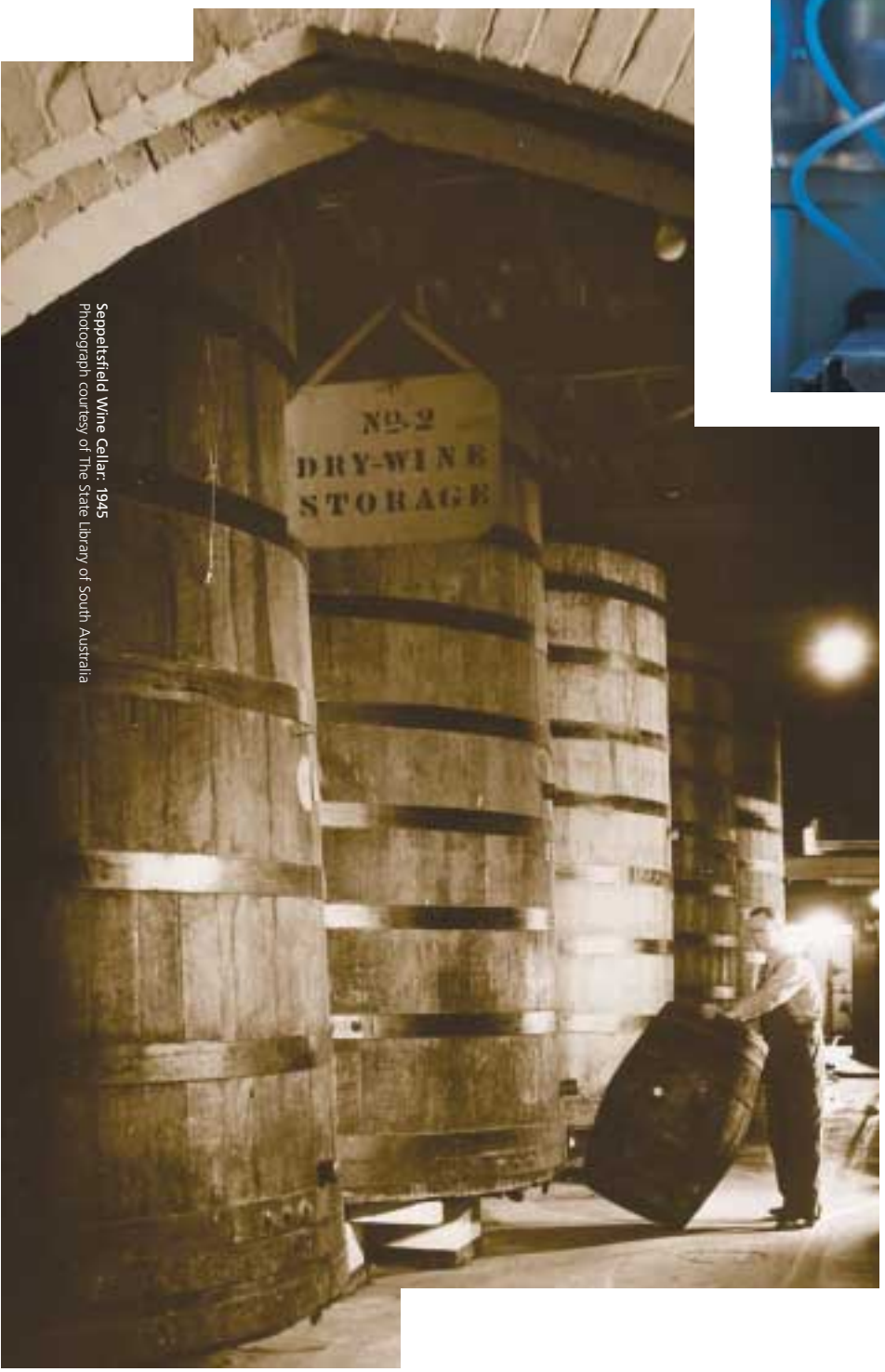
Industry evaluation of selected novel flavour yeasts

Replicated winery scale fermentation trials were undertaken during the 2001 vintage with the aim to consolidate the preliminary trials undertaken previously on a laboratory scale and to focus on the key attributes that these non-conventional yeasts can offer wine makers in search of increasing the flavour diversity of wines.

Saccharomyces bayanus *trials*

In addition to the barrel fermentation trials referred to in the past annual report, a winery scale trial using *S. bayanus*, and *S. cerevisiae* as a reference wine yeast, with Chardonnay and Cabernet Sauvignon has been undertaken in the Hickinbotham Roseworthy Wine Science Laboratory in

Atomic absortion spectrometer



Seppeltsfield Wine Cellar: 1945
Photograph courtesy of The State Library of South Australia

collaboration with Mr Stephen Clarke during the 2001 vintage. The Wine Science Laboratory offers the opportunity to have the wines contract made essentially under winery conditions but the close proximity to the Institute also allows us to closely monitor fermentation progress. We are grateful to BRL Hardy for generously providing 4000L of Chardonnay juice and 4 tonnes of Cabernet Sauvignon grapes for use in these trials.

The main purpose of the Chardonnay trial was to evaluate the performance and to determine the organoleptic properties of *S. bayanus* AWRI 1375 under larger scale conditions using 260-280L fermentors. As in previous trials *S. cerevisiae* AWRI 838 was the reference yeast. In addition, treatments were included which were designed to evaluate different methods for ensuring that wine with low residual sugar was achieved after fermentation with non-conventional yeasts, in this case *S. bayanus* 1375. The treatments involved:

- Sequential inoculation with *S. cerevisiae* AWRI 838 when the *S. bayanus* ferment was approximately 70% completed,
- Aerobic pumping-over of a stuck *S. bayanus* ferment, and
- Inoculation of a stuck *S. bayanus* ferment with an *S. bayanus* AWRI 1375 rescue culture prepared by the Institute recommended procedure.

The reference wine yeast, *S. cerevisiae* AWRI 838, completed fermentation at 15°C in 18-24 days whereas *S. bayanus* AWRI 1375 ceased fermentation with a residual sugar of 40 g/L after 35 days fermentation. Sequential inoculation of the *S. bayanus* ferment with *S. cerevisiae* AWRI 838 prevented lagging of the *S. bayanus* ferment which completed in 39 days. Both the aerobic pumping over treatment and the use of the *S. bayanus* rescue culture successfully removed the residual sugar from the stuck *S. bayanus* ferment. The latter procedure gave an especially interesting result because the rescue procedure not only worked well with the same yeast that caused the stuck fermentation but that the Institute's recommended rescue procedure was successful with the less robust *S. bayanus* fermentation yeast compared with the normally recommended *S. cerevisiae*. The effect of these treatments on wine chemical composition and sensory characteristics is being determined as a guide to recommending treatment preference.

Based on encouraging results obtained with *S. bayanus* AWRI 1375 in a previous laboratory-scale Shiraz trial, a larger scale trial using six 800 kg capacity rotary fermentors located in the WSL was undertaken during the 2001 vintage. *S. cerevisiae* AWRI 838 was the reference yeast. Mechanically harvested Cabernet Sauvignon fruit, which had been treated with SO_2 during harvest, was crushed and inoculated with *S. bayanus* or *S. cerevisiae*. Problem-free ferments with both yeast completed in 13-14 days, however, starter culture-induced MLF was delayed in the wines fermented with *S. bayanus* compared with *S. cerevisiae*.

All trial wines are currently being filtered and bottled in readiness for extensive chemical, instrumental and sensory analysis. In particular, we plan to quantify the main yeast-derived flavour compounds of these wines with GC-MS analysis combined with the newly developed stable isotope dilution assays developed by the team of Dr Mark Sefton and Dr Leigh Francis to establish the chemical basis for the flavour attributes of this yeast and the impact of fermentation conditions on their formation.

Several of the winery trial wines will be selected for use in the *Winemaking with non-conventional yeasts* workshop being planned in conjunction with the Eleventh Australian Wine Industry Technical Conference to be held in Adelaide in October 2001. The team is quietly confident that the soon to be performed rigorous analysis and results will point to the AWRI 838 and AWRI 1375 strains giving very different wines with differing positive attributes.

***Candida stellata* and *Candida krusei* trials**

Due to tank limitations in the Hickinbotham Roseworthy Wine Science Laboratory, 18L Chardonnay (BRL Hardy) fermentation trials were performed in glass with the experimental *Candida* strains, *C. stellata* AWRI 861, 1159 and *C. krusei* 863, at the Institute. The Chardonnay juice provided by BRL Hardy was also used for these trials. The ferments were superinoculated with *S. cerevisiae* AWRI 838 at approximately 50% fermentation progress to ensure the production of wines with low residual sugar. The *S. cerevisiae* AWRI 838 ferment failed to complete and was rescued by reinoculation with AWRI 838. The wines were cold-settled, racked, treated with SO_2, membrane filtered and bottled. Formal sensory descriptive evaluation and chemical analysis has commenced. Preliminary analysis confirms our previous findings (Institute staff publications #614 and #625) that the wines made with different yeast have marked differences especially in the ester profile (floral versus banana versus fruity versus ethyl acetate). Chemical analysis is recorded in the Table 1.

The chemical and sensory data confirm the potential of novel yeasts for developing wines with novel attributes.

Dr Peter Costello and

Optimisation of C. stellata *inoculation for inducing fermentation*

Some non-*Saccharomyces* yeasts are known to exhibit greater sensitivity than *S. cerevisiae* to growth inhibition by SO_2, which is often added to grape juice/must prior to inoculation with a selected wine yeast to inhibit indigenous yeasts and to prevent browning; this observation was noted in the Institute staff publication #625 in relation to *C. stellata*. Since this property may impact on the effective deployment of this strain by industry, the sensitivity of *C. stellata* to SO_2 has been quantified.

The inhibitory affect of SO_2 on yeast growth was determined in a freshly prepared grape juice by monitoring the delay in the onset of fermentation. *C. stellata* 1159 showed an extended lag phase of 2-4 days when inoculated into sultana juice containing free/total SO_2 of at least 32/50 mg/L (pH 3.2) whereas *S. cerevisiae* commenced noticeable growth within one day. By contrast with *C. stellata*, *C. krusei* was more tolerant and commenced good growth with a minimal lag phase. The low tolerance of *C. stellata* to SO_2 is likely related to low production of SO_2 and acetaldehyde by this yeast.

In addition to the use of H_2O_2 to reduce the SO_2-induced growth lag by oxidation of the SO_2, the lag phase in grape juice was also abolished by the addition of acetaldehyde, which presumably acted by binding the toxic free SO_2 , even at a concentration of 65 mg/L SO_2. Recent research from Prof. Pamment's laboratory, University of Melbourne, has shown acetaldehyde to stimulate fermentation independent of reaction with SO_2 so it is possible that both mechanisms have stimulated yeast growth.

These data confirmed that *C. stellata*, like most indigenous yeasts, is more susceptible than *S. cerevisiae* to SO_2 requiring either reduced SO_2 addition before inoculation, prolonged storage of juice at low temperature to ensure maximal binding of the free SO_2 or a reduction in free SO_2 content by oxidation with H_2O_2 or aeration, or by acetaldehyde treatment. The treatment with acetaldehyde is experimental and is not permitted under existing wine regulations.

Yeast impact on wine flavour and aroma—mixed culture versus monoculture fermentation

It is universal practice to induce the alcoholic fermentation of grape juice/must with a selected wine strain of *Saccharomyces cerevisiae* in order to produce a reliable fermentation and a wine with expected chemical and organoleptic properties. However, a relatively small, but increasing proportion of wines are made without inoculation thereby allowing the indigenous yeasts to proliferate, to give a so-called 'natural ferment.' This practice, which some winemakers believe gives wines preferred sensory characteristics, can be unreliable and its application is limited because the wine making properties of the indigenous yeasts are not known in advance.

A key difference between inoculated and non-induced fermentation relates to the initial population of *S. cerevisiae* present. Non-induced ferments typically initially contain a mixture of indigenous non-*Saccharomyces* yeasts which dominate the early stage of fermentation but then give way to a mixture of *S. cerevisiae* strains which complete the fermentation of the sugars whereas induced ferments, while also having a similar population of indigenous yeast species are generally dominated by the inoculated *S. cerevisiae* yeast culture from the outset. This project was undertaken to evaluate the use of a mixed culture strategy to simulate 'natural' ferments and to investigate the interaction between strains. As a simplified model, the mixed culture ferments were performed with three strains of *S. cerevisiae* wine yeast.

Five different strains of *S. cerevisiae* in three different combinations of three strains were tested under nutrient limiting and non-nutrient limiting conditions in a Chardonnay juice. Wines were also made with each of the five strains. These treatments resulted in three wines made by mixed culture ferments (M1-M3) and five wines made by monoculture ferments (T1-T5) under the two nutrient conditions. Three blended wines were also prepared by mixing in equal proportion the monoculture wines which corresponded to the yeasts used in the mixed culture ferments (B1-B3). The wines were tested for organoleptic differences by duo-trio aroma testing.

The proportion of each yeast strain in the mixed culture ferments was monitored by microsatellite-PCR analysis. The microsatellite sequences, present in the yeast chromosomal DNA, were demonstrated to be sufficiently polymorphic to permit reliable strain identification.

Duo-trio testing of the resultant wines showed that in some cases multiple strains of *S. cerevisiae* interacted to produce wines that were sensorially different to:

- Any of the wines produced by monoculture fermentation with the corresponding yeasts, or
- A wine produced by blending in equal proportion the three wines produced by the corresponding monoculture fermentations.

These preliminary results need verification, but nevertheless suggest that wine with an aroma profile different from wines of single yeast ferments, or their blends, can be produced by mixed culture fermentation only when the yeast strains interact and no-one strain dominates, as appears to normally be the case for 'natural' ferments. This suggests that mixed cultures of *S. cerevisiae* in wine fermentations could be used to modify the aroma of wine reminiscent of natural ferments but with a minimal risk typical of inoculated monoculture fermentations.

Treatment	Alcohol (% v/v)	Glycerol (g/L)	pH	TA (g/L)	VA (g/L)	Succinate (g/L)	free SO_2 (mg/L)	total SO_2 (mg/L)
Juice			3.24	7.5			13	35
***S. cerevisiae* 838**	13.5	5.9	3.39	7.5	0.41	0.5	22	112
***C. stellata* 1159 + Sc 838**	13.4	14.8	3.28	9.5	0.94	0.8	24	107
***C. stellata* 861 + Sc 838**	13.4	10.0	3.39	7.6	0.21	1.0	26	131
***C. krusei* 863 + Sc 838**	(13.8)	6.6	3.37	7.3	0.16	0.8	28	102
Range for malate = 2.3-2.5 g/L, lactate <0.1 g/L, tartrate=4.0 g/L, and citrate=0.3-0.4 g/L								

Table 1. Comparison of the chemical composition of Chardonnay wine made by fermentation with S. cerevisiae AWRI 838, C. stellata AWRI 861, 1159 and C. krusei 863.

Photograph courtesy of The State Library of South Australia

Orbital shaking water bath with small scale fermentations

Ms Kate Howell has performed this work at the Institute as part of her PhD studies undertaken through the University of NSW with supervision by Dr Eveline Bartowsky, Professor Graham Fleet (University of New South Wales) and Dr Paul Henschke.

Role of malolactic bacterial glycosidase enzymes in the development of wine flavour
Nadia D'Incecco, a PhD student from The University of Padua, Italy, working at the Institute under the supervision of Dr Eveline Bartowsky and Dr Paul Henschke has completed her studies on determining the role of ß-glucosidase activity, produced by malolactic bacteria, on the release of glycosylated flavour precursor compounds isolated from Chardonnay wine. This study complements her work in Italy where she is characterising the ß-glucosidase activities of yeast.

The role of malolactic bacteria in the development of wine flavour is not clear but is believed to involve at least three mechanisms, i) deacidification, by which L-malic acid is decarboxylated to L-lactic acid and CO_2, ii) metabolism of wine substrates to form either flavoured or flavourless products, such as the metabolism of citric acid to form diacetyl with a 'buttery' flavour, acetic acid, and 2,3-butanediol which has no flavour significance in wine (reviewed by Bartowsky and Henschke 2000, staff reprint #637), and iii) the hypothesised release of grape derived glycosylated flavour precursor compounds by excreted glycosidic enzymes (GWRDC project UCS 92/4).

An experiment was designed and conducted to establish whether or not malolactic bacteria have the ability to hydrolyse and release glycosylated flavour precursor compounds isolated from Chardonnay wine. The flavour precursor compounds present in wine are typically ß-glucosides or diglycosides in which an arabinose, rhamnose, apiose or xylose residue is linked to the inner glucose residue (Institute publication #458). An extract of glycosides was made from a Chardonnay wine, added to a model wine, and after MLF, the glucose content of the wine was measured by the glycosyl-glucose (GG) assay to indicate the extent of hydrolysis and a GC-MS analysis performed to reveal the extent of liberated flavour aglycons. Control experiments included no bacterial addition to test for acidic hydrolysis, and addition of various combinations of glycosidic enzymes as reference positive controls.

Using synthetic substrates, the two strains of *Oenococcus oeni* studied were shown to exhibit low α-L-arabinofuranosidase and α-L-rhamnosidase activities, endoglycosidases, which are necessary to cleave the outer sugar, often arabinose or rhamnose, from the diglycoside. These strains had significant ß-glucosidase activity, which cleaves glucosides formed by the action of the previously mentioned endoglycosidase enzymes or are directly derived from the grape, to release the flavour aglycon.

Monitoring the total a-a content of the model wine containing the glycosidic extract showed that purified ß-glucosidase only provided limited hydrolysis of the wine glycosides and confirmed that α-L-arabinofuranosidase and α-L-rhamnosidase were required for efficient hydrolysis of the glycosides. MLF conducted by the *O. oeni* strain only led to limited hydrolysis of the wine glycosides, suggesting that this bacterium does not elaborate sufficient activity of the enzymes necessary for the hydrolysis of disaccharides. Addition of α-L-arabinofuranosidase and α-L-rhamnosidase to the wine during MLF demonstrated that these enzymes are required for efficient hydrolysis of grape glycosides during MLF.

This work has provided important new information on the somewhat uncertain role of MLF in wine flavour development. This investigation, which requires confirmation with additional strains, suggests that the ability of ML bacteria to release glycosylated flavour precursor molecules is relatively restricted and probably confined to the ß-glucosides, unless yeast derived or extraneous glycosidases, such as rhamnosidases and arabinofuranosidases, are present.

Malolactic fermentation and wine flavour

Staff: Dr Paul Henschke, Dr Eveline Bartowsky, Dr Peter Costello, Holger Gockwiak, Jane McCarthy
Collaborators: Dr Leigh Francis, AWRI, and Dr Andrew Markides, Adelaide University

Sensory attributes associated with the malolactic fermentation of wine - diacetyl formation by commercial MLF bacteria in wine
The flavour compound, diacetyl, is a major contributor to the 'buttery' attribute of wine which has undergone malolactic fermentation (MLF). The ML bacterial strain can affect the diacetyl content of wine (Institute publications #546 and #637), however, limited comparative information on commercially available strains is available to winemakers. This project aims to systematically characterise these strains under similar conditions in a red and white wine (alcohol 12.5-13.0% v/v, malic acid 2.5 g/L, citric acid 0.5 g/L, pH 3.4, SO_2 negligible). The present report relates to diacetyl production by four ML bacteria cultures, Chr. Hansen Viniflora oenos, Lallemand EQ54, Lallemand OSU, and Condimenta/Lallemand Bitec/Viacell proVino, in a 2000 Chardonnay wine, the results pertaining to the red wine (1998 Cabernet Sauvignon) trial were summarised in the previous annual report.

Completion of MLF ranged 2 - 8 weeks for the four strains in the Chardonnay wine whereas the same four strains completed MLF within two weeks in the Cabernet Sauvignon wine, suggesting a nutrient limitation or an inhibitory metabolite may have selectively delayed the growth and fermentation activity of two of the strains in the Chardonnay wine. The peak diacetyl concentrations were lower and of greater range in the white wines (1-4.5 mg/L) compared with those obtained with the red wines (8-10 mg/L). As for the red wines (Figure 2), diacetyl peaked after the consumption of malic acid and when approx. 75% of the citric acid had been metabolised (Figure 2). After the complete removal of citric acid, diacetyl had fallen to less than 50% of the peak value.

These data agree with our bottled wine survey that red wines carry a higher diacetyl concentration than whites (Institute staff publication #546). These data also showed a correlation between peak diacetyl concentration and duration of MLF, although further work is needed to confirm this observation. The kinetic pattern of malic and citric acid degradation and diacetyl evolution was qualitatively similar for all strains in each wine.

In conclusion, these results suggest that although strains vary in ability to produce diacetyl, other factors, such as duration of MLF, and the stage of MLF at which the wine is stabilised (SO_2 addition or membrane filtration) appear to give relatively greater differences in diacetyl concentration. The choice of ML bacterial strain is important to the extent that growth response in a particular wine is variable, thereby significantly influencing the final diacetyl concentration.

Most importantly, these data confirm the result obtained previously with red wine that diacetyl is a transient metabolite and that the timing of wine stabilisation (e.g. sulfite addition) for controlling the desired level of diacetyl in wine is important.

Preliminary evaluation of a chemical sensor with wines having undergone MLF

A small project has been undertaken to evaluate the ability of a chemical sensor to determine the aroma profile of wines which had undergone MLF. The chemical sensor instrument, a Hewlett Packard model HP4440, located in the Department of HVO of Adelaide University, is a mass spectrometry based 'electronic nose' for the characterisation of wine volatiles, where the fragmentation patterns may provide a fingerprint of the volatile compounds present in the headspace of the wine sample. This instrument comprises an autosampler (capacity for 44 samples) and a mass sensor where the wine volatiles are ionised and fragments detected, and the abundance of ions

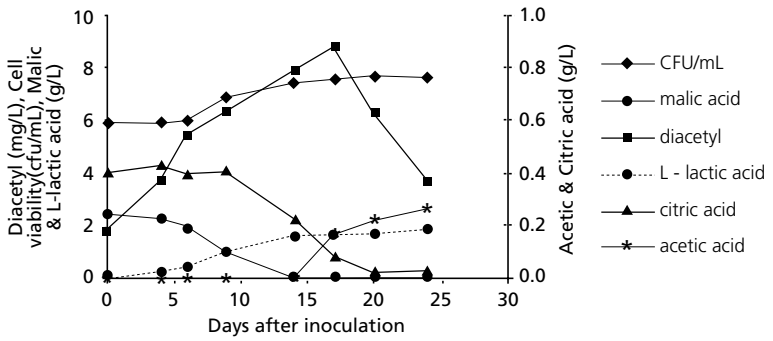


Figure 2. Relationship between diacetyl and organic acid metabolism by *Oenococcus oeni* in Cabernet Sauvignon wine

determined. The instrument has a major advantage over conventional instruments in that it can rapidly analyse many samples.

Two wine types (Chardonnay and Cabernet Sauvignon) from three different viticultural regions (Chardonnay – Eden Valley, Sunraysia and Padthaway; Cabernet Sauvignon – Eden Valley, Coonawarra and Swan Hill) were subjected to MLF with two commercial bacteria preparations (Viniflora oenos and Lalvin MCW). Malolactic fermentation was carried out at 20ºC in an oxygen-free atmosphere.

The chemical sensor could distinguish between both the red and white wines, and pre and post MLF wines, and in some cases was also able to distinguish the wines on the basis of origin, possibly on the basis of fermentation esters. There was evidence that the bacterial strain used to conduct the MLF could be distinguished. However, the chemical sensor was unable to distinguish wines on the basis of the flavour compound, diacetyl, due to the diacetyl ion fragments overlapping those of ethanol. Further work is required to establish the identity of the volatile compounds which allowed for the differentiation of wines pre and post MLF with a particular bacteria strain. To unequivocally assess the use of the chemical sensor for discriminating wines from different regions, further work would required, for example, standardised wine making of several examples of fruit from several viticultural regions.

This project was undertaken by Ms Helen McCarthy as an Honours project in the Department of Horiculture, Viticulture and Oenology, with supervision by Drs Eveline Bartowsky and Leigh Francis. Helen was awarded a first class honours degree, and commenced as Graduate Winemaker with Southcorp Wines at Karadoc in December 2000.

This work has provided an insight into the potential use of the chemical sensor as a means to analyse wines, and may prove useful as a quick method for the initial examination of wines for MLF character, and the technique could give information on the factors which allow the wines to be distinguished. However, this sensor cannot detect diacetyl, the main compound conferring the 'buttery' character, in the presence of alcohol.

Model studies on the effect of timing of O. oeni *inoculation on the induction of MLF*
Based on the chemically defined grape juice/wine medium reported on in the 1999 Annual Report, a trial has been undertaken to evaluate this medium for the ability to model aspects of the MLF. This investigation focused on the effect of timing of *O. oeni* inoculation on the initiation and duration of MLF and diacetyl production. A compatible yeast (Lalvin CY-3079) and ML bacteria strain (AWRI Lc5p) were chosen. The ML bacteria were inoculated at different stages during alcoholic fermentation (AF) (mid exponential yeast phase and at approximately 5 ºBrix sugar), immediate post AF and 8 weeks post AF, in the presence or absence of yeast, to examine the effect on MLF duration and the effect of yeast lees on diacetyl content.

It was found that the timing of *O. oeni* inoculation affected the initiation and duration of the MLF as reported by others for wines. MLF was found to initiate and complete more rapidly when *O. oeni* was inoculated towards the end of the stationary phase and during the decline phase of yeast growth, particularly if the yeast was removed before inoculation. When *O. oeni* was inoculated in the presence of fermenting yeast, the MLF was delayed until near the completion of the alcoholic fermentation. Insufficient diacetyl data have been obtained to date to show any clear association with inoculation for MLF and diacetyl content.

This project was undertaken by Ms Marie Pearce as an Honours project in the Department of Horticulture, Viticulture and Oenology, with supervision by Drs Eveline Bartowsky and Andrew Markides, The University of Adelaide. Marie was awarded a first class honours degree, and commenced as Graduate Winemaker with Southcorp Wines at Coonawarra in January 2001.

This work has demonstrated that the recently defined model grape juice medium can support yeast growth and completion of alcoholic fermentation and the model wine supports MLF and completion in a timely fashion which is reflective of a 'real' grape juice/wine. The model also reflected grape juice studies with respect to the effect of timing of inoculation with ML bacteria on the time course of MLF.

A test system for predicting the stimulately and inhibitory affects of wine yeast on malolactic bacteria
As stated previously, the aim of this project is to develop a test system for detecting stimulatory and inhibitory interactions between wine microorganisms, and to use this system to characterise the interaction between pairs of popular wine yeast and ML bacteria so that winemakers can choose the most appropriate combination for their wine process. Previously, we have described the growth response of one *Oenococcus oeni* strain to four fermentative yeast strains using the model test system. Because this test system uses a chemically defined medium to reproducibly simulate the important nutritional and chemical properties of grape juice, work is in progress to validate this test system with several different grape juices.

Juice prepared from hand-picked, disease free, undamaged grapes has been ameliorated to closely match the chemical composition of the model medium, with respect to sugar, acidity, nitrogen and so on. Wines have been produced from one juice with the test yeasts and the growth response of the test ML bacterium has been determined. Quantitatively, the growth response was lower in terms of biomass yields when compared with the model medium, but qualitatively similar results were obtained, indicating that the test system acts as a grape juice model, based on these results derived from a single juice. Following validation with the additional grape juices, the model system will be used to screen various combinations of popular commercial wine yeasts and ML bacteria.

Aspects of this work have recently been communicated to industry via i) Institute Roadshows to winemaker groups in South Australia and North-western Victoria during November/December 2000, ii) presentation to the Department of Horticulture, Viticulture and Oenology Grape and Wine Industry Outreach Seminar, University of Adelaide, November 2000, and iii) 'Can interactions between wine yeast and lactic acid bacteria be more predictable?' formed part of an article called *Clearer answers sought for old questions* which appeared in the Aust. N.Z. Wine Ind. J., Nov./Dec. 2000. This project was undertaken as a collaboration with Dr Andrew Markides of The University of Adelaide (GWRDC Project UA 92/3).

The model test system shows promise for the relatively rapid laboratory assessment of the inhibitory, neutral or stimulatory affect of wine yeasts on the growth and fermentation of a ML bacterial strain based on the results obtained with a single grape juice sample. Validation of the test system with additional juices is in progress.

Preliminary characterisation of an MLF stimulatory fraction from wine

Based on recent reports from the literature that a yeast mannoprotein fraction could be stimulatory for MLF, a small project was undertaken to investigate this hypothesis using a chemically defined grape juice medium to simplify analysis. Wines were made by fermentation with AWRI 838, an MLF stimulatory yeast, and AWRI 839, a yeast with neutral affect on MLF. The wines were clarified by filtration, and contained <5 mg/L total SO_2. Various factions of these wines were prepared with 30,000 and 3,000 molecular weight cut-off ultrafilter membranes. The effect of these fractions on the growth of a test strain of *O. oeni* has been assessed by supplementation of a synthetic wine medium.

Growth and malic acid degradation was greatest in the unfractionated wine, followed by the 3K eluate, 3K retentate, 30K retentate, and synthetic wine medium. This result suggested that the low molecular weight material was most stimulatory to bacterial growth, more so than the high molecular weight fraction, known to contain (manno)proteins. HPLC analysis of the fractions revealed that the yeast-derived protein content was highest in the unfractionated wine and the 30K retentate. With the exception of the unfractionated wine, the protein content of the fractions gave a negative relationship to bacterial growth and malolactic activity.

This study was undertaken by Ms Nina Viergutz as an Honours project in the Department of Horticulture, Viticulture and Oenology, with supervision by Drs Peter Costello and Andrew Markides, The University of Adelaide. Nina has been awarded an honours degree, and commenced as an Assistant Winemaker with Andrew Peace Wines, Victoria, in early February 2001.

These results suggest that stimulation of malolactic bacterial growth may be related more to yeast produced growth factors, such as vitamins, amino acids and peptides than to the macromolecular fraction. Our future work will aim to characterise which factors, if any, are responsible for MLF stimulation. Such knowledge could be used to manager malolactic fermentation better.

Microbiological analysis of industry technical problems

Staff: Dr Paul Henschke, Dr Eveline Bartowsky, Dr Peter Costello, Jeff Eglinton, Holger Gockowiak, Jane McCarthy, Dr Leigh Francis, Mark Gishen, Dr Michael Esler, Dr Bob Dambergs

In addition to investigating specific winery microbiological problems this project also provides resources for maintaining the Institute's culture collection which provides yeast and bacteria for teaching and research, performing advanced wine microbiological analysis, undertaking dried yeast quality testing, and providing microbiological consultation to industry personnel. A workshop on the identification and oenological characteristics of wine spoilage microorganisms is being planned for the 11th Australian Wine Industry Technical Conference in October 2001.

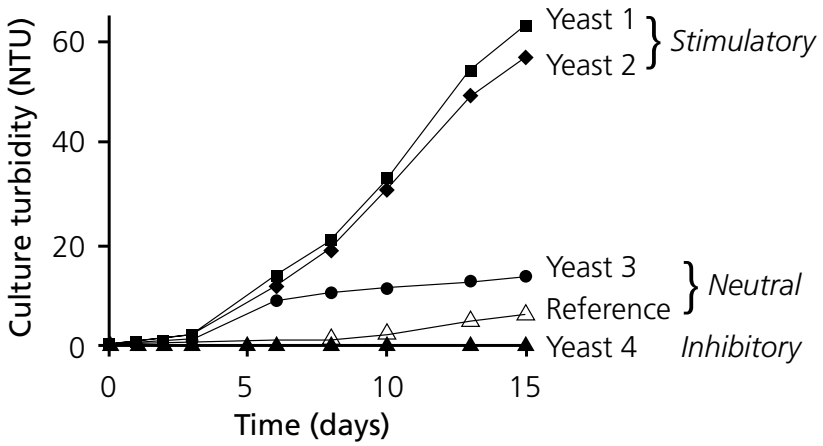


Figure 3. Growth of *Oenococcus oeni* in model wines made by fermentation with four different yeast strains and the unfermented reference model wine. Relative to bacterial growth in the model wine, yeast 1 and yeast 2 are designated to be stimulatory, yeast 3 neutral and yeast 4 inhibitory.

The suitability of Near Infrared Spectroscopy for measurement of yeast assimilable nitrogen content of grape juice

As reported previously, this project, which is being pursued in collaboration with GWRDC AWR 98/2, has the objective to develop and evaluate near infrared spectroscopy (NIRS) for estimating the yeast assimilable nitrogen content (YAN) of grape juice as measured by established reference chemical methods. The poor correlations previously observed between NIRS and chemical measures of YAN were thought to relate to low signal due to the low relative concentration of free amino acids in grape juice. Work on evaluating several techniques to enhance the weak NIRS signals has recommenced with Dr Bob Dambergs recently joining the Institute's NIRS project team.

Restarting stuck ferments

This subproject, detailed in previous annual reports, has been concluded with a communication to industry (Eglinton and Henschke [2001] Aust. N.Z. Wine Ind. J. 16: 77–81. Institute staff publication #657). This paper relates to the effect of acetic acid concentration on the ability to restart a stuck fermentation. Aspects of this work with flavour implications have been taken up in project GWRDC AWR 2. A *S. bayanus* Chardonnay ferment which stuck in the Hickinbotham Roseworthy Wine Science Laboratory was successfully restarted with a rescue culture of *S. bayanus*, prepared by the Institute's recommended method, indicating that yeast other than certain strains of *S. cerevisiae* can be used to restart stuck ferments under appropriate conditions. This wine, and others rescued by aeration/stirring or with *S. cerevisiae* are undergoing sensory evaluation to determine the effect of the rescue procedure on wine flavour.

Mousy off-flavour occurrence and formation in wine

Two manuscripts communicating the microbiology of mousy off-flavour formation in wine to industry have been published:

- Grbin and Henschke (2000) Mousy off-flavour production in grape juice and wine by *Dekkera* and *Brettanomyces* yeasts. Aust. J. Grape Wine Res. 6: 255–262 (Institute publication #650);
- Costello, Lee and Henschke (2001) Ability of lactic acid bacteria to produce N-heterocycles causing mousy off-flavour in wine. Aust. J. Grape Wine Res. (in press).

The main points of these papers are that all species of *Dekkera/Brettanomyces* yeast and many species of the heterofermentative lactic acid bacteria, including some strains of the wine malolactic bacterium O*enococcus oeni*, are capable of producing mousy off-flavour N-heterocyclic compounds under certain conditions. Aspects of *Brettanomyces/Dekkera* management in

winemaking in relation to mousy off-flavour and formation of volatile phenols was presented to the Southern Pinot Noir Workshop held in Methven, Canterbury, New Zealand, 18-21 January 2001.

Yeast interaction with red grape phenolics and effect on wine sensory properties

Staff: Dr Eveline Bartowsky, Simon Dillon, Dr Paul Henschke

A collaboration between the Institute and Lallemand has been established to investigate the interactions between yeast and grape phenolics during red wine fermentation.

Lallemand is contributing a substantial sum of money for our investigations, for which we are most appreciative.

Red grape must contains a variety of phenolic compounds which contribute to the colour, aroma, flavour, texture and stability of wine. The principal groups of phenolic compounds are the monomeric (flavanols, proanthocyanidins, anthocyanidins and flavonols) and polymeric phenolics (tannins). Fermentation yeasts are believed to interact with many of these compounds in a complex manner, involving physical, chemical and enzymatic mechanisms. These interactions may include: liberation of phenolics by chemical/enzymatic maceration of grape tissues; loss from wine by adsorption to the yeast cell wall; intracellular metabolism (uptake, enzymatic modification, excretion); extracellular metabolism (enzymatic); and production of chemically and physically reactive metabolites (such as, carbonyls and polysaccharides). Many of these reactions and their implications for wine properties are not well researched, and more specifically at the applied level, the specific properties of individual wine strains are not well characterised.

A laboratory scale fermentation procedure using replicated quantities of red grape must (0.25-1.0 kg) has been shown to give statistically reproducible fermentation profiles and wine chemical composition. Wine colour density measurements were also found to reproduce well.

Comparison of 17 commercial wine yeast for their ability to effect extraction and or retention of various grape phenolic compounds into wine was examined using triplicated 1 kg lots of Shiraz grapes (Clare Valley). The grape must was adjusted to pH 3.5, 25 °Brix soluble solids, 250 mg/L YAN and 25 mg/L SO_2, and fermentation was conducted at 25ºC with no protection from the air during cap plunging. All 17 yeast strains completed alcoholic fermentation within 23 days. On the basis of wine colour density analysis, the yeast strains were ranked into three groups which generally reflects industry observations, as reported by Lallemand.

Colour density comparisons of wines made with grapes obtained from three viticultural regions (Clare Valley, Langhorne Creek and Adelaide Hills) and fermented with six yeast strains showed a similar ranking for each yeast. One yeast consistently demonstrated an ability to allow the production of wines with low colour density whereas another consistently enabled the elaboration of wines with high colour density. These results suggest that yeast strains have a significant influence on the phenolic content of wines. Studies are currently in progress to better understand the nature of this observation with the view to make recommendations about the suitability of individual yeasts for specified fermentation outcomes.

Simon Dillon and Dr Eveline Bartowsky

Selection and improvement of wine yeasts by application of molecular biology

Staff: Dr Miguel de Barros Lopes, Dr Paul Henschke, Jeff Eglinton, Anthony Heinrich, Jenny Bellon and Professor Peter Høj

In the past few years a number of molecular genetic projects studying different aspects of wine yeast have been carried out, mostly within the program of the previous CRCV-1. These included studies on hydrogen sulfide production, killer yeasts, nitrogen utilisation, acetic acid formation and glycerol production. Many of these projects have been concluded, and the students researching these areas have recently completed their thesis. During the past year Chris Smyl received his PhD and is currently doing post-doctoral research on tannins at The University of Adelaide. Nicholas Yap also received his PhD and is presently employed in the Australian wine industry. Two of the projects, acetic acid formation and glycerol production, have been combined in order to successfully produce a yeast that accumulates less ethanol during fermentation.

Construction of a wine strain producing less ethanol

In previous annual reports a commercial wine yeast overexpressing the glycerol synthesis gene (*GPD2*) has been described. These yeasts (*GPD2*-OP) accumulate two to three fold more glycerol than non-modified strains during grape juice fermentation, and divert sugar away from ethanol production. These modified strains, however, also increase the acetic acid concentration to amounts generally unacceptable for winemaking (Institute publication #649). Several strategies have been tested to decrease the amount of acetic acid. The strategy that appears to be the most successful is the modification of a second gene *ALD6*, which encodes for an aldehyde dehydrogenase. Deletion of the *ALD6* gene (*ald6*Δ) in an otherwise wild-type strain decreased the amount of acetic acid more than three-fold, demonstrating that this is an effective strategy of decreasing acetic accumulation during fermentation. Importantly, the concentration of acetic acid produced

when the cells overexpress the *GPD2* gene was also diminished when the *ALD6* gene was deleted (from 1.42 g/L to 0.36 g/L). In fact, the accumulation of acetic acid with the strain modified at both genes (*GPD2*-OP / *ald6*Δ) was lower than that of a non-modified strain (0.66 g/L). The biosynthesis of glycerol remained elevated (increasing from 6.0 g/L to 16.3 g/L), with a simultaneous decrease in the concentration of ethanol (from 31.1 g/L to 27.3) (Note: these ferments were done with an initial sugar concentration of 80 g/L).

Gas chromatography/mass spectrometry (GC/MS) was used to demonstrate that *GPD2/ALD6* modification affected the biosynthesis of a number of secondary metabolites of fermentation, including acids, esters, aldehydes and higher alcohols, many of which are flavour-active. The concentration of acetaldehyde, acetoin and 2,3-butanediol are the compounds most affected by the increase in *GPD2* expression. Also, the concentration of a number of ethylidene glycerols (acetals of glycerol and acetaldehyde), that have been previously found in other wines that contain high concentrations of acetaldehyde such as sherry, increased more than ten-fold in a *GPD2* overproducing strain. The formation of secondary metabolites was influenced by the inclusion of oxygen, which also affected the growth rate, final cell number, and sugar consumption of the culture. The results indicate that modification of *GPD2* and *ALD6* expression represents an effective strategy to increase glycerol concentration and decrease ethanol and acetic acid concentrations in wine. These modifications alter the chemical composition of the wine such that, potentially, novel flavour diversity is possible. A double modification of a yeast by means of molecular biology gives a remarkable demonstration of the power of the technology. A paper describing some of this work has been published (M. de Barros Lopes, A.U. Rehman, H. Gockowiak, A.J. Heinrich, P. Langridge, P.A. Henschke. *Australian Journal of Grape and Wine Research*, 2000, Institute publication #649). A second manuscript describing the more recent work has been submitted for publication.

Use of non-molecular techniques for the improvement of wine strains

In order to produce novel wine yeasts with unique characteristics, hybrids between commercial wine strains and other species with winemaking potential are being made. Hybrids between strains of *S. cerevisiae* and other *Saccharomyces* species have been successful. These hybrids generally share properties of both parents, including ethanol tolerance and the ability to grow in media containing high levels of sugar from the wine yeast, and improved growth at low temperature from the second parent. A commercial wine yeast X *S. paradoxus* hybrid has been used in a winemaking trial. The hybrid fermented grape juice at the same rate as the commercial wine yeast parent, even though the *S. paradoxus* parent was unable to ferment grape juice at all. Results indicate that the genome of these hybrid yeasts is stable during fermentation. Chemical analysis demonstrates that the composition of the wine is changed when the hybrid yeast is used. For example, a significant increase in the glycerol concentration, without a corresponding increase in the acetic acid concentration, was seen in the wine made with the hybrid yeast. Further chemical studies and sensory analysis are necessary to evaluate the winemaking properties of the strain. It is also being tested if this hybrid yeast ferments more efficiently at low temperature, a characteristic derived from the non-*S. cerevisiae* parent. These results demonstrate that producing hybrids between *S. cerevisiae* wine strains and other species is a useful strategy for acquiring yeasts with novel winemaking characteristics from species that are less suited for grape juice fermentation. One of the main advantages of this method is that it is considered safe and not classified as genetic modification.

Jeff Eglinton, Dr Miguel de Barros Lopes and Alan Pollnitz

Compound	*GPD2 ALD6*	*GPD2-OP ALD6*	*GPD2 ald6Δ*	*GPD2-OP ald6Δ*
Glucose (g/L)[1]	3.3	4.25	10.95	2.9
Glycerol (g/L)[1]	5.1	13.4	6.0	16.3
Ethanol (g/L)[1]	34.1	26.0	31.1	27.3
Acetic acid (g/L)[1]	0.66	1.42	0.20	0.36

Table 2. Concentration of some metabolites for wild-type and modified strains

Identifying genes for wine yeast improvement

Saccharomyces cerevisiae is a model biological organism and the first eukaryote to have its chromosomal DNA completely sequenced. This has been followed by a systematic approach to study the function of each of its more than 6000 genes. The strains used in these studies are laboratory strains of *S. cerevisiae*, which have important physiological differences when compared to commercial wine strains. For example, phenotypic studies have confirmed that wine strains tolerate an increased sugar and alcohol concentration, and generally multiply more rapidly. Also, wine yeast produce secondary metabolites during fermentation that are positive for wine flavour. The gene and regulatory sequences that specify these key wine yeast properties are unknown. A DNA approach (amplified fragment length polymorphism - AFLP) has been used to identify genes that differ between wine and laboratory strains. These results demonstrate that the genome of commercial wine strains are quite different to those of laboratory strains with several genes duplicated and novel sequences present.

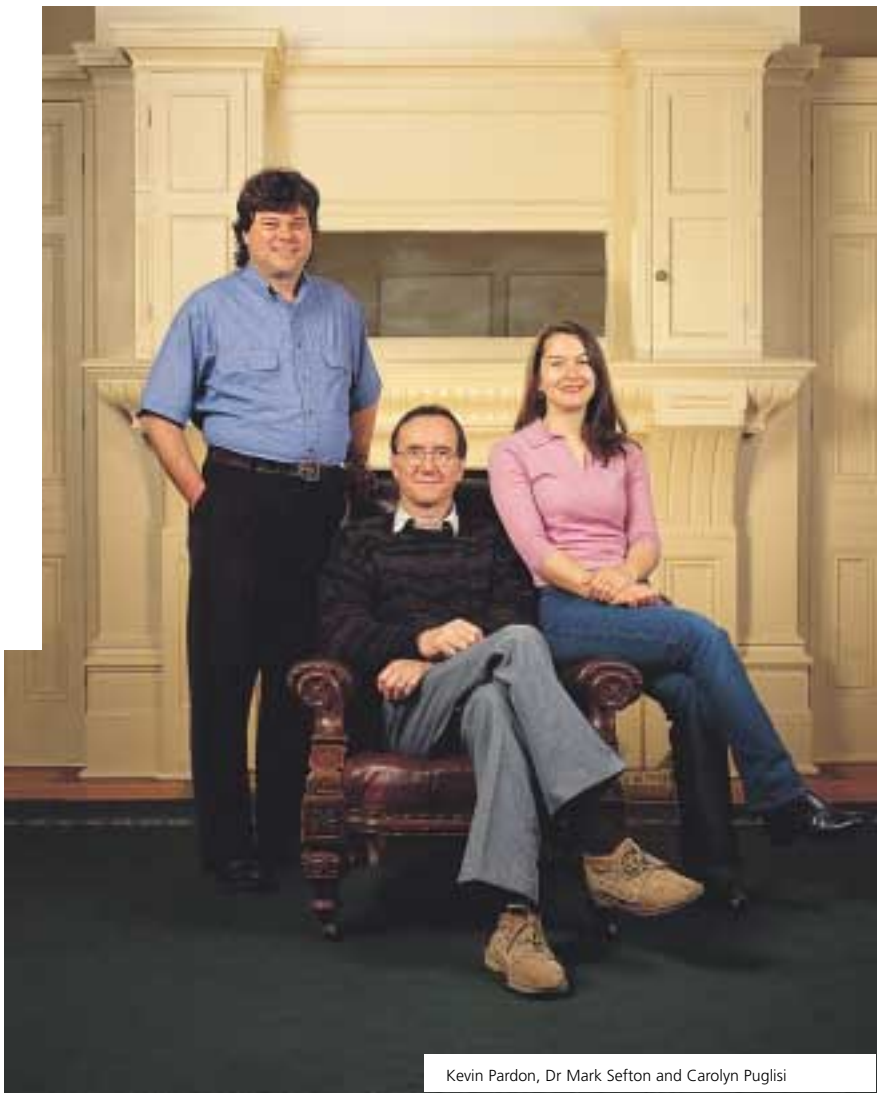
To further understand the mechanisms behind the phenotypic differences between yeasts, and hence to understand what makes a wine yeast perform well under different fermentation conditions, two-

dimensional gel electrophoresis (2D gels) has been employed. This system allows proteome comparisons of different yeast strains under different growth conditions. The wine and laboratory strains display distinct protein maps, which supports the AFLP results showing that there are important differences between the two types of strains. Initially, this technique is being used to identify proteins that may provide improved ethanol tolerance of commercial strains. The results show that the expression of several proteins are increased in response to ethanol stress. A subset of proteins also appear to be destabilised by ethanol. Proteins of interest are being isolated from the gel, digested with specific proteases, and the resulting peptides analysed by matrix-assisted laser ionisation/ desorption time-of-flight spectrometry (MALDI-TOF) in collaboration with Dr Graeme Currie at the University of Melbourne. The proteins can then be identified by comparison to databases that exist for the well studied laboratory yeast. Characterising proteins that are altered during wine fermentation will be beneficial for tracking wine fermentations, predicting strain attributes and selecting and producing new improved strains.

Grape composition and wine flavour

Staff: Dr Mark Sefton, Dr Leigh Francis, Yoji Hayasaka, Stella Kassara, Gayle Baldock, Professor Peter Høj, Wies Cynkar, Dr George Skouroumounis, Kevin Pardon, Dimitra Capone, Dr Markus Herderich, Dr Gordon Elsey, Carolyn Puglisi

This project has the long-term objective of understanding how the combination of viticultural and winemaking practices determines the aroma and flavour characteristics of wine. While some aroma and flavour compounds present in finished wine are derived unchanged from the grape, the majority are formed by biological and chemical processes from flavourless grape metabolites. To achieve the aims of this project thus requires not only an understanding of the compounds that are responsible for wine flavour but also the processes by which they are formed. Only then can viticultural practices be linked to flavour outcomes in finished wine.



Kevin Pardon, Dr Mark Sefton and Carolyn Puglisi

The program is divided into three broad areas: the identification of grape-derived wine components that affect wine aroma and flavour; studies of the formation and degradation of these wine flavour constituents including the identification of their grape-derived precursors; and, ultimately, studies on how viticultural variables affect the production of wine flavour compounds and their precursors in the grape. In order to achieve these aims, it is necessary to have available routine, sensitive and accurate analytical methods for determining the concentration of these grape and wine components in various matrices. The development of such methods is, and continues to be, a high priority of the program.

Black pepper aroma in Shiraz wines

Earlier work on this project had shown that the black pepper character can be tasted and scored in Shiraz grapes from the 1999 vintage. Analytical data had indicated that certain compounds, that are reported to be involved in the aroma of peppercorns, occur in higher concentration in the high-pepper Shiraz samples, than in the low-pepper Shiraz samples.

Further grape lots were obtained from vineyards identified from historical data as producing wines with peppery characteristics. Fruit from the 2000 season from four separate vineyards in Coonawarra, and two lots of grapes from a central Victorian vineyard, have been studied. Following informal tasting of the berry samples, only one lot of fruit (from the Central Victoria vineyard) was characterised as high in pepper flavour, and this plus a low pepper sample has been analysed by GC/MS. From data collected from the 1999 samples analysed, it was intended to confirm whether the compounds identified previously were also present in the 2000 season samples, and carry out more detailed investigations. The project is now on hold as a result of the key researcher, Ms Stella Kassara, having taken maternity leave and subsequently resigned. This project will resume once a suitable replacement for Stella is recruited. With the consent of the GWRDC, salary savings from this project were employed to further boost the Institute's NIR-research by securing the services of Dr Bob Dambergs.

Synthesis of potential wine flavour compounds formed from grape-derived glycosides

In the past, we have tentatively identified several compounds (four megastigmatrienones and four maleimides) in mild-acid hydrolysates of grape-derived glycosides. These mild-acid hydrolysates have been demonstrated, by formal sensory studies, to confer honey, lime and woody characteristics to base wines to which they have been added (Institute publication #420). Enzyme hydrolysates

Nicholas Brooks at 'Brookside': 1930
Photograph courtesy of The State Library of South Australia

GC/MS with tray samples

which do not confer these characteristics, do not contain these compounds. They are patented as flavour additives for other products, but are not commercially available. In order to assess the impact of the individual compounds on wine flavour it has been necessary to produce them by synthesis.

The four megastigmatrienones has been successfully prepared, while the synthesis of the four substituted maleimides is well advanced. Following some difficulties with the final step of the first two sequences that were attempted, an alternative route was successful, and two of the target compounds were obtained. These compounds will be prepared in sufficient quantities for sensory investigation. Progress towards the synthesis of the remaining target compounds, based on the successful strategy towards preparing the first two, is well advanced, and this task should be completed shortly. A sensory evaluation of all of these compounds will then be undertaken – to ascertain their importance for wine sensory characters. The compounds that are found to have very strong sensory characteristics at concentrations typically found in wine will be the target of future viticultural and oenological experimentation.

Precursors to damascenone

ß-Damascenone is one of the most important flavour compounds known to science; it is found in many fruit and vegetable products, including wine where it imparts 'stewed apple', 'fruity' and honey-like characters, and tonne quantities are produced commercially for the perfume and flavouring industries. Recent studies by us have indicated that ß-damascenone in wine results from the hydrolytic breakdown of complex grape-derived secondary metabolites formed from carotenoids such as neoxanthin (Institute publication #630).

Two synthetic sequences towards an acetylenic damascenone precursor were developed in parallel, and the target compound has now been obtained. Consequently, we have been able to identify this precursor as an intermediate in the conversion of a grape-derived norisoprenoid to damascenone in fruit, and a preliminary hydrolytic study in model wine has shown that this precursor gives damascenone in high yield. Once grape samples from this year's vintage have been processed, extracts of these samples will be examined to determine whether glycoconjugates of this precursor are components of ripe fruit, and whether it will be desireable to develop techniques for measuring these compounds in fruit as quality parameters. Further material is being prepared for detailed hydrolytic studies at room temperature. The more efficient of the two synthetic sequences is now being employed towards the synthesis of a glycosidic derivative of this acetylenic precursor. The reactivity of this glucoside at wine pH will then be determined.

Analysis of other wine flavour compounds and their grape-derived precursors

Precise, sensitive and relatively simple analytical methods, employing stable isotope-labelled internal standards, are continuing to be developed for quantifying known key grape-derived wine flavour compounds. These methods once completed will enable future research on viticultural and oenological controls of flavour formation, and will be added to over time with further compounds identified from the literature or from on-going research. Following the completion of stability studies of deuterium-labelled analogues, fully validated methods have been developed for measuring ß-damascenone (fruity and honey like character) α- and ß-ionone (berry, violets), isobutylmethoxypyrazine (capsicum/vegetative), nerol, geraniol and linalool (floral/citrus), 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN, kerosine character in aged Riesling wines) and cis-rose oxide (lychee character, particularly in Traminer wines). Deuterium-labelled ß-phenylethanol, which has a floral/rose-like aroma, and labelled ß-phenylethyl acetate (fruity) have been successfully prepared.

Two grape-derived precursors to the important wine aroma compound known as 'wine lactone,' which has woody and coconut-like aromas in concentrated form, have been synthesised on a large scale. These compounds will be studied to determine the rate at which they can be converted to wine lactone under typical bottle storage conditions. A parallel synthesis of deuterium-labelled analogues of these compounds has also been completed. The deuterium-labelled analogues will be used in future viticultural trials for measuring the variability in concentration of these precursors in grapes.

Ms Agnieszka Janusz from Flinders University has recently taken up an offer of a CRCV postgraduate scholarship to work on the identification of new wine flavour compounds formed from glycosides derived from Shiraz and Cabernet Sauvignon grapes. Ms Heather Smyth (ne Pain) has taken up an Institute postgraduate scholarship to study the relationship between wine composition and flavour in young Riesling and Chardonnay wines.

The influence of oak cooperage on wine composition

Staff: Dr Mark Sefton, Dr Alan Pollnitz, Kevin Pardon

Several experiments on shelf-life and extraction rates have been conducted with commercial sized French and American oak chips, and also with fine oak shavings. No significant change in amounts of extractable oak lactones, vanillin, guaiacol or 4-methylguaiacol were observed after six months storage of the commercial-sized chips in plastic bags.

Extraction of volatile oak components from finer shavings was faster than from chips over the first week of extraction, but the amount of volatile compounds extracted over a longer period (28 days) was similar. Soaking for this longer period is necessary to extract most of the flavour from commercial sized chips.

Earlier work in the Institute had shown that the presence of oxygen can influence the effects of heating on oakwood composition. Lignin breakdown by heat is more extensive when oxygen is present and this results in greater amounts of vanillin, guaiacol, and 4-methylguaiacol as well as *cis*- and *trans*-oak lactone being formed in the oak compared to when oak is heated in an inert atmosphere. This indicated that the composition of heated shavings, which have a high surface area to volume ratio, will be quite different to that of larger pieces of heated oak, a supposition supported by recent experiments.

When oak which had been chipped or shaved to different sizes was heated in air, the finer fragments (shavings) were richer in extractable volatile constituents than were the coarser fragments. The effect of heating the finer shavings in the absence, compared to the presence of air, was similar to that of heating coarser versus finer shavings. The effects of air and chip size on the composition of heated oak pieces was not the same for all compounds.

Thus the relative proportions as well as absolute amounts of oak volatiles was affected by these parameters, and smoky/toasty aromas are more likely to be predominant when smaller pieces of wood are heated, especially if heated in air.

As previously stated, when oak volatiles are totally extracted into a wine from oak shavings and the shavings are then removed, these volatiles can continue to be generated hydrolytically, presumably from precursor forms, also extracted from the oak samples. Further studies on exhaustively extracted oak shavings have shown that when these shavings

are resuspended in a fresh sample model wine, vanillin can continue to be formed over time. The formation of vanillin was greater when air was present. This demonstrates that oak contains non-extractable precursors of vanillin as well as precursors which can be extracted into wine. The accumulation of oak components in wine during barrel ageing is therefore not only a function of extraction rates but is also dependent on this hydrolytic generation. This new insight has implications for the potential development of 'oaky' characters in wine following bottling.

Precursors to oak lactone

Despite their sensory importance, the origin of *cis*- and *trans*-oak lactone remains unclear. These compounds are already present in green oakwood, but additional quantities of these compounds can be generated in the wood during the drying (seasoning) and coopering processes, in oak extracts heated to 50°C, and even in the injector block of a gas chromatograph during analysis of oak extracts. These observations indicate the presence of one or more precursor forms of oak lactone in oak. Several potential precursors have been observed. More than 20 years ago, scientists from the whisky industry isolated, from oak wood powder, a compound which they termed a 'precursor' to oak lactone. On the basis of rather limited degradation studies, they assigned a structure (a methylated gallate ester) to this compound. Despite the limited evidence presented, this compound as a precursor to oak lactone has gained credence through literature citation.

We, therefore, set out to synthesise this methylated gallate ester in an unambiguous fashion. Because of the possibility that the methylation on the aromatic ring might also have occurred as an artefact of the original isolation, the corresponding straight gallate ester was also synthesised. Monomethylgallic acid derivatives are relatively uncommon in nature, whereas gallate esters are major constituents of oak and other woods.

Both the gallic acid ester of *cis*-oak lactone and its monomethyl analogue have now been successfully synthesised. The work has shown that the monomethylgallate ester structure originally proposed for the oak lactone precursor isolated and studied 20 years ago and accepted in the literature thereafter is in fact in error. A French group, with whom we have established a collaboration, are using the sample of the straight gallate ester we have synthesised as a reference to determine whether this compound is an oak component and, therefore, potential oak lactone precursor. Understanding of the precursor pool in oak is an important prerequisite for optimal use of oak in cooperage and winemaking.

4-ethylphenol in red wines – implications for microbial problems

In previous staff publications (Institute publications #624, #627), we pointed out that a survey of Australian red wines indicated a relatively high concentration of 4-ethylphenol, a product of *Brettanomyces* activity, was found in some Australian red wines at concentrations above 500-600 mg/L. 4-ethylphenol can impart an undesirable 'medicinal'/ 'bandaid' character. Using our stable isotope dilution assay, our research group measured the concentration of 4-ethylphenol in wine stored in 44 American and 47 French new and used oak barrels from several suppliers. Wine stored in shaved and refired oak barrels contained up to 85% less 4-ethylphenol and 4-ethylguaiacol than wine stored in normal barrels of the same age that were not shaved. The concentration of 4-ethylphenol found in 61 bottled commercial Australian red wines of various ages ranged from 2 µg/L in a Merlot up to 2660 µg/L in a Shiraz, with a mean concentration of 795 µg/L. The highest level of 4-ethylphenol observed in a 'problem wine' submitted to the Institute was 8000 µg/L! Further analyses by our Analytical Service of more than 700 different samples submitted by industry, paints a very similar picture. It appears that wineries would be well advised to analyse their products for the presence of 4-ethylphenol in order to pinpoint any microbial problems that might occur, and to track the efficiency of corrective measures. Our experience shows that the 'problem' is widespread and that it can occur suddenly in wineries which previously have not had such problems.

Studies on unstable wine proteins involved in haze formation

Staff: Dr Elizabeth Waters, Dr Leigh Francis, Ken Pocock, Dr Miguel de Barros Lopes, Yoji Hayasaka, Shauna Brown, Professor Peter Høj

Previous work in this project focussed on characterising the proteins responsible for wine haze and the effect of irrigation and mechanical harvesting on the protein concentration of juice and has been communicated in many publications. The project has now entered its final stage of developing alternative methods to prevent haze formation and of exploiting the properties of the proteins to discriminate juices and wines varietally.

Use of mass spectrometry to differentiate varieties

Since subtle differences between the molecular weights of proteins are readily detectable by electrospray mass spectrometry, it is theoretically possible to identify cultivars by the protein profile of berries, must and wine. Such an identification technique would complement and extend the identification service currently available, since DNA fingerprinting is valid for berries and must only. This has been explored and led to a novel approach for varietal discrimination of fruits.

Proteins with a wide range of masses (13 to 33 kDa) were found in the juices of 19 different varieties of grape and were identified as mostly thaumatin-like proteins and chitinases. Small consistent differences in molecular masses were noted when otherwise identical proteins were compared from different varieties. These differences persisted through different harvest years and in fruit grown in different locations. Based on the definition of 4 different masses for thaumatin-like proteins and 12 different masses for the chitinases and using statistical analysis, the methods developed could be used for varietal differentiation of grapes on the basis of the protein composition of the juice. A refereed paper describing this work has been published (Institute publication #663). The next step is to determine whether the method can be applied to fined wines where the concentration of proteins is much lower.

Proteolytic enzymes and/or heat as alternatives to bentonite

As reported previously, work at the Institute and elsewhere has shown that juice and wine proteins are resistant to proteolytic attack at temperatures below 25°C. At higher temperatures, other groups have reported losses of proteins and reduced bentonite requirements for juices and wines treated with proteases. Nevertheless, the use of proteases combined with heat treatment is not widespread in industry, possibly due to the perception that heating under any conditions is detrimental to wine quality. This appears to be unfounded because previous work at the Institute (Institute publications #444, #462) showed that short time (2 or 10 minutes)/high temperature (~90°C) treatment had no clear sensory effect on wine.

Two unstable wines were processed with two different enzymes on industrial scale heat treatment equipment at the Highett facility of Food Science Australia. The enzymes were added to the wines immediately before heating at 90°C for 1 minute. The wines were rapidly cooled to 18°C after heating.

This combined heat and enzyme treatment reduced the protein levels in both wines to about 40% of the original levels (see an example in Figure 5). Heat treatment alone reduced the protein levels to about 75%. The bentonite fining requirement was reduced to approximately 35% in the combined heat and enzyme treated wines, compared to the heat alone treatment that reduced the requirement to approximately 60%, of the bentonite requirement for the untreated wines.

Dr Elizabeth Waters and Tracey Siebert

Importantly, and in line with previous Institute work, sensory evaluation of the wines have shown that the treatments resulting in reduced bentonite fining requirements had negligible effect on aroma and palate in the timeframes and storage period employed in this study.

The long-term stability of the treated wines looks promising. After six months:

- All wines subsequently fined with bentonite after heat and or enzyme treatment were stable after storage at typical cellar temperatures (15°C) and typical retail temperatures (25°C).
- Hazes were not detected in any wines after storage at 15°C.
- At 25°C hazes were only detected in untreated wines and wines treated with one of the enzymes. The heated wines and wines treated with the other enzyme have not given hazes so far even though these wines contain unstable protein.

These experiments were repeated in June 2001 with a Yarra Valley Sauvignon Blanc from the current vintage to test whether the protein reductions are reproducible and whether the sensory impact of the treatment is negligible for premium wines as well as for the commercial wines tested in the previous vintage.

Treatment of an unstable Sauvignon Blanc wine with and without enzymes at 45°C for longer time periods has also been assessed. Unlike short term high temperature treatment, this process has been shown by others to have a significant sensory impact. After seven hours (the first sampling time), the combined heat (45°C) and enzyme treatment reduced the protein level to about 30% of the original levels. Heat treatment alone reduced the protein levels to about 80%. After two days, the combined treatment had reduced the protein levels to 10% with the heat alone reducing the level to 70% of the original. With one enzyme there was no protein left after treatment for 14 days. The sensory impact of this treatment is not yet known.

Our results show that for some wines, it is, in principle, possible to reduce bentonite fining requirements drastically, apparently without gross short-term sensory impacts.

A poster describing these experiments will be presented at 11AWITC to communicate these results to industry.

Haze protective mannoproteins

Haze protective mannoproteins or factors (HPFs) were discovered at the Institute in 1991 and are yeast cell wall mannoproteins present in wine (Institute publications #439, 443, 460, 464, and 471). HPFs have the potential to prevent haze formation. Previous work has examined methods to extract HPFs from yeast cells or fermentation media, and two manuscripts describing this work have been published in this reporting period (Institute publications #624 and #639). Due to low yields these appear not to be commercially viable. The project is now focused on producing yeast strains that over express HPFs.

HPFs have the potential to prevent haze formation through their ability to change the particle size of haze. In order to understand mechanism better a bid to augment the HPF project through collaboration within the CRC for Bioproducts was mounted and has been successful. Dr Filomena Pettolino was appointed as a postdoctoral fellow to work with Professor Tony Bacic (University of Melbourne) on the physiochemical basis for HPF action. Apart from the Institute's in-kind contribution the funds to employ Dr Pettolino are derived from CRC for Bioproducts and Dr Bacic's group is of outstanding quality.

Vanessa Stockdale submitted her PhD thesis in April 2000 and qualified for the award of the degree of Doctor of Philosophy in November 2001. The thesis will not be

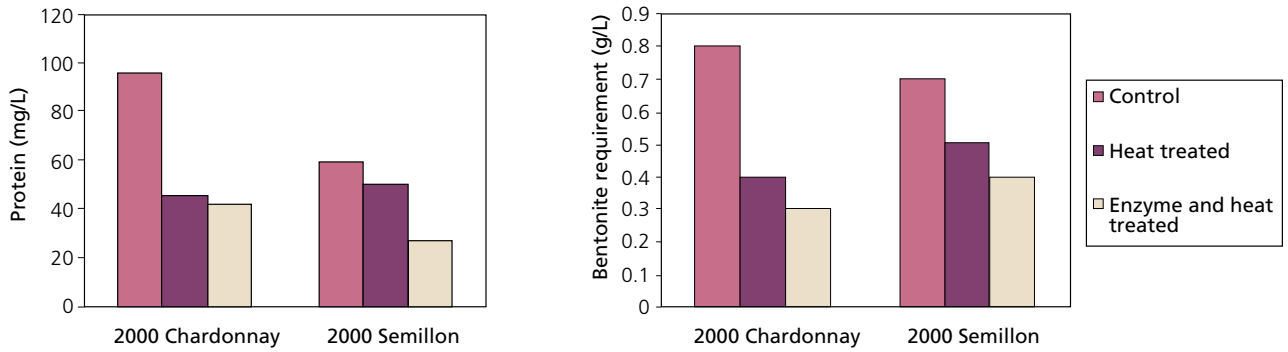


Figure 4. The effect of heat and or enzyme addition on the concentration of protein and the bentonite required to stabilize a Chardonnay and Semillon wine.

available in the public domain until the IP regarding the identity of the HPF genes has been protected.

Shauna Brown has cloned the yeast HPF genes (HPF1, HPF1' and HPF2) and successfully deleted a single copy of all three in haploid and diploid lab yeast. Shauna is now assessing the relative importance of each gene for the production of HPF activity. Preliminary phenotypic analyses have suggested that the HPF mannoproteins may have some impact on the ability of yeast to tolerate certain fermentation stresses. If this survives closer scrutiny, this is an unexpected bonus from the HPF project and illustrates the serendipitous nature of research.

Studies on random oxidation of bottled wines

Staff: Dr Elizabeth Waters, Dr George Skouroumounis, Dr Mark Sefton, Dr Leigh Francis, Dr Zhong Kui Peng, Mariola Kwiatkowski

Random post bottling oxidation shows itself in white wines as an obvious browning in a proportion of the bottled wine typically after six to 18 months' storage, and is accompanied by loss of SO_2 and ascorbate as well as the development of oxidised flavour. The estimated costs to the industry is $160 million per year in spoiled wine. We have worked in this area since 1995 and, excluding oxygenation of wine due to poor bottling procedures, have identified variable oxygen permeability of the closure as a major cause of the problem. It is possible that other winemaking techniques such as use of ascorbic acid, and upright storage, contribute to the manifestation of random oxidation (see e.g. Institute publications #577 and #595). This aspect and also an understanding of why closures are permeable have been the focus of the project during the past 12 months.

The effect of ascorbic acid, bottle position and wine type on oxidation

A Riesling and a wooded Chardonnay wine, for an experiment undertaken with assistance from Southcorp Wines to evaluate the effect of ascorbic acid, bottle position and wine type on the extent of oxidation, were bottled in late August 1999. The wines are stored under relatively stable temperature and humidity conditions in the Hickinbotham Roseworthy Wine Science Laboratory (HRWSL, annual temperature range from 16.9 to 22.1°C and humidity range from 45 to 84%). Browning measurements on at least 30 replicate bottles for each treatment have been taken throughout their storage period and will continue for another 6 months at least. Other analyses have and will be done at selected times. After 18 months storage of the bottled wines at HWSL the following conclusions were drawn:

- The phenomenon of random oxidation was not yet evident.
- Storage position (upright versus inverted) had generally no impact on the level of browning in either wine type under the stable temperature and humidity storage conditions used in this experiment. For one of the natural cork closures there was greater browning if bottles were stored upright. The difference in browning between bottles stored upright or inverted was small (maximum of 0.006 at 420 nm after 18 months) and likely to be undetectable by eye. In the inverted storage position, this closure is showing considerable seepage. It is possible that the higher moisture levels in the cork and/or in the cork/bottle interface in the inverted storage position relative to the upright position is contributing to these closures in the inverted storage position being a more effective barrier to oxygen.
- For both wines, and at all analysis times, there was greater browning if ascorbic acid had been added to the wine (see an example in Figure 6). The difference in browning between wines with and without ascorbic acid was small (maximum of 0.014 AU at 420 nm after 18 months) and was expected to be difficult to pick by eye. This is to be confirmed.

Figure 6. View of the top of the natural cork 'window' bottles. Araldite was applied to the top of the bottle to cover the closure bottle interface. A circle of approximately 30% of the original cork surface area was left exposed in the centre of the closure.

A much smaller and incomplete set of bottles is stored in an office at AWRI. The temperature is controlled at 23°C during business hours but uncontrolled at other times, and there is no humidity control. These wines are consistently browner than the equivalent wines stored at HRWSL (for example see Figure 6). Similar to the wines in HRWSL, the difference in browning between wines with and without ascorbic acid was small (maximum of 0.017 AU at 420 nm after 18 months). The relative importance of discussed variation and absolute temperature to the enhanced browning of the office-stored wine, is yet to be elucidated. It should be noted that the brown colour in the office-stored wine would now be judged too intense by most individuals (Institute publication #577).

In summary, this trial has demonstrated that

- Upright or inverted storage position had only a small or little impact on wine oxidation for either wine style when bottles are stored under 'best practice' conditions (humidity and temperature control) for 18 months.
- Addition of ascorbic acid to both wine styles increased the level of browning.

The sensory impact of ascorbic acid addition after two years storage will be determined in August 2001. Immediately following bottling and after six months storage there was no discernible difference in nose or palate attributes for wines with and without ascorbic acid addition.

It has not been possible to determine the impact of ascorbic acid addition, wine style and storage position on the incidence of

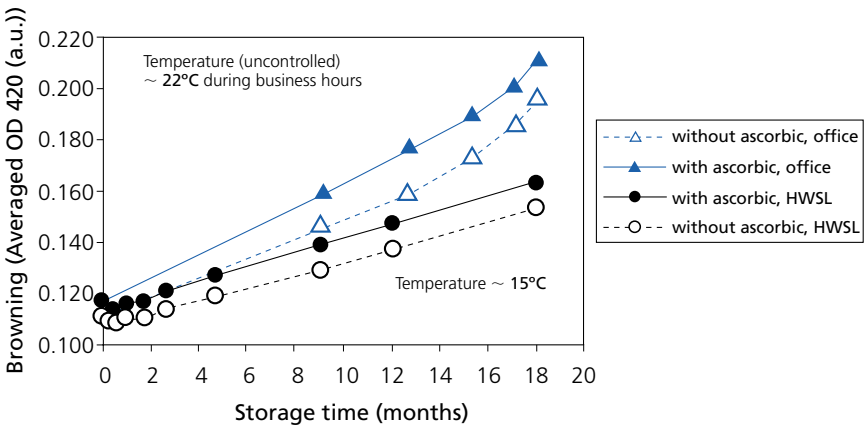
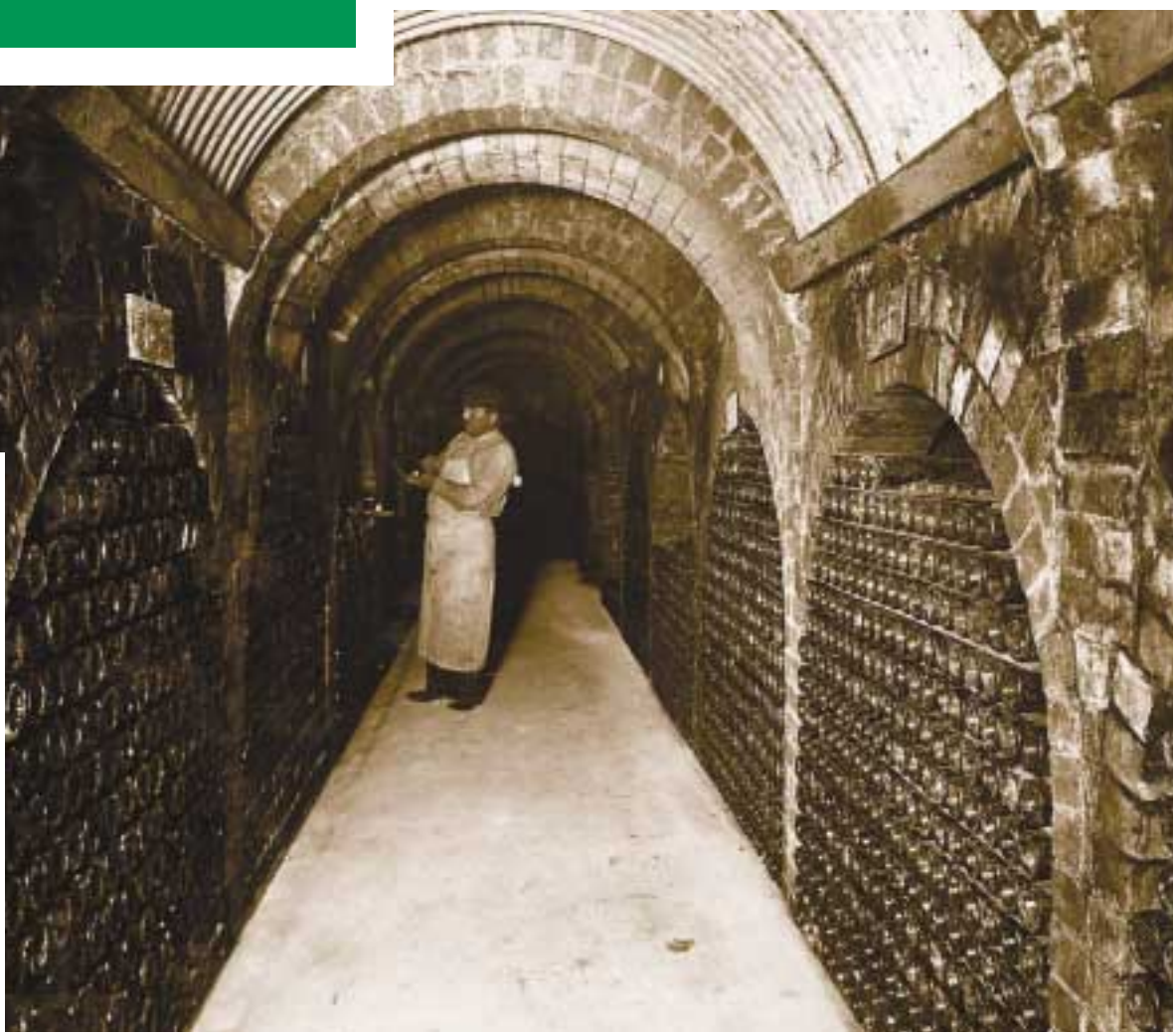
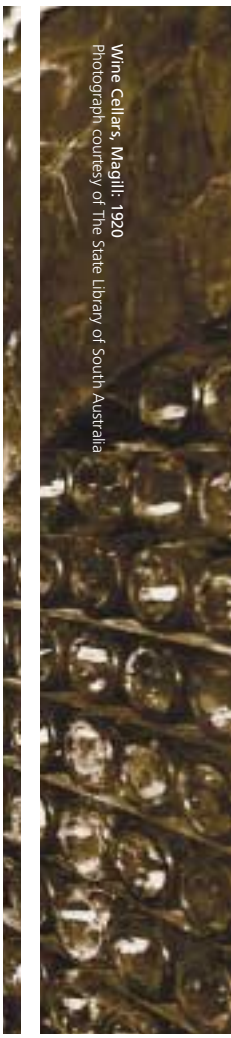


Figure 5. The effect of ascorbic acid addition and storage location on the browning of a Wooded Chardonnay wine sealed with a synthetic closure.

TSQ Mass spectrometer





Wine Cellars, Magill: 1920
Photograph courtesy of The State Library of South Australia

random oxidation because random oxidation, as judged by browning, has not been evident in this trial.

The reader should note that the amount of ascorbic acid added at bottling to these wines was relatively low (60 ppm) and the concentration of SO_2 relatively high (29 ppm). This is particularly important to bear in mind when comparisons to earlier experiments are made. In these studies (e.g. Institute publication #595) the most dramatic pro-oxidative effects of ascorbic acid were seen at 200ppm addition levels with low concentrations of SO_2.

The inherent permeability of corks to oxygen

As discussed previously, a large effort has gone into developing a method that would allow us to reliably measure permeability of oxygen through closures in wine bottles, in a reasonable time. A new water-soluble trap of singlet oxygen has been synthesised by Kevin Pardon under the supervision of Dr George Skouroumounis. This compound should be appropriate to measure oxygen permeating through closures into model wine. George Skouroumounis and Kevin Pardon have improved the published synthetic method and currently have more than one gram of the material. Due to delays in delivery of equipment, no further progress has been made on assessing the compound in this period.

A small subset of wines from the bottling trial described above have been used in a pilot experiment to determine whether the closure or the closure glass interface is the main route for oxygen permeation. Araldite, a material well known for its oxygen impermeability, was applied to the tops of bottles over the glass and the closure to either completely cover the closures of two replicate bottles, or to partially cover the closure so that a 'window' remained in the centre of the closure for four replicate bottles (Figure 7). The amount of closure surface exposed in these 'windows' was between 20% and 30% of the control closures and was in the centre of the closure. These samples have been stored at HRWSL for nine months. In general the highest level of browning was shown by the uncovered controls and the lowest level by the covered samples (Figure 8). These results are further evidence that wine oxidation is at least partially due to oxygen permeation through closures.

In addition these data suggest that coverage of the tops of the bottles and closures with an easily removed material with good oxygen barrier properties could reduce wine oxidation. The browning of wines in bottles with closures with the 'windows' was similar to that in the bottles with the fully covered closures for the natural closures. For the synthetic closures, browning in bottles with a window was higher than that shown by wines in bottles with the fully covered closures (Figure 8). Data interpretation must be made with caution due to the small sample size, however, the results suggest that the relative importance of distinct routes for oxygen permeation into wine might differ between natural corks and synthetics. These conclusions need to be confirmed with a larger sample set in a custom designed experiment.

Chemical analysis of industry technical problems

Staff: Dr Mark Sefton, Yoji Hayasaka, Dr Alan Pollnitz, Gayle Baldock, Dimitra Capone

Problem solving work carried out by the group is carried out in collaboration with, and under the direction of the Industry Services section. Accordingly, the results of this work are given in the report by Industry Services as are extensive studies into the adsorption of flavour active constituents by bag in box material.

A breakdown of industry technical problems investigated by the group over the past 12 years has been carried out in order to prioritise targets for the development of new analytical methods for wine taints and other contaminants. Nearly half of all such problems are associated with chloroanisoles, and these are now dealt with using routine analytical methods developed earlier by the group for both problem-solving and research purposes.

Other than chloroanisoles, the most common contaminants identified in wine are simple aromatic compounds, accounting for approximately one quarter of the problems investigated. These are mainly associated with contamination caused by paints, resins used for tank lining, and some instances of oil contamination. Rapid and accurate analytical methods using solid phase microextraction techniques are therefore being developed for these aromatic compounds, which include styrene, ethylbenzene, isomers of xylene, trimethylbenzene, tetramethylbenzene and diethylbenzene, as well as benzyl alcohol, benzaldehyde and isomeric monomethyl- and dimethylnaphthalenes. Once these methods have been fully validated, the group will be able to conduct a survey of local wines to determine the 'background' level of these compounds in wine.

General activities of the Industry Services team

Staff: Peter Godden, Mark Gishen, Adrian Coulter, Peter Valente, Ella Robinson, Greg Ruediger, Heather Donnell

The continuing growth of the Australian wine industry is reflected in the level of demand for the services provided by the Institute's Industry Services team (Tables 3 and 4). However, following several years of rapid growth, the level of demand has stabilised to some extent over the last twelve months, albeit at what could be considered as historically high levels. The Institute is convinced that the provision of these services add value and a competitive edge to the industry, but also recognise that maintenance of a quality

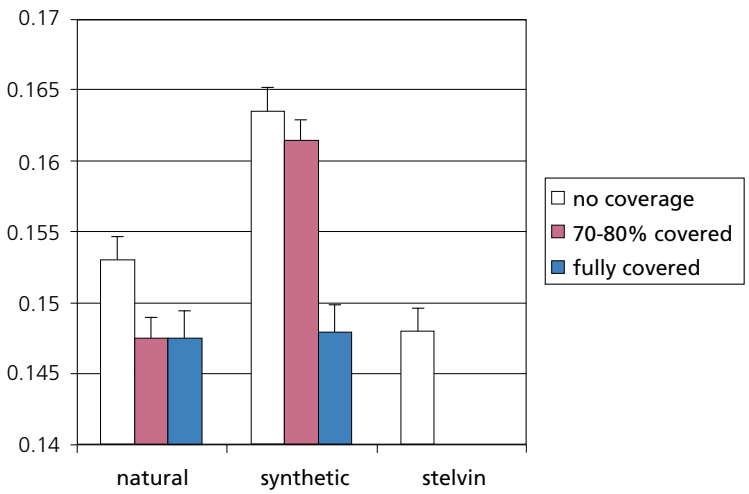


Figure 7. The effect of Araldite coverage of two closure types on the browning of wooded Chardonnay wines containing ascorbic acid after 18 months storage at HRWSL. Closures were covered with Araldite after nine months initial storage. Half of the bottles from each coverage and closure type were stored upright, the remainder were stored inverted. Data from both storage positions were pooled. Sample size of no coverage for natural and synthetic closures was 60 and for the Stelvin closure was 12, sample size of 70-80% covered for naturals and synthetic closures was 8, and sample size of fully covered closures for both natural and synthetic closures was 4.

service will require increased resources, in order to maintain its value and responsiveness, and therefore relevance to the industry and its development of human capital. In addition to the direct services provided to winemakers on an individual basis, the Industry Services team is conducting several projects as described below, and also contribute to many other Institute projects described elsewhere in this report.

Technical problem solving and consulting

Staff: Peter Godden, Adrian Coulter, Peter Valente, Ella Robinson

The provision of technical support service to the Australian wine industry, primarily in the form of an advisory service which disseminates a wide range of technical information, and a problem solving and analysis service, represents a significant proportion of the workload of the Industry Services team. In addition, the team manages a major research trial that is examining the technical performance of various types of wine closure, and a project which is developing a web-based technical reference manual for the Australian wine industry, with associated workshops which are presented in conjunction with Institute Roadshow seminars. During the year, the first sections of this web-based manual went live, providing levy paying wineries with a comprehensive trouble shooting and diagnostic guide for wine instability problems (www.awri.com.au).

The Industry Services Laboratory analyses several hundred samples each year (Table 3), using a wide range of routine and sophisticated analytical techniques. The majority of samples are wine, the analysis of which is supplemented by detailed sensory evaluation by a panel of experienced tasters. Of the remaining samples analysed the majority consist of wine additives, closures, or compounds which are suspected to have caused taints and or deposits in wine. The aim of the service is to offer remedial and preventative advice based on the cumulative problem solving experience of the staff, and the practical winemaking experience of the team Manager, Oenologist and other staff members, rather than providing a simple diagnosis of the cause of the problem. Increasingly, staff regard their role as educational, seeking to disseminate information in a variety of ways in order to prevent the recurrence of particular types of problem. The Industry Services team also provides technical support to the Institute's Analytical Service, particularly in the maintenance and auditing of the quality management system, contract trial-winemaking services, and the interpretation of analytical results. The Analytical Service also supplies chemical analysis on problem solving and research samples to the Industry Services team, on a contractual basis.

Confidentiality is an important aspect of the services provided, and is strictly maintained at all times. Assisted by lawyers, strict Terms and Conditions for the provision of problem solving services have been developed during the year, which reinforces the confidentiality of the work performed. This facilitates a frank exchange of information between the Institute and its clients, which in turn allows the maximisation of the knowledge gained from the provision of these services. When a particular problem is considered to be of interest to the wider industry, the results of investigative work are made available through relevant publications. The Industry Services team has contributed six Technical Notes and six other articles to *Technical Review* during the past year. When preparing material for publication, great care is taken to ensure that, under no circumstances is the name of the client, or any possible identifying reference, revealed.

As outlined in the previous Annual Report, sensory evaluation is an important analytical and research tool, and is commonly used in problem solving investigations, and commercial work conducted by the Analytical Service. The quantity of sensory analysis performed has increased substantially during the last year, and the team manager and the Institute's sensory chemist have continued to develop protocols for the training and assessment of an enlarged sensory evaluation panel. A summary of the number and type of investigations conducted by the Industry Services team over the past three financial years is presented in Table 3. The manner in which problem solving investigations are reported has been changed from previous years, in order to more accurately reflect the nature of investigations conducted, and the workload of Industry Services staff. The total number of samples analysed increased by 33% from the previous year, while the number of investigations (a figure not previously reported) actually declined slightly.

The number of samples which were found to exhibit the combined and related problems of hazes and deposits and microbiological instabilities is disappointing, and as with previous years, the number of these wines that have a high pH, a low concentration of sulfur dioxide, and in some cases incomplete primary or malolactic fermentations, remains unacceptably high. The issues related to these wine instability problems are the first to have been addressed by GWRDC-funded project, *Targeted training of wine industry personnel: Compilation of a technical reference manual and delivery of complementary workshops*, which is discussed in more detail later in this report. In addition, the issues were also discussed in an article written by the team manager in December 2000: *Persistent wine instability issues* in the *Australian Grapegrower & Winemaker* (Institute publication #645), a seminar paper presented in Melbourne in July 2000, and lectures given to Oenology students at the University of Adelaide in October 2000 and May 2001.

The types of investigations recorded in Table 3 as 'other investigative analyses' are varied, and some particularly interesting and unusual cases have been investigated this year.

- two separate investigations (one conducted for an overseas company on a fee-for-service basis) concluded that the deposits in bottled white wines consisted of a protein-phenolic complex, the phenolic material having originated from the corks and the protein from the wines. In both cases the deposit had formed at the cork/wine interface. It should be noted that it would be considered as quite usual for phenolic compounds to be

	Samples received (98/99 and 99/00) and investigations conducted (00/01)[a]		
	1998/99	1999/00	2000/01
Identification of hazes and deposits	79	114	135
Microbiological investigations	48	85	50
Sensory assessments	52	29	51
Taint problems	117	186	66[b]
Other investigative analyses	245	252	128
Closure-related investigations	154	122	24[b]
Total	**695**	**788**	**454[c]**

[a]the manner in which sample numbers have been calculated for Table 3 has changed from previous reports. Some investigations require the analysis of multiple samples, while, in some situations, one investigation is conducted on several different wines from the same winery which all exhibit the same problem, and all become the subject of a single report. Therefore, in future, the actual number of investigations conducted will be listed in the body of the Table, while the number of samples analysed will be provided as a footnote.
[b]much of the decrease in the number of 'taint problems' and 'closure related investigations', many of which involve the analysis of multiple samples, can be attributed to a portion of this work now being conducted by the Institute's Analytical Service on a commercial contractual basis.
[c]a total of 1048 separate wine samples were analysed as part of these investigations

Table 3. Summary of the number and type of problem solving investigations conducted by Industry Services during the past three years

extracted from corks into wine, and therefore the issue lies with the need for winemakers to take this factor into account when protein stabilising their wines. Some older winemaking texts advocate the addition of 'tannic acid' when conducting heat stability tests, presumably to simulate the conditions in which wine may be placed once it comes into contact with corks after bottling.

- the previous Annual Report stated that three cases of oxidative pinking had been investigated, two of which were in bottled wine. In each case, the wineries concerned had not previously experienced a pinking problem in wines made from fruit from the same vineyards, as those that were being investigated. It was ascertained that, in each case, the only change in winemaking procedures that had occurred was the reduction or elimination of the use of ascorbic acid. The incidence of this problem increased during the remainder of 2000, with a total of ten such cases being reported, equal to the total number of cases investigated during the previous nine years. In seven of these cases the wineries indicated that they had reduced or eliminated the use of ascorbic acid in the vintage in which the wines were made. While only two cases have been reported in the first half of 2001, previous experience suggests that oxidative pinking is a seasonal (and perhaps vineyard specific) problem and is more likely to be reported in bottled white wines from the 2001 vintage in the second half of the year. Winemakers are, therefore, encouraged to note when bottling their wines, that the conclusions to be drawn from the Institute's previous research into ascorbic acid use (Institute publications #577 and #595), are not that the use of ascorbic acid necessarily needs to be eliminated. Rather, everything else being equal, the use of ascorbic acid requires a higher concentration of SO_2 to be present in the wine, if accelerated long-term browning is to be avoided.

- the previous Annual Report also described a Riesling wine that was found to contain a concentration of 4-ethylphenol, the major spoilage compound formed by *Brettanomyces* yeast, of 2050 micrograms per litre (parts per billion). This was considered an unusually high concentration, especially in a white wine, based on previous work conducted at the Institute (Institute publication #623). This type of spoilage is poorly documented in white wine, and is almost exclusively associated with red wines. During the last year the same compound has been found in a batch of white, bottle fermented sparkling wine, the concentration in most samples analysed being between 350 and 400 micrograms per litre, with the highest concentration being approximately 600 micrograms per litre. These concentrations would almost certainly be above the sensory detection threshold for this wine type.

- the number of wines that are found to be contaminated with unintentional substances that are not permitted additives to wine is of concern. During the first six moths of 2001, nine cases of refrigerant brine contamination and six of hydraulic oil contamination have been investigated. Once again, the issues related to the minimisation of the risks of this type of contamination are addressed in the workshops described above, and at least two of the wineries affected by the recent cases had personnel who attended the workshops held in November and December 2000. These wineries have since reported that they have now implemented the advice given in the workshops, and that if they had taken this action sooner, then their particular cases of contamination would not have eventuated. As discussed below, a new gas chromatographic analytical method for the identification and quantification in wine of the concentration of propylene glycol, a common coolant used in winery refrigeration systems was developed during the year, to aid these investigations.

- it is pleasing to note that the number of stuck fermentations reported to the Institute has declined dramatically over recent years. However, stuck or sluggish fermentation is still considered an important issue in the industry, and it is interesting to note that five out of seven regions chose this as a topic for discussion at the Roadshow seminars presented during November and December 2000. This was in spite of the fact that similar presentations had been made at the previous two seminars to be held in each of these regions.

  The first case of stuck fermentation reported in 2001 was particularly interesting. A Shiraz fermentation had virtually stopped after approximately 80 grams of sugar had been fermented, and the must at that stage contained a concentration of 1.91 g/L of volatile acidity (VA) expressed as acetic acid. Analysis of the wine further revealed the presence of numerous, viable, catalase positive, rod-shaped bacteria. The cell morphology and catalase activity of the bacteria was consistent with the behaviour of acetic acid bacteria, but it is difficult to distinguish between *gluconobacter* sp. and *acetobacter* sp. However, since *acetobacter* are more tolerant of ethanol than *gluconobacter*, and since *gluconobacter* are normally isolated from grapes and must, have a preference for sugar and appear to be intolerant of the concentration of ethanol present in the wine in question, it is likely that the bacteria isolated from the wine were *acetobacter* sp. It is further possible that that both *gluconobacter* and *acetobacter* species were present on the grapes, and that *gluconobacter* predominated early in the fermentation causing the high VA, and then *acetobacter* became dominant

- another case of high VA investigated during the year was also of interest, and a Technical Note based on the investigation was published in the June 2001 issue of *Technical Review* (issue #132). This case involved a Shiraz wine, one barrel of 25 of which had developed a concentration of VA above 3 g/L. The wines in ten other barrels that were sampled all contained a concentration of approximately 0.6 g/L. The barrels in which the wine was stored were predominantly new, and when the wine had last been racked out of barrel and blended, approximately four months earlier, the winemaker had not noted an unusually high concentration of VA. Numerous viable acetic acid bacteria, the bacteria most usually associated with VA production in wine, were isolated from each of the eleven barrels which were sampled. Measurements of stave thickness, the 'ovality' of the bunghole and the degree of ullage in each barrel were also made, and the degree of taper of each bunghole was also noted, but not measured. It was found that the problem barrel differed from the others inspected only by the ovality of the bunghole, which was also noted to exhibit the greatest taper. A small crack in the bung-stave on

	1998/99	1999/00	2000/01
Wineries	980	1152	1155
Government organisations	70	99	117
Other	419	413	359
Students	35	36	26
Total	**1504**	**1700**	**1657**

Table 4. Enquiries received by advisory staff during the past three years

one side of the bunghole was also noted, but the ullage in the barrel was less than that in many of the other barrels. It was, therefore, speculated that the combination of imperfections of the bunghole, and the crack in the bung-stave, had allowed a greater concentration of oxygen to enter this barrel, and thus allow the acetic acid bacteria to become more active. Importantly, the imperfections in the barrel would not have been identified by casual inspection.

- three cases of cork-boring insects were investigated during the year, bringing the total number of cases of this problem to four in the last eighteen months. In each case the insects were found to be infesting the corks in bottled wines that were being stored without capsules or labels, in bins, in what could be considered as standard warehousing conditions. In one case, a brand new purpose-built warehouse was involved. The four cases involved three different insect species, which were identified with the assistance of CSIRO Entomology.

- an investigation was conducted for a winery which produces sweet wines from grapes infected with the mould *Botrytis cinerea*, in order to ascertain if precipitates that tended to appear in the bottled wines over time, and which were thought to consist of calcium tartrate, could be related to the concentration of calcium in the wines. If this had been found to be the case, then those batches of wine with high calcium levels and thus a greater propensity to form a deposit could be identified, and processed in a way to reduce the risk of deposit formation. A 1993 Semillon wine made from botrytised Semillon grapes was analysed as part of this investigation. The wine contained a large amount of a white deposit that appeared to consist of crystals, but existed in clumps rather than the more usual crystalline shape of salts such as calcium tartrate or potassium bitartrate. After isolation, microscopy revealed the deposit did consist of crystals with a rhombic-like morphology, and the crystals left a white-coloured residue when ignited in the flame of a Bunsen burner, indicating the presence of calcium. However, the infra-red (IR) spectrum of the material did not match that of any of the calcium salts contained in the Institute's IR spectral library. As the wine was made from botrytised grapes, it was suspected that the crystals might have been calcium mucate. Mucic acid is a by-product of botrytis mould growth and reacts with calcium to form insoluble precipitates, that deposit slowly. To confirm that the crystals were indeed calcium mucate, a sample of mucic acid was obtained and reacted with calcium carbonate to form calcium mucate. The IR spectrum of the artificially induced calcium mucate precipitate was identical to that of the crystals from the wine.

- during the year there were two cases where winemakers were concerned about the possibility of metal fragments from winemaking equipment entering their wines, and subsequently dissolving and causing irreversible contamination.

  In the first case, small fragments of foreign matter from a sulfitometer (a pressurised container from which sulfur dioxide, a commonly used preservative, can be added to wine), were isolated on gauze and submitted to the Institute for identification. The winemaker was concerned that the fragments might contain heavy metals. The isolated material was analysed with the assistance of staff from the Inductively Coupled Plasma Atomic Emission Spectrometry (ICPAES) unit, at the Waite Campus of the University of Adelaide. The results indicated that the material contained a large proportion of iron and some nickel and molybdenum, all components of stainless steel. No heavy metals were detected. Unfortunately, the ICPAES scan does not detect chromium, another important component of stainless steel. However, the results do suggest the fragments originated from stainless steel. It is possible that this could occur if water were to come into contact with the inside of a sulfitometer; causing the sulfur dioxide to react to form sulfurous acid, which could cause corrosion of the stainless steel.

  In the second case, metal fragments were found to be flaking off nickel-coated components of a bottle-filling machine. Identification of the fragments was requested, as well as analysis of some wines that had recently been bottled using the filling machine, in order to determine if similar fragments had contaminated any of these wines. The wine samples and a portion of the fragments were also analysed for the presence of metals at the University of Adelaide ICPAES facility, and the staff are thanked for their help in these investigations. The fragments were found to contain approximately 85% nickel and 10% phosphorus, which indicates that the material was likely to be a type of nickel alloy. The range of concentrations of nickel in wine reported in the literature from various countries is large, and all of the wines analysed contained nickel concentrations within this range. However, one of the samples contained a concentration of nickel approximately seven times higher than the average concentration of nickel in the other samples. It was concluded that this may have been due to contamination by fragments from the filling machine, or may have been due to differences in variety, soil and viticultural practices between the different vineyards from which the grapes were sourced, even though these were neighbouring vineyards owned and managed by the same company.

Winemaking consultation

Staff: Peter Godden, Adrian Coulter, Mark Gishen, Greg Ruediger

The Industry Services team provides a winemaking consultancy service principally through the Manager, Peter Godden, a qualified and experienced winemaker, Adrian Coulter, a Graduate in the Diploma in Oenology from The University of Adelaide, and Mark Gishen. Greg Ruediger, the Trace Analysis Laboratory Supervisor, also joined the Industry Services team on a 0.25 basis during the year, and also holds the Graduate in the Diploma in Oenology from The University of Adelaide. Adrian Coulter gained nine weeks of practical winemaking experience during the 2001 vintage, working at a winery close to Adelaide. Greg Ruediger has gained similar experience in both the 1999 and 2000 vintages.

Most queries received are technical in nature and arise predominantly from Australian winemakers. However, many queries are also received from wine industry suppliers and Government bodies, as well as a relatively small number from the general public and secondary and tertiary students. Where appropriate, the query is answered over the telephone, by facsimile or e-mail, and Industry Services staff supply approximately five hundred technical papers or other pieces of relevant literature to callers each year. Similarly, the support facilities provided by research and library staff are important in supplying relevant information to callers, and the analytical capacity of the Industry Services Laboratory plays an important role in responding to many of these enquiries. In addition, most of the investigations recorded in Table 3 result in a full written report being prepared for the client. These reports contain detailed technical information relating to the problem being investigated and are written in a way which seeks to explain the underlying causes of the problems encountered, and provide advice on how to prevent them re-occurring. The reports are often accompanied by a number of technical references relating to the area of investigation.

The Institute often acts as a referral service, increasingly for Government bodies, wine industry suppliers and wine journalists, having links to Australian and international wine research and political bodies. The vast store of information, both formal (in the John Fornachon Memorial Library) and informal, is a valuable resource to the wider industry.

During the year, the Institute provided assistance to the developers of public displays for the new National Wine Centre. In previous years the Institute's Director, Sensory Scientist (Dr Francis) and winemaking staff have provided advice to companies involved in this project, with a view to ensuring that the displays are accurate from a technical perspective. During the current year, Greg Ruediger has advised on the principles and practices of winemaking for typical white and red wine styles, and has reviewed and edited flow charts for white and red winemaking processes. Many of the displays at the Centre will be interactive, and the public will have the opportunity to make their own 'virtual wine.' To this end, multiple choice questions, along with appropriate weighting for the importance of various winemaking techniques for the creation of a range of wine styles, have been reviewed and edited. In addition, digital images of typical wine microorganisms have been supplied for use in the displays.

A summary of the enquiries received by Peter Godden, Adrian Coulter, Mark Gishen and Greg Ruediger during 2000/2001 is presented in Table 4. The figures show a 2.5% decrease in the number of enquiries received in the previous year, following steady increases totalling 35% over the previous five years. Thus, the number of enquiries has apparently stabilised at a very high level, with the increased demand for this service clearly reflecting the expansion of the Australian wine industry over the same period. The proportion of enquiries received from wineries increased to 70%, continuing a trend that has been highlighted in previous Annual Reports.

The Consultation and Investigative and Advisory Services are supported by vineyard and winery visits and seminar tours to all major wine growing regions, generally organised in conjunction with local vignerons' associations. The Institute aims to visit each major Australian viticultural region through such formal visits and tours every second, or in some cases third year, as the number of wine growing regions continues to increase. Routine shorter visits by key staff are also made, as opportunities arise—frequently in conjunction with industry events such as capital city wine shows, and seminars held by other industry bodies.

Formal 'Roadshow' visits were made to South Australian and Victorian regions in November and December 2000 (see Appendix 1). Up to six senior Institute staff presented seven full-day seminars, each seminar consisting of a minimum of twelve presentations focussing on current areas of Institute research, or topical issues in the wine industry. Each regional winemaker's association was asked to select the presentations to be made at their seminar, from a list of approximately forty areas of current Institute activity, in order that each seminar was closely tailored to the interests of the audience in each region. In addition, a workshop developed under GWRDC project 99/1 *Targeted training of industry personnel: compilation of a technical reference manual and delivery of complementary workshops* was delivered by Industry Services staff in five regions on the day following the seminar, thus a total of 12 days of Roadshow activities were presented.
The 14th Advanced Wine Assessment Course was held in July 2000, giving another 30 participants the opportunity to develop and test their sensory evaluation performance. This was the second Course presented under a four-day format, which includes over 40 hours of activities over the four days. Fifteen leading wine show judges, journalists and winemakers, assisted in the presentation of the Course. The demand for the Course continues to be extremely strong, with many repeat customers, despite the fact that it has not been promoted or advertised, which is an indication of the need for practical training of industry personnel away from their individual work environment. As in the past, several Associate Judges for the 2000 Adelaide Wine Show were selected from the most successful recent participants in the Course, and other shows have expressed interest using Course results as part of their selection criteria for new Associate Judges. The Course continues to attract interest from the production, marketing, sales and educational arms of the industry, from all States of Australia, New Zealand, South Africa and the United Kingdom.

Evaluation of new analytical techniques and of processing aids for winemaking

Staff: Peter Godden, Mark Gishen, Adrian Coulter, Peter Valente, Ella Robinson

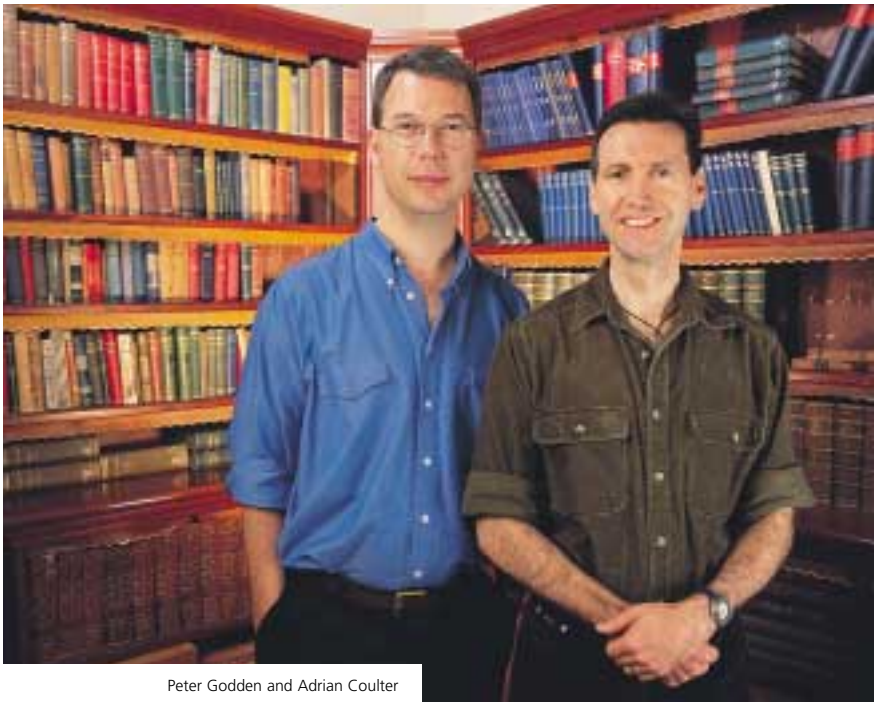
The Industry Services Laboratory maintains a GWRDC funded project for the improvement, development and evaluation of methods of wine analysis, and the evaluation of winemaking processing aids and additives. The evaluations take one of two forms: the relative performance of commercially available products and the evaluation of new materials marketed to the industry. Industry Services staff also provide advice to the Institute's Analytical Service on the development of protocols which relate to trials being conducted on a fee for service basis for various wine companies and industry suppliers.

During the year, the vast majority of resources allocated to this project were used for the Closure Trial, a wide-ranging trial that is examining the technical performance of 14 different closure types. The closures comprise a roll-on tamper-evident (ROTE) screw-cap closure, two grades of natural conventional cork (Reference 2, 44 mm length and Reference 3, 38 mm length), two 'technical cork' closures (cork-based closures that also contain a synthetic component: Sabaté 'Altec' and Amorim One plus One 'Twintop'), and 9 closures manufactured from synthetic materials, three of which are produced by extrusion processes (ECORC, Nomacorc and NuKorc) and 6 by moulding (Aegis, Auscork, Betacorque, Supremecorq, Tage and Integra). Various aspects of the performance of the closures are being examined, including those relating to each closure's physical characteristics and extraction from the bottle, chemical analysis of the wine in order to examine apparent sealing performance, and sensory analysis. In addition, Dr Mark Sefton's team will perform chemical analysis using GC/MS, to examine any effect the closures may have on wine aroma and flavour.

As demonstrated by Table 3, problems associated with the use of both natural cork or synthetic closures continue to represent a large proportion of the problem solving investigations conducted by the Industry Services team, and an increasing amount of commercial work performed by the Analytical Service. However, until now little published information has been available regarding the performance of synthetic closures, or little data available regarding the relative importance of various parameters of closure performance, by which natural cork, screw caps, currently available synthetic closures and closures which may become available in the future, can be reliably compared. The broad aim of this trial is, therefore, to elucidate these factors, by examining a large number of parameters of closure performance for a broad cross section of currently available closure types, on an ongoing basis for up to ten years. From this data, the relative importance of various performance parameters will be determined, and this knowledge can then be applied to the development of testing protocols by which the future performance of closures can be rapidly and reliably evaluated and possibly predicted. In addition, the trial will also produce data on parameters relating to bottling and storage, which may then be used to elucidate the importance of those parameters.

Photograph courtesy of Orlando Wyndham

Robotic arm with needle



Peter Godden and Adrian Coulter

Senior personnel from the Australian wine industry with extensive prior experience in the evaluation and purchase of wine bottle closures have had significant involvement in the design of the trial. In addition, extensive consultation with closure suppliers ensured that the closures examined were representative of those being used by the wine industry, and ensured that the bottling procedures were optimal for each closure, as far as practical. The wine was bottled under controlled conditions at an ISO 9002-certified commercial contract bottling facility, with personnel who had prior experience with the use of many of the closures. The design of the experiment was motivated by the need to generate data of practical relevance, with all aspects of the protocol matching as closely as possible current industry practice, with due regard for careful experimental design and appropriate replication. The wine used for the trial was produced during the 1999 vintage, from sound Semillon grapes grown in the Clare Valley of South Australia, was fermented to dryness and was made utilising common Australian winemaking practices for wines of this style. Importantly, measured variables of composition at bottling indicate that the wine had a low pH (3.1), and contained adequate SO_2 (free 30 mg/L total 95 mg/L) to prevent premature oxidation. In addition, the presence of laccase, an enzyme which can cause accelerated oxidation of wine, was not detected.

The study is ongoing, and sufficient wine was bottled so that the testing of many variables may continue for up to ten years. Results will be published periodically, and testing will continue for as long as it is considered that useful data is being generated.

The published testing protocol (Australian Grapegrower & Winemaker [425] p 59-64, 1999, Institute publication #593), was expanded at the 24 months post-bottling time point to include the testing of a higher number of replicates for measurements of wine composition (SO_2, ascorbic acid concentration and browning), and for sensory analysis. In addition, sensory analysis was conducted on bottles that had been stored in both an upright, and inverted orientation, and SO_2, ascorbic acid and browning were also measured in these same bottles. Testing was also conducted at 18 months post-bottling (concentration of free SO_2) and at 21 months (free and total SO_2 and sensory evaluation on the same bottles), which was additional to the testing foreshadowed in the protocol. This extra testing was conducted, because it appeared from previous results that the development of the wine had reached a crucial stage.

The large amount of work that has been involved in all aspects of the planning and implementation of this project culminated in the submission of a large publication late in the reporting year. The paper, which reported the results obtained up to the 20 month time point after bottling, was scheduled to appear on July 12, 2001 published as the entire contents of the Volume 7 issue #2) of the *Australian Journal of Grape and Wine Research* (AJGWR) (Institute publication #666). This trial has been a major team effort, and Industry Services staff acknowledges the assistance of the many members of Institute staff, and personnel from other organisations, who have been involved in the project. Dr Leigh Francis, the Institute's Senior Research Chemist and sensory evaluation expert, has been responsible for the sensory evaluation work conducted as part of the trial, and is the second named author on the published paper – in recognition of his very strong and sustained contribution to the project.

Because the testing at the 24 month point of the trial was conducted at the very end of the reporting period, there was insufficient time for a full analysis of the data. However data obtained at the 21 month time point confirm the trends of relative closure performance which are discussed below, and published in detail in the AJGWR [Vol 7, #2], which the reader is recommended to consult in order to be fully informed of all relevant trial details. Inspection of the 24 month data suggests that these trends also continued to that time point.

- Wine under the screw cap closure retained the greatest concentration of sulfur dioxide (SO_2) and ascorbic acid and had the slowest rate of browning, at all testing intervals (including 21 and 24 months). For other closures the trend of SO_2 loss relative to the screw cap closure was apparent from an early stage of testing, and was most evident in the group of synthetic closures, intermediate in the conventional corks, and least evident in the technical cork closures. For several closures upright storage tended to accelerate loss of SO_2 from the wine, but in many cases this effect was marginal up to and including 18 months. However, at 12 months there was greater variability in the data for bottles that had been stored upright, as opposed to inverted.

  The loss of SO_2 was in general highly correlated with an increase in wine browning (OD_{420}) and the concentration of SO_2 in the wine at six months was a strong predictor of future browning in the wine, particularly after eighteen months. Neither the concentration of dissolved oxygen at bottling (0.6–3.1 mg/L), nor the physical closure measures were predictors of future browning, and the concentration of dissolved oxygen at bottling was not a strong predictor of later SO_2 loss from the wine.

  Seven of the closures displayed a strong correlation between the concentrations of free and total SO_2 and ascorbic acid ($P < 0.001$) at 12 months, but with the other closures the correlation was weak, or did not exist at all. In addition, wine sealed with many of the closures contained a higher concentration of ascorbic acid when stored upright as opposed to inverted, although there was only a statistically significant difference for three of the closures. These observations may imply that some closures themselves play a role in the degradation of ascorbic acid.

- The closures differed widely in regard to physical characteristics. All but one of the synthetic closures, and one of the technical corks, were found to elongate to a greater extent than the natural corks after insertion into the bottle. One of the synthetic closures was found to contract upon insertion. The elongation observed raises possible issues related to achieving a suitable headspace (the space between the closures and the wine) when bottling wine.

  In general, synthetic corks appeared least 'consumer-friendly' in terms of the higher extraction forces and energies required to remove them from the bottle, and ease of closure re-insertion into the bottleneck after extraction. In addition, three of the synthetic closures required a torque to remove them from a corkscrew that was approximately double that required for the natural corks (while some synthetic closures required less torque than the natural corks). However, there was a trend for natural cork closures to exhibit larger variability in these physical characteristics than technical cork and synthetic closures.

  All of the closures were found to expand in diameter by a similar amount during the 30 minutes following extraction, and there was no relationship between degree of post extraction expansion or the chemical and sensory properties of the wine sealed with each of the closures. Likewise, there was no relationship between the physical measurements of maximum extraction force, total extraction energy, and the torque to remove the closure from the corkscrew, and the chemical and sensory analysis of the wines sealed with each of the closures, when data for a closure which displayed notably high extraction force, and notably poor SO_2 retention, (and lowest density) was removed from this analysis. In addition, when data from another closure which displayed notably high torque, extraction force and density was also removed, there was found to be no relationship between the density of the material from which the closure was manufactured, and the chemical or sensory analysis of the wines sealed with each of the closures.

- Sensory analysis indicated large differences in wine flavour properties, with closures which tended to result in the best retention of free SO_2 having wine sensory scores for citrus that were generally high whilst scores for the attributes *developed* / *oxidised* were low. The situation was reversed for wine under closures that performed poorly in the retention of free SO_2. It was found that below a critical level of free SO_2 remaining in the wine (10 mg/L) closures exhibited substantially higher oxidised aroma. These trends continued, and tended to be reinforced for all closures, at 21 and 24 months.

  Trichloroanisole (TCA)-type taint was a noticeable problem for some cork and technical cork closures, at all testing intervals up to and including 24 months. Any plastic-type taint appeared not to be a problem with most synthetic closures up to 18 months, but ratings for the attribute *glue-like* / *plastic* by our highly skilled panel, were notably higher for many of the synthetic closures at 24 months.

  Whilst the ROTE closure was rated as highest for the fruit attributes at all testing intervals up to and including 24 months, a *reduced* (rubbery or sulfidic) character began to be evident in wine sealed with some closures (in general those which had tended to retain the greatest concentration of SO_2) at 18 months, and was significantly higher in wine sealed with the ROTE closure compared to all other closures. These trends continued at the 21 and 24 month time points, with the intensity of the *reduced* attribute increasing.
  Closures that scored highest for the *oxidised* attribute at 18 months also tended to display the greatest bottle-to-bottle variation in the rating of this attribute.

Conclusions

Differences in bottled wine composition that result from the use of closures of different types have been assessed, and importantly, the study has allowed the compositional measures of SO_2 and degree of browning to be directly related to the sensory properties of the wine. It is apparent that many of the closures included in the study are suited to short term (approximately 6 to 12 months) storage of wines, but for longer time periods there is doubt over the abilities of various closure to act as an adequate seal, with consequent effects on antioxidant levels, browning and on oxidised aroma. It was apparent early in the trial that the closures showing lowest SO_2 concentration were rated as sufficiently high in *oxidised* aroma to consider the wine from these bottles as markedly lower in quality. These issues become particularly important when it comes to managing stock on shelves.

The apparent importance found in this work of a critical concentration of free SO_2 (approximately 10 mg/L) below which wines are perceived as substantially affected by oxidised aroma is of considerable interest. This observation requires confirmation to determine whether this might be a generally applicable cut-off value, or at the least might be appropriate as a guide for white wines of the style used in this study. If this observation were confirmed, the periodic measurement of SO_2 in bottled wine during storage could be used by wine companies, distributors and retailers, to identify batches of wine that are approaching this critical level, and thus take the appropriate action in the marketing of those wines. It is possible that by increasing the concentration of SO_2 in wines at bottling to compensate for the losses over time identified in this study, winemakers could increase the longevity of their bottled wines.

For a broad range of performance measures, variability among bottles from the same closure was not a serious issue, except for the TCA-affected samples, but there was some variability evident in the SO_2 concentration, where several closures had relatively large variation. Whilst no closures could be considered to be showing unacceptable variation in SO_2 concentration, indicating that so-called 'random bottle oxidation' was not apparent up to 18 months of testing, there was greater variability in the SO_2 concentration in bottles stored upright. In addition, the closures that did exhibit higher *oxidised* aroma at 18 months also tended to display greater bottle-to-bottle variation on the rating for *oxidised*.

It will be of interest to note if this observation is manifested as greater variability in wine development at later time points.

Overall, technical cork closures were found to exhibit less variability than synthetic closures, which in turn tended to be less variable than natural cork closures, when assessed for a number of the physical and wine composition variables. However, there did not appear to be a trend for either moulded or extruded synthetic closures, when considered as groups, to be more or less variable than each other.

One objective of this study was to identify the most important variables of closure performance, and then apply this knowledge to the development of protocols that can be used by the wine and closure industries for the rapid and reliable testing of new or existing closures. Sulfur dioxide concentration was found to be a particularly important measurement when assessing the comparative performance of closures, and short-term storage followed by simple SO_2 or browning measurements provided an excellent indication of later sensory performance.

No single closure tested in this study was considered entirely suitable by all criteria assessed, for the long term storage of wine, although the ROTE was the best performing closure in terms of chemical measures of wine composition, and sensory evaluation. It is possible that the *reduced* character which was particularly identified in wine sealed with this closure, was at least in part exacerbated by the manner in which this

closure was used which, while still complying with the closure's specifications of use, did seek to exclude oxygen from the headspace.

The incidence of 'cork taint' (TCA contamination of the wine) was considered an important factor with all four of the cork-based closures tested. Wine sealed with one closure was affected by a styrene-like off-flavour when assessed at the six month time point. However, due to the very poor retention of SO_2 displayed by this closure, it was excluded from further testing.

Note: Readers are asked to keep in mind that the current study is ongoing, and that the results reported here generally represent the performance of the closures under examination only up to 20 months post bottling. It is possible that, over time, the relative performance of various closures may change, or that closures which have apparently performed well to this point may be found to perform less well in future testing. Readers of the Annual Report should also refer to the full publication of the closure study (Institute publication #666) to understand the full context in which this study was performed.

Analytical method development and evaluation

Staff: Peter Godden, Mark Gishen, Adrian Coulter, Peter Valente, Ella Robinson

Although the majority of resources allocated to the evaluation of methods and processing aids project has been spent on the closure trial during the last year, some development of methods has also been conducted.

During the year, the Industry Services team commissioned a new *Perkin Elmer* Fourier transform - infra-red (FT-IR) spectrometer, a new *Agilent* 1100 Series High Performance Liquid Chromatography (HPLC) system, and a reconditioned Hewlett Packard 5890 Series II gas chromatograph (GC). The assistance of the GWRDC in providing capital funding for this equipment is acknowledged as is the very substantial contribution of Analytical Service.

The Institute's internal Quality System (based on NATA requirements) required that the analytical assays previously performed using the old HPLC and GC systems, were validated for the new equipment. Validations of key assays required for the closure trial and problem-solving investigations, ascorbic acid and the 'organic acid profile,' were the first validated for the HPLC, whilst the methods for methanol, ethanol and ethyl acetate were validated for the new GC system, and validation of the GC method for acetaldehyde has commenced.

In response to the high number of cases of refrigerant brine contamination reported to the Institute, as described above, a method for the determination of propylene glycol in wine, using gas chromatography (GC) has been developed and validated. Previously, the chief analysis available to Industry Services staff when investigating cases of this sort was the concentration of methanol, a common component in refrigerant brine solutions. However, this was considered unreliable in identifying low levels of contamination. Two of the five cases of suspected contamination analysed since the method was developed were found to contain concentrations of propylene glycol far in excess of the normal levels reported in the literature.

Good progress has been made on the development of an assay for the identification of isovaleric acid in wine. Isovaleric acid is reported in the literature as an important spoilage compound produced by *Brettanomyces* yeast, and a number of wines submitted to the problem solving service are thought to possibly contain this compound.

Many wines are also submitted to the problem solving service that apparently contain sulphur compounds which cannot be removed by the addition of copper sulphate, a legal and relatively common technique employed worldwide for the removal of hydrogen sulfide and mercaptans from wine. Therefore, the development of methods for the identification and quantification of these compounds would be of great benefit. During the year a literature review has been conducted on the analysis of sulphur containing compounds in wines, ranging from the simple volatile compounds such as sulphides and mercaptans to more complex and less volatile thiol containing compounds, with a view to the development of such methods.

The superiority of the new FT-IR spectrometer compared to the previously used dispersive IR instrument has been demonstrable. Spectra are obtained in a matter of seconds using the new FT-IR and are stored as digital information. This facilitates a range of interpretive techniques including matching with reference spectra from spectral libraries, identification of mixtures of dissimilar materials, and quantification programs. The Industry Services team is continuing to compile its own spectral library using the new instrument, which has been useful in identifying a number of deposits and increasing the confidence of positive identification of samples, compared with the previous system.

In conjunction with the Institute's Analytical Service, the following analytical methods have been validated during the year, to the standards required by the Institute's internal Quality System, which is based on NATA requirements and ISO Guide 25: Malathion and Fenitrothion in grapes and marc by GC-MS; Fludioxonil, pyrimethanil, fenhexamid, tebufenozide and cyprodinil in wine, juice, grapes and marc by solid phase extraction followed by HPLC, Eugenol in wine and wood products by GC-MS; Spiroxamine, indoxacarb, trifloxystrobin, axoystrobin in wine, juice, grapes and marc by GC-MS; Trifloxystrobin, axoystrobin in wine, juice, and grapes by GC-MS; Fludioxonil in juice and wine by GC-MS, and, Cyprodinil in wine and juice by liquid-liquid extraction followed by HPLC. In addition, NATA accreditation was obtained for the following methods: Determination of ochratoxin A in wine by HPLC, and, Malathion and Fenitrothion in wine and juice (pending final NATA approval). This work is conducted by the Institute's Quality Liaison Manager, Mr Mark Gishen, who is part of the Industry Services team.

Flavour scalping

Staff: Dr Mark Sefton, Alan Pollnitz, Dimitra Capone

A detailed study on the capacity of two-litre bag-in-box packaging to absorb wine flavour compounds has been completed. Changes in wine composition resulted both from hydrolytic reactions in the wine (determined by measuring changes to the wine stored in sealed glass ampoules) and from contact with the packaging. Some wine components were absorbed rapidly and near-completely from wine by the plastic components of the packaging, while others were essentially unaffected by this process.

The volatile wine components that were the most efficiently removed were the least polar. Thus, 1,1,6-trimethyl-1,2-dihydronaphthalene (TDN), which has a kerosene-like aroma and can be an important contributor to the aroma and flavour of Riesling wines in particular, was slowly generated from precursor forms in a Semillon wine stored in the sealed glass ampoules for 32 days, but more than 97% of this compound was absorbed when the same wine was stored under identical conditions in bag-in-box packaging (figure 8). Seventy five percent of this absorption took place following the first 24 hours of contact. Naphthalene, which is occasionally found as a trace taint compound in wine and has a structure similar to that of TDN was less efficiently absorbed. Nevertheless, some 70% of this compound was removed by the packaging, again, mostly within the first 24 hours.

A series of ethyl esters produced during fermentation and including several which are major contributors to the fruity character of wine were included in the study. The concentration of these compounds changed during storage of the Semillon wine in sealed

Dimitra Capone and Andres Hakkensson

glass containers for 32 days, but not in a systematic way – most decreased slightly in concentration during glass storage, while the concentration of ethyl octanoate increased slightly. For the most part, more substantial decreases in concentration resulted from storage in the bag-in-box packaging, and these decreases were greater for the longer chain (and therefore less polar) esters. In the case of ethyl decanoate, a combination of a 50% reduction in concentration resulting from hydrolysis and an 85% absorption of the remaining ester by the packaging resulted in most of this compound being removed from the wine over 32 days. Decreases in concentration, attributable to absorption of ethyl octanoate, ethyl hexanoate and ethyl butanoate were 55%, 30% and 10% respectively.

Absorption played less of a role in the changing concentration of several other wine components. Thirty-five percent of ß-ionone, which can give an aroma of violets to red wine and is found in highest concentration in Pinot Noir wines, was absorbed over 32 days, and 27% of *cis-rose* oxide, which is associated with a lychee aroma, particularly in Traminer wines, was absorbed over the same period. A more than 50% decrease in ß-damascenone took place in both glass and bag-in-box packaging. In this case absorption phenomena were not responsible for the change in concentration.

A variety of flavour compounds associated with oak products were unaffected by storage in the bag in box packaging. These were the *cis-* and *trans*-isomers of oak lactone which, depending on concentration, give coconut, vanilla and woody characters to wines, vanillin which is the principal aroma component of natural vanilla, guaiacol and 4-methylguaiacol (smoky aromas), and 4-ethylphenol and 4-ethylguaiacol which are commonly formed by *Brettanomyces* yeasts during barrel maturation and which are associated with elastoplasts, medicinal and clove-like aromas.

This study with the bag-in-box packaging clearly demonstrates the capacity of some plastics to absorb flavour compounds from wine in a relatively short time. Earlier research had also shown that natural cork-bark closures could similarly absorb chloroanisoles from wine, albeit slowly (Institute publication #616). This indicated that wine bottle closures, be they natural cork bark or synthetic, might be able to modify wine flavour by absorption, and that such absorption could contribute to flavour

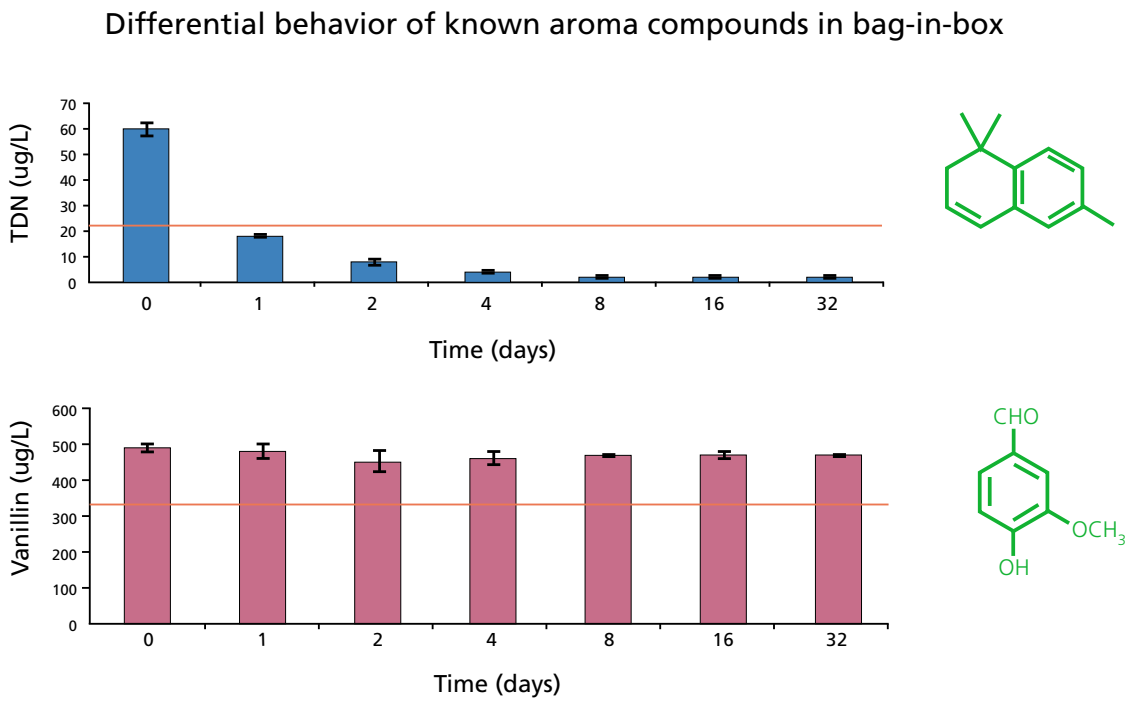


Figure 8. Drastic differences in the behaviour of known aroma compounds is observed when stored in bag-in-box containers. Known amounts of trimethyldihydopthalene (TDN) responsible for the 'aged Riesling/kerosene' character, and vanillin (a known volatile compound extracted from oak) were added to a 1999 Clare Valley Semillon and their concentration followed over time as indicated. The published aroma thresholds for the two compounds are indicated with the red line.

Cork extraction test

Photograph courtesy of Orlando Wyndham

changes associated with bottle-ageing. A long term study of flavour absorption from bottled wine by various types of closure has therefore been undertaken and the wines are approaching the end of the second year of storage. A report on changes during these first two years of storage will be made in the following Annual Report.

Links between viticultural and oenological research

Staff: Dr Sally-Jean Bell

During 2000/2001, the Viticulturist responded to 592 enquiries, 86 more than in the previous 12 months. The majority of these enquiries related to agrochemicals. The Viticulturist participated in several *Research to Practice* workshops, in different states, and Institute Roadshows during the year (details provided in Appendix I).

Nine thousand copies of the Institute's annual publication, *Agrochemicals registered for use in Australian viticulture 2001/2002* were produced and the information duplicated on the Institute's website. The booklet was distributed with the *Australian Grapegrower & Winemaker, Technical Review* and in the *Research to Practice*™: IPM and Spray Application manuals. The tables are featured in *Australian Viticulture*. The 2001/2002 MRLs for Australian export markets was also updated for the Institute's website.

As a direct result of the Institute's agrochemical publication and its Residue Analysis Service, the Viticulturist and Greg Ruediger have been devoting an increasing amount of time in liaising with major chemical companies. Companies are increasingly aware of the importance the wine industry places on meeting export market specifications. Thus, they are keen to work more closely with the industry and the Institute to ensure that their products can be used in viticultural pest and disease control programs in such a way that the maximum residue levels set by Australian export markets will not be exceeded.

With short notice, the Viticulturist coordinated and prepared the application for National Registration Authority (NRA) approved emergency use permits for four insecticides on behalf of the wine industry for the control of locusts, and these permits are held by the Winegrape Growers' Council of Australia Inc.

The investigation into the effect of plant water status and canopy management on the phenolic profiles of grapes and wine in collaboration with Dr Patrick Iland and Dr Peter Dry (University of Adelaide), Orlando Wyndham (Langhorne Creek) and Professor Enrico Peterlunger (Universita di Udine, Italy), commenced in October 2001. This trial was conducted by the Viticulturist and a visiting PhD student Mr Paolo Sivilotti (visiting for seven months from Universita di Udine). To date it has been confirmed that water stress in both VSP and Scott Henry vines resulted in reduced leaf water potential, stomatal conductance, leaf area, canopy density, shoot length, pruning weight and berry weight. Fruit exposure consequently increased and preliminary assessment indicates that skins and seeds from exposed fruit had lower quantities of tannin and anthocyanin than skins and seeds from less exposed fruit. Wine was made from the fruit and the first stage in the sensory analysis is scheduled to start towards the end of November 2002.

Preparation of information on wine and health issues

Staff: Creina Stockley

This project has been funded since 1990. Ms Creina Stockley, a clinical pharmacologist, assumed this position in 1991 as part of her responsibilities as Health and Regulatory Information Manager. A database of research on the beneficial and detrimental health effects of alcohol and in particular, wine, was established on the internal computer network of the Institute and as part of the John Fornachon Memorial Library. This was facilitated by the subscription to relevant medical and scientific journals, and by the formal and informal exchange of information between complementary organisations, both national and international. During 2000/2001, 74 independent information requests were received on wine and health issues from industry, government and the general public by the Health and Regulatory Information Manager.

Subscription to relevant medical and other journals has continued. The journals have been regularly scanned, the database of research on the health effects of wine has been added to and articles have been prepared for inclusion in the Institute's publication, *Technical Review* and for other Australian wine industry and international alcohol industry newsletters. Articles and other material have also been prepared for the electronic and print media, for example, six articles have been prepared for the bimonthly international publication, *AIM—Alcohol in moderation*, and four articles for the quarterly newsletter of the Australian Society of Wine Education. The Health and Regulatory Information Manager is also a member of the editorial board of AIM—Alcohol in moderation. A national radio interview and a television interview were also undertaken related to the issue of potential blood products in winemaking. A review of the draft Training Procedures Manual for the National Wine Education and Training Centre was also undertaken, as well as preparing material to support the WFA-funded *Costs and benefits of drinking wine–A discussion and review of Collins and Lapsley* by the Centre for International Economics.

Submissions prepared in support of the Australian wine industry include:

- *Draft Australian Alcohol Drinking Guidelines* of the National Health and Medical Research Council of Australia;
- *National Alcohol Research Agenda* of the Federal Department of Health and Aged Care;
- *Foetal Alcohol Syndrome Draft Scoping Paper* of the National Expert Advisory Committee on Alcohol; and
- PRELIMINARY DRAFT RESOLUTION ECO/REGL/96/23/Stage 3 on ingredient labelling and PRELIMINARY DRAFT RESOLUTION OENO/ASP/99/129/Stage 3 on a health warning for the of the Nutrition and Wine Expert Group of the Office International de la Vigne et du Vin (OIV) (Creina Stockley is a member of the Australian OIV delegation—Nutrition and Health Sub-commission).

The Health and Regulatory Information Manager has been instrumental in organising and developing the program for the wine and health symposium session of the 26th World Congress and 81st General Assembly of the Office International de la Vigne et du Vin to be held 11–18 October 2001. The theme of this session is "Current wine and health information: the issues in perspective". There are also three sub-themes: 1) The current and future status of wine and health information; 2) The need for consumers to relate this information to their diet and lifestyle; and 3) Responsible communication of wine and health information.

Institute and industry consumer health and safety information and support system

The Health and Regulatory Information Manager has also prepared the Institute's and industry's consumer health and safety information and support system, which is accessible on the Institute's web-site www.awri.com.au.

Project coordination

Through Creina Stockley, the Institute has played a coordinating and a participating role in three GWRDC-funded research projects on medical aspects of wine consumption entitled AWR97/2 Potential cardio- and cancer-protective effects and mechanisms of wine. GWRDC pays the Heart Research Institute, The University of Western Australia and CSIRO Health Sciences and Nutrition directly. The respective project titles are: Potential cardioprotective activities of wine components based on synergistic interaction with vitamin E; Grape antioxidant phenolics: absorption and inhibition of lipid peroxidation in humans; and Reduction of

damage to LDL and DNA from oxidative free radicals by the regular and moderate consumption of wine. All three projects have completed their third year of funding.

Technical and regulatory support to the Australian wine industry

Staff: Professor Peter Høj, Creina Stockley and Rae Blair

Information requests

One of the activities of the Institute has been to provide legal/regulatory and technical advice and assistance to the Australian wine industry, through the Health and Regulatory Information Manager, the Industry Services team and the Director. During 2000/2001, 110 independent information requests on technical and regulatory issues from the government and industry were fielded by the Health and Regulatory Information Manager.

Industry committee membership

Additional support to the industry is derived from the Director's membership on the AWBC Compliance and Technical Advisory Committee, International Trade and Technical Advisory Committee and the WFA Technical Committee. One of the important aspects of the Institute's support of the Australian wine industry is its pivotal role in facilitating the triennial Australian Wine Industry Technical Conference (AWITC), in conjunction with the Australian Society of Viticulture and Oenology. The Director is the Chair, and the Personal Assistant to the Director is the Conference Manager and Treasurer. Professor Høj and Ms Blair are also members of the Australian Organising Committee of the 26th World Congress and 81st General Assembly of the Office International de la Vigne et du Vin to be held 11-18 October 2001.

At present, the Health and Regulatory Information Manager is a member of the following committees: the AWBC Legislative Review Committee; the WFA Technical Committee; and the National Reference Committee-Environment Strategy Development (SAWBIA). Ms Stockley was also elected Vice-President of the Nutrition and Wine Expert Group in March 2001.

Reviews and publications

During 2000/2001, supplementary to the Standard 2.7.4 for wine and wine product of the [joint] Australia New Zealand Food Standards Code that was gazetted in December 2000, a draft industry standard for wine, sparkling wine, fortified wine, wine products, reduced and low alcohol wines was prepared by the Health and Regulatory Information Manager in conjunction with the AWBC and WFA, for potential inclusion in the *Australian Wine and Brandy Corporation Act 1980 and Regulations*.

Significant efforts, energies and resources have also been directed towards ensuring the establishment of realistic and reasonable maximum limits for the additives and processing aids approved for use in winemaking by the Codex Alimentarius Commission, on behalf of the AWBC, WFA and AFFA (Wine, Vine and Citrus Section). Technical information and/or issues that have been reviewed, and discussion or position papers prepared or contributed to during 2000/2001 include: the national system of gene technology regulation of the Therapeutics Goods Administration and Australia New Zealand Food Authority; ochratoxin A; silver nitrate; tannin and tannin-like additives; current status of country of origin labelling; acidification with lactic, malic and tartaric acids; wine as a source of dietary sodium; BSE and the Australian wine industry; and factors influencing the sodium and potassium content of Australian wines.

Technical Information is also disseminated to the Australian wine industry, including all grape and levy payers, by the institute's bimonthly publication, *Technical Review*, of which the Health and Regulatory Information Manager is editor, and by the Institute's *Annual Report* edited by the Director and Communication and Publicity Manager.

Australian Wine Industry Technical Conference

Several Institute staff members, namely, Professor Peter Høj, Peter Godden and Rae Blair, serve on the Conference Planning Committee of the Eleventh Australian Wine Industry Technical Conference, and Professor Høj and Mr Godden served on the Program Sub-Committee. The formal program has been developed and will cover eight two hour sessions from Sunday, 7 October to Wednesday, 10 October. Some 74 workshops are being planned, and the workshop program, being convened by Mr Godden, will run from Sunday, 7 October to Thursday, 11 October. Institute staff will conduct several workshops and prepare more than 30 posters. Jeremy Hack acts as the poster coordinator.

This project of technical and regulatory support to the Australian wine industry is ongoing, as technical and regulatory issues are regularly raised by the government or by industry, both in Australia and internationally. Furthermore, these issues often span several years.

Provision of technical information

Staff: Rae Blair, Catherine Daniel, Ingrid Oats

The John Fornachon Memorial Library holds the largest collection of wine technical literature in Australia. The Library's principal responsibility is to provide technical information to the Australian wine industry and to the researchers of the Institute. The Library is also used extensively by other groups such as students, government bodies and private companies (see Table 5).

Information and document delivery services

The Library has excellent access to international databases, particularly in the fields of science, technology and medicine. If requested, the Librarian, Catherine Daniel, will carry out online searches on commercial databases on any appropriate topic (on a fee-for-service basis). Alternatively, Library staff can provide, free of charge, a report of relevant articles indexed on the Library's in-house databases. Requests for information from Library staff continue to rise significantly with a 108% increase in information requests for 2000/2001 over 1999/2000. Most of the Library's services showed increases in demand, with the exception of online search requests and requests for articles from *Technical Review* (-3.8%). This minor decrease in requests for articles from *Technical Review* has been more than compensated for by a 40.5% increase in other articles forwarded over 1999/2000 figures. Database searches undertaken on internet and CD-ROM networked databases, now recorded as Information requests, offset the continuing decline in online database searches. These specialist databases are now only used to access information not available in the public domain and to deliver specialist current awareness services. The funding approved for the third Library staff member (whilst primarily to assist with the loading of records for the web-accessible database) has facilitated an appropriate level of service and response time to the steady increase in requests received by the Library.

Document delivery

The Library can supply either books or photocopies from its collection or obtain such items for wine industry clients through the interlibrary system. Patents or standards can also be ordered. Electronic ordering and delivery services mean that most interlibrary requests are fulfilled within five days. Charges apply for the supply of some items.

Specialised information services

The Library staff continue to be actively involved in the production of specialised information products for the benefit of the wine industry, such as the annual and web-based editions of the *Agrochemicals registered for use in Australian viticulture*, the bimonthly *Technical Review* (where the Librarian is Acting Editor for editions #133 and #134), and several in-house technical information databases.

Library collection

A total of 79 monographs and seven conference proceedings were added to the library collection in 2000/2001. The Library

subscribes to 54 journals and receives approximately 70 annual reports, journals and newsletters through exchange and donation. The Library also maintains a collection of over 19,000 reprints.

Library databases

Apart from a computer-based catalogue of books and journal holdings, the Library has several specialist in-house databases, which index over 38,700 scientific and technical reprint articles; over 2,000 articles on the medical aspects of alcohol consumption; and the bibliographic details of the Library's collection of the European Union wine legislation.

The Librarian, Catherine Daniel, provides reports, either on particular subjects or authors, listing the records retrieved from any of the Library's in-house databases. A summary of the size of the Library's catalogue and information databases is given in Table 6.

The Library provides access to its databases, 24 hours a day, via the internet, to Wine and Grape Levy payers only. The restriction in access is enforced to comply with copyright approvals obtained from the various publishers. Edited database records continue to be posted onto the Library's web database, with the total number of searchable records available in excess of 10,000.

Agrochemicals Grid

As reported elsewhere in this Annual Report, Dr Sally Bell and Catherine Daniel prepared the twelfth edition of the *Agrochemicals registered for use in Australian viticulture*. All levy payers receive a printed copy of the revised edition automatically, and the web-based edition (accessible on www.awri.com.au) is updated on a regular basis.

	Wine industry	Staff	Other	Total	% inc/(dec) over 1999/2000
Information requests	515	484	1155	2154	108%
Online database searches	5	14		19	(47.2%)
Interlibrary loans					
• requests sent[1]	159	496		655	2.8%
• requests received[2]			159	159	117%
Technical Review requests[3]				167	(21%)
Technical Review articles forwarded[4]				907	(3.8%)
Articles forwarded[5]				662	40.5%
Number of Institute publications forwarded				440	18%
Articles photocopied			3795		n/a

[1] Staff at the JFML sent a request to another library for an article.
[2] Requests received by the JFML from other libraries for articles from our collection.
[3] Number of requests received for articles published in the *Technical Review*.
[4] Number of articles forwarded (usually more than one article is requested).
[5] Number of articles forwarded, excluding staff publications (usually more than one article is requested).

Table 5. Summary of information requests during 2000/2001

Technical Review

Technical Review is received by all Wine Levy paying wineries in Australia and, through subscription, by government and other organisations and individuals, both in Australia and overseas. *Technical Review* provides progress reports to the industry on the Institute's research as well as updates on relevant conferences, regulatory amendments and medical issues. *Technical Review's* 'Current Literature' section provides citation details and abstracts of recently published technical and scientific articles. Recipients of *Technical Review* may order articles featured in the 'Current Literature' section via a request form available within each issue. The Librarian, Catherine Daniel, was Acting Editor of *Technical Review's* June issue, #133. Dr Barbara Hardy AO continues to support the publication of *Technical Review* through financial contribution to the Thomas Walter Hardy Memorial Trust.

Library catalogue databases	
BOOKFILE: Books, conferences and theses	3,304
ARTICLES: scientific papers	38,737
MEDIC: medical papers	2,304
JOURNALS: journals, newsletters, statistics and annual reports	428
Library information databases	
REGS: European Community wine legislation	372
ISYS – full text retrieval database covering	
United States of America Federal Register	689
Web accessible database	**10,309**

Table 6. Number of records on the Library's catalogue, information and web-accessible databases

Rae Blair, Catherine Daniel and Heather Donnell

Email service

The Email advice and information on technical issues service continues to be a fast and cost-efficient way of disseminating important technical information to interested members of the Australian wine industry. There are over 530 email addresses recorded to receive the email bulletins to date. Twelve email bulletins were issued during the year and are shown in Table 7.

18/7/00	Launch of new Agrochemical booklet
18/7/00	Use of *Shirlan* for control of *Phomopsis*
19/9/00	Notice of First National Environment Conference
3/10/00	*Switch* Fungicide registered for control of Botrytis
4/10/00	Chemical Control of Australian Plague Locusts in Viticulture
6/11/00	Fenitrothion emergency use permit
6/11/00	Flint fungicide
6/2/01	AWRI denies claims of endorsement of NuKorc
16/2/01	Blood products for sale from Yeruva SA, Argentina
2/5/01	Non thermal food processing seminar advice
10/5/01	Eleventh Australian Wine Industry Technical Conference – updates and early registration closing date notification
16/5/01	Eleventh Australian Wine Industry Technical Conference – advice of new workshop added to program

Table 7. Email bulletins sent during 2000/2001

The John Fornachon Memorial Library Endowment Fund

The Institute acts as the Trustee of this fund, which was established in 1969 by donations from winemakers and friends of the late John Fornachon, the first Director of Research of the Institute. The Library is funded by an annual grant from the Grape and Wine Research and Development Corporation, together with the income generated from investment of the Endowment Fund.

Acknowledgments

The Institute wishes to thank all individuals and companies who contribute to the collection through donations or exchange agreements. The support of the following persons and organisations that have donated books or journals is acknowledged: Australian Bureau of Agriculture and Research Economics, Australian Dried Fruits Corporation, Australian Wine and Brandy Corporation, Australian Wine and Brandy Producers' Association, Commonwealth Scientific and Industrial Research Organisation, K.F. Pocock, Dr B.C. Rankine, Viticultural Publishing Inc., Winemakers' Federation of Australia Incorporated, Yalumba Winery.

Targeted training of wine industry personnel: compilation of a technical reference manual and delivery of complementary workshops.

Staff: Peter Godden, Mark Gishen, Adrian Coulter, Peter Valente, Ella Robinson

The primary aim of this project is to produce flexible and updateable information packages on selected technical subjects, which will be delivered to winegrape levy payers via the world-wide-web, and workshops to be held in all major grape growing areas of Australia in conjunction with the existing Roadshow seminars.

The Institute has a considerable amount of collective knowledge pertaining to grape and wine production, much of it generated over the years by research and Industry Services' projects that have been supported by industry research-levy funding. Although this research has produced many technical publications, there is also a great deal of information generated and recorded in a more informal manner by the staff concerned. This project, therefore, seeks to make a record of this information, so that it can be delivered to the industry in a manner in which it is both readily useable, and relevant to those involved in day-to-day wine production. It is envisaged that the project will be ongoing, and that many areas of interest to winery technical personnel will be addressed in due course. During the year, the first two major objectives of this project were achieved; the posting of technical information and associated detailed problem solving guide onto the Institute's web-site, and the delivery of the first workshops developed under this project, in five wine production areas in South Australia and Victoria. Both the web-based information and the workshops relate to wine instability issues. As discussed elsewhere in this report the combined and related area of hazes and deposits and microbiological instabilities continue to represent a significant proportion of the problem wine sample investigations conducted by the Industry Services team. In many cases it is apparent that the problems could have been avoided if winemakers were more aware of the causes of many types of instability, and were able to access practical and relevant tools with which to trouble-shoot the problems, before significant wine spoilage had occurred. The web site and workshops have addressed these issues, providing practical trouble shooting information and simple diagnostic tests, with which to isolate and identify a wide range of hazes and deposits in wine. The text is supported by a gallery of images of deposits in wine that have been generated from routine problem solving investigations. In addition the web site provides extensive background information on the causes of a wide range of instabilities, written by a contracted technical writer and based on the collective information generated by various teams within the Institute. The workshops also contain a large amount of practical winemaking advice regarding not only the causes of problems, but also their avoidance, and remedial advice on how to deal with them if they occur.

During the year, work commenced on new sections of the web site, particularly those which will deal with wine microbiology in more detail, with particular emphasis on the identification of both wanted and unwanted wine microflora, and good laboratory and winery practice with regard to wine microbiology issues. It is envisaged that this section will be expanded to encompass the issue of stuck fermentation, thus addressing another cause of potential wine instability, as discussed elsewhere in this report. A further section on which work has commenced is the design of a winery laboratory, an area that has obvious synergies with those already addressed, and the subject of an increasing number of advice calls to the Industry Services team.

The information on the web site (www.awri.com.au) is password-protected, for use only by Australian grape and wine research levy payers. The password has been supplied to all levy payers via personal letter. Levy payers who are unsure of the password may obtain it by contacting either the Institute's Librarian or Communications and Publicity Manager. The posting of the technical information generated by this project onto a web-site was a large undertaking, and required a substantial amount of design and development of the website in order to make it both as practical and relevant as possible, while at the same time aesthetically pleasing. It was, therefore, decided that concurrent development of the Institute's overall site would generate substantial efficiencies, rather than addressing this as a separate issue at a later date. Industry Services staff, therefore, had a high level of involvement in the development of a new web site for the Institute, in conjunction with the Institute's Communications and Publicity Manager, Director, and two external design and web-site development companies. The template design developed for material generated under this project has been successfully applied to many other areas of the site.

Waite Campus Mass Spectrometry Facility

Staff: Yoji Hayasaka, Gayle Baldock

The four important roles of the Waite Campus Mass Spectrometry Facility are to act 1) as a leader in the application of mass spectrometry to grape and wine research; 2) as an investigator to solve the problems facing the wine industry and individual

winemakers, using mass spectrometric techniques; 3) as a collaborator with The University of Adelaide in the research and teaching activities involving mass spectrometry; and 4) as a provider of versatile and advanced mass spectrometric techniques and expertise to the scientific community including public as well as private institutions. Much of our work is, therefore, conducted on a fee-for-service basis.

The Facility's instruments

The Facility accommodates four instruments namely a ThermoQuest TSQ gas chromatography tandem mass spectrometer (GC-MS/MS) installed in February 2000, a PE Sciex ionspray tandem mass spectrometer (LC-MS/MS) installed in October 1995 and two Agilant gas chromatographs with mass selective detectors (GC-MSD) installed in March 1997 and January 2001, respectively. The GC-MSDs are used mainly for grape and wine flavour projects under the supervision of Dr Alan Pollnitz, and one has been fitted recently with a Gerstel MPS2 autosampler which also has solid phase micro extraction and headspace capability. The GC-MS/MS and LC-MS/MS are used for various purposes and appropriate financial arrangements for all users are in place to recover the running cost of the Facility. Table 8 details the time usage of the GC-MS/MS and LC-MS/MS.

Problem solving work

During the reporting period, thirteen cases of chemical analysis of either juice or wine were conducted using GC-MS, in collaboration with staff of the industry services.

As mentioned in the previous annual report, the development of a taint screening method has commenced using GC-MS. This development is conducted for the projects of AWR 19 *Chemical analysis of industry technical problems and AWR 10 Technical problem solving and consulting*. In order to investigate the common contaminants frequently encountered in the wine industry, the problem solving work investigated by the Institute using GC-MS in the past 12 years was reviewed.

> *The review revealed that toluene, xylene, styrene, alkyl (C3-C4) benzene, naphthalene and alkyl (C1 and C2) naphthalene were common as contaminants in the rare faulty juices and wines submitted. These compounds are not normal constituents of grape juice and wine but they are volatile components of some petroleum products. Therefore, these compounds were nominated as target compounds of the taint screening of juice and wine. The method development is in progress.*

Research activities

Involvement in the research activity of the Institute is one of the most important roles of the Facility and its demand has been increasing in not only classic applications like volatile flavour analysis but also characterisation of macro, involatile and labile compounds like proteins, tannins and glycosidic conjugates. The following research activities highlight this function.

- The use of mass spectrometry to differentiate varieties of juice was investigated under project AWR 8 'Studies on unstable wine proteins involved in haze formation.' Varietal differentiation of juice was successfully achieved based on the composition of PR-proteins determined by electrospray mass spectrometry. This work was published in the *Journal of Agricultural and Food Chemistry* (Institute publication #663) and an oral presentation was made by Yoji Hayasaka at the 18th Conference for the Australia and New Zealand Society for Mass Spectrometry (4-9 February 2001). The application of this technique for wine is under investigation.

- Search for wine pigments in red wine using nanoelectrospray tandem mass spectrometry was conducted as part of project AWR 96/1 'Wine grape tannin and colour specification,' in collaboration with Dr Robert Asenstorfer of the University of Adelaide. Wine pigments such as a pyruvic acid adduct (vitisin A), a 4-vinylphenol adduct (pigment-A) and a vinylcatechin adduct of malvidin-3-monoglucoside have been reported in the past five years. All wine pigments are formed during and after vinification. They are substituted at the C-4 position of malvidin-3-glucoside and thought to be more chemically stable than malvidin-3-glucoside. Nanoelectrospray was used for the screening of wine pigments (anthocyanin derivatives) in red wine. The advantages of this technique over conventional electrospray are the better sensitivity, the smaller size of a sample required (1 µL) and that sufficient time for the application of various tandem mass spectrometric techniques – is available due to very low flow-rates.

The screening method for wine pigments was successfully developed using nanoelectrospray tandem mass spectrometry. This method was applied to search for new possible wine pigments in red wine extracts. Apart from the wine pigments reported above, the possible presence of malvidin-3-monoglucoside hydroxy, vinylalcohol, 4-vinylcatechol and vinylsyringol adducts were demonstrated. A manuscript outlining this study is well progressed.

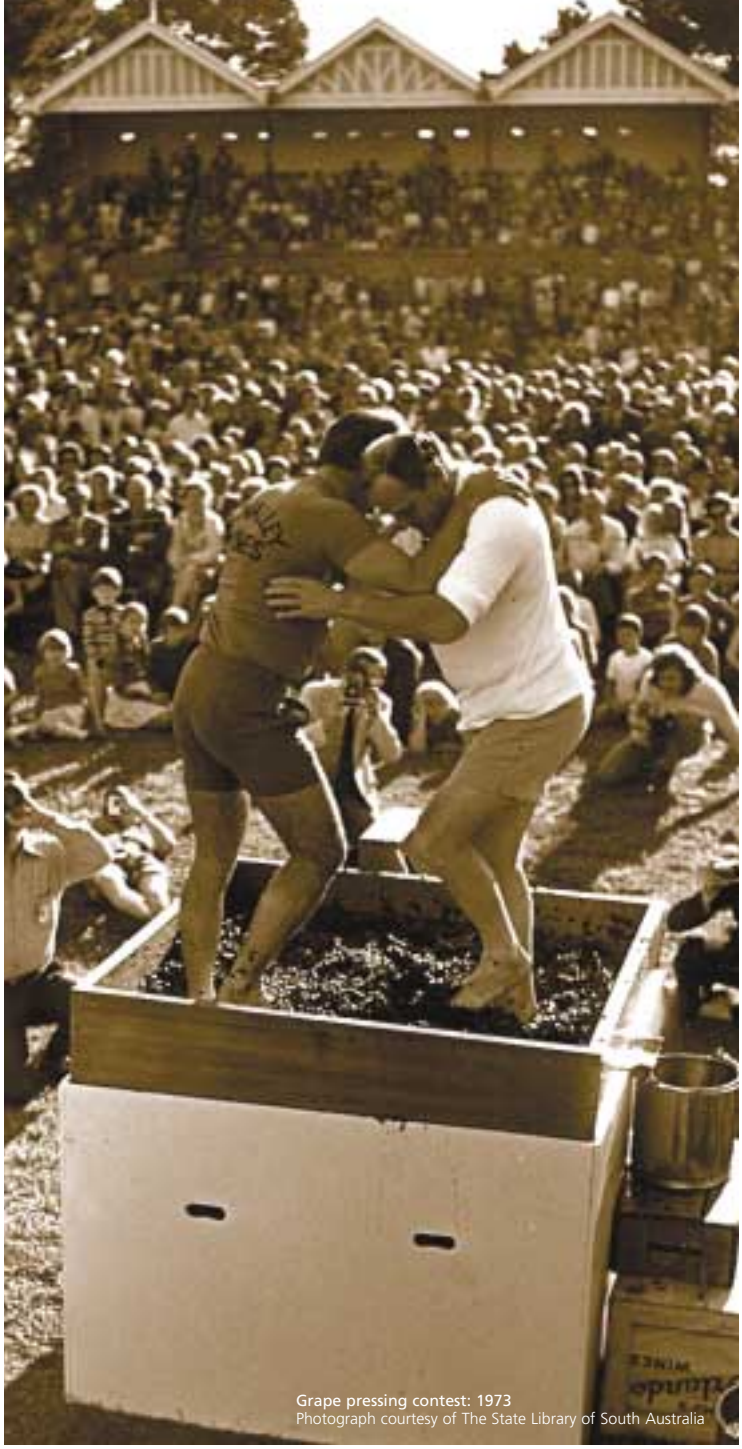
	GC-MS/MS	LC-MS/MS
Staff/students of AWRI	90%	82%
Adelaide University	10%	15%
Other Institutes	0%	3%

Table 8. Time usage of the GC-MS/MS and LC-MS/MS for 2000/2001

Characterization of highly polymerised seed tannin by electrospray mass spectrometry was conducted under the projects of AWR 96/1 and CRCV 1.2 in collaboration with Dr Stephane Vidal. The characterization of highly polymerised tannin (proanthocyanidins) is a challenging task and has been attempted using chromatographic and analytical techniques including mass spectrometry. To date, the sizes of proanthocyanidins determined by mass spectrometry were pentamer in grape seeds by liquid secondary ion mass spectrometry (Vivas 1996), heptadecamer in apple (Guyot, 1997) and docosamer in litchi (Roux, 1998) by electrospray mass spectromery and undecamer in grape seed (Kruger, 2000) by matrix assisted laser desorption ionisation mass spectrometry. In our studies, grape seed tannins were fractionated into two fractions by column chromatography according to their molecular sizes. The two fractions, LDPS (lower degree of polymerisation) and HDPS (higher degree of polymerisation), were analysed by electrospray mass spectrometry in the negative mode. The LDPS and HDPS fractions were well characterised and the presence of more than pentacosamer of procyanidin gallates in the HDPS fraction was demonstrated. These analytical techniques are sophisticated but much needed to understand better the tannin properties of wine. A manuscript outlining this study is in progress.

The considerable contribution of the Facility to the CRCV tannin projects (Program 1.2) was made in collaboration with staff of The University of Adelaide. The following outcomes were achieved.

- Asenstorfer, R.E.; Hayasaka, Y.; Iland, P.G.; Lambert, S.G.; Jones, G.P. Wine phenolics: the development of pigments in red wine. Steans, G., ed. Proceedings of the 1999 conference of the New Zealand Society for Viticulture and Oenology; 4-5 November 1999; Auckland, New Zealand Society for Viticulture and Oenology; 1999: 83-78.
- Asenstorfer, R.E.; Hayasaka, Y.; Jones, G.P. Isolation and structures of oligomeric wine pigments by bisulphite-mediate ion exchange chromatography. J. Agric Food Chem. Submitted.
- Kennedy, J.A; Hayasaka, Y.; Vidal, S.; Waters, E.J.; Jones, P.J. Composition of grape skin proanthocyanidins at different stages of berry development. Manuscript in preparation.



Grape pressing contest: 1973
Photograph courtesy of The State Library of South Australia

HPLC instumentation

Gayle Baldock and Yoji Hayasaka

Quality Liaison Manager

Staff: Mark Gishen

The major output of the Institute's activities in the provision of advice on quality management techniques to industry remains the *From Grapes to Glass* program jointly run with the South Australian Wine and Brandy Industry Association. The program was published in August 1997 and enhanced with a simple HACCP module in 1999. Industry interest remains greatest in the HACCP module — a simple program delivered in a one-day course that incorporates an HACCP-type (hazard analysis and critical control point) food safety plan. This module was designed to satisfy the requirements of the proposed (now delayed) changes to the food hygiene regulations, and meet the needs of the smaller scale businesses in the industry. Three courses for the HACCP module were conducted throughout the year, adding 20 more to the growing list of companies having attended (now totalling 73). The *From Grapes to Glass* program provides a simple and relatively cheap program that uses a staged approach in the attainment of internationally recognised standards, starting from the *Codex* HACCP principles and leading to the full ISO 9000 quality management standard. The program may soon be upgraded and simplified to reflect the changes brought in with the new year-2000 version of the ISO 9000 standard.

The *Code of good manufacturing practice* that was developed during the development of the HACCP module of the *From Grapes to Glass* program, was also published on the Institute's new web site to enhance its circulation throughout the industry.
Mark Gishen is assisting in the staging of events of interest to the wine industry including workshops on objective grape quality measurement, quality management options, HACCP plan development, and NIR spectroscopy calibration for the 11th Australian Wine Industry Technical Conference (Adelaide, October 2001), and is contributing as part of the speaker program for the same conference.

Mark Gishen takes primary responsibility for the internal quality management systems of the Analytical Service, overseeing management reviews, documentation, auditing, and corrective actions. An external reassessment of the laboraotry's quality system was conducted by NATA during this year. The method for the determination of ochratoxin A was successful in gaining accreditation, and several changes to signatory status of staff were approved. It was pleasing to note that improvements to the system that have been implemented recently meant that the system already complies with many of the elements of the revised standard, ISO 17025, ahead of the requirements of NATA. The Analytical Service continues to participate and excel in both national and international proficiency testing programs for routine wine analysis and for agrochemical residue testing. The Analytical Service continues to manage its quality management system with the aid of the *Paradigm Quality* software package and will be upgrading to a newer version release of the software in the coming year. Following the upgrade, the software will be introduced throughout the Institute, primarily as a means of assisting control and availability of policy and procedural documentation, as part of a general move toward quality management systems implementation.

As reported elsewhere, Mark Gishen remains heavily involved in the collaborative research project evaluating the use of near infrared spectroscopy (NIRS) for the rapid determination of a number of compositional parameters in grapes, must, wine and grape spirit, and recently assumed primary responsibility for the project. The details of this project are reported elsewhere in the Annual Report.

Publications and seminars given by the Quality Liaison Manager and staff working on the NIR project are shown in Appendix 1.

Communications and publicity

Staff: Rae Blair

The Communication and Publicity Manager's role is a part-time position for Rae Blair, who is also the Personal Assistant to the Director and Conference Manager of the Eleventh Australian Wine Industry Technical Conference. As Communication and Publicity Manager, she is responsible for ensuring that industry and stakeholder groups clearly comprehend the positioning (value) of the Institute. This positioning is developed in line with the Institute's mission statement and business plan objectives. Part of her role is to coordinate the Institute's printed material and other non-technical communications, and to act as a conduit for media. The Communication and Publicity Manager is also responsible for the performance and output of the John Fornachon Memorial Library. The report of the activities of the Library can be found elsewhere in this Report.

Technical Review

The Communication and Publicity Manager has overall fiscal responsibility for the production and supply of *Technical Review*, and the pricing structure of *Technical Review* was assessed in line with the introduction of GST as at 1 July 2000. During the reporting period the cost of subscribing to *Technical Review* was further considered and no price rise was deemed necessary, although the cost structure of recouping postage costs was streamlined. The subscription period was also set at expiring on 30 June each year. The Communication and Publicity Manager met with the Director, Health and Regulatory Information Manager (as Editor of *Technical Review*) and the Librarian to discuss changing the format of part of the *Technical Review* to ensure its copyright-compliance.

Coordination of media

Institute staff were interviewed by representatives from various forms of media

over the year as shown in Table 9.
On Monday and Tuesday, 5 and 6 February, the Communication and Publicity Manager circulated a press release (prepared by the Manager Industry Services and the Communication and Publicity Manager) to correct some information relevant to NuKorc, to 62 wine industry organisations and wine media Australia-wide, and to 358 email addresses via the Institute's email bulletin. The release was also published in the February edition of *Technical Review*.

Editing

The Communication and Publicity Manager assisted the Analytical Service staff by proofing their 2000-2001 Analytical Fee Schedule and with the Director she also compiled and edited the 2000 Annual Report. The Communication and Publicity Manager coordinated the production of *Agrochemicals registered for use in Australian viticulture 2001/2002*.

Institute's website

Much of the year was spent on the evaluation and implementation of the design of the new Institute website and preparation of information to be loaded onto the website. It was decided that the Institute needed a separate domain from the Adelaide University's server, and accordingly, the Communication and Publicity Manager arranged for the Institute to purchase server space from Internode, and has registered its own domain name awri.com.au

Accordingly, the address for the new website is www.awri.com.au

The sitemap was designed using four simple, user-friendly boxes: 'Research,' 'Services,' 'About Us' and 'Information.' Each box contains various sub-sections and can be accessed by rolling the cursor over the box to reveal a slide-out menu of sub-sections.

The Director had strong input into the final product, along with the Communication and Publicity Manager, the Manager – Industry Services and his staff. The site was designed by Geoffrey Reed Communications, and was facilitated by WineBiz.

Website maintenance

Various staff within the Institute are charged with ensuring the website is kept up to date. The 'Research' section is maintained by Leigh Francis, Paul Henschke, Michael Esler, Bob Dambergs and Miguel de Barros Lopes. Peter Godden (and his team), Don Buick and Sally Bell maintain the 'Services' section. Rae Blair maintains the 'About Us' section, and Creina Stockley is responsible for the 'Information' section. Dr Alan Pollnitz undertakes the actual updating of the www files in his role as part-time Computer Systems Officer.

Date	Staff	Discussed	Media
3/8/00	PWG	Brettanomyces	Tim White/*Australian Financial Review*
13/10/00	PWG	Addition of tannin to wine	Dan Sogg/*Wine Spectator*
Several media interviews were conducted by Peter Høj and Mark Gishen as a result of a stand-alone press release issued by BRL Hardy regarding the Near Infrared Spectroscopy project			
14/10/00	PBH	Research in the Australian Wine Industry	Sky TV (UK)
8/8/00	MG	Grape quality/NIR	ABC Regional SA (Country Hour)
9/8/00	MG	Grape quality/NIR	ABC Tropical North (Drive)
11/10/00	SJB	Chemical control of locusts	ABC 5CK the North & West Report
27/10/00	ADC	'Sour sob' character in Sauvignon Blanc wine	Philip White/ *The Advertiser*
6/11/00	ILF	AWRI	Stefan Maus/ *Wein-Wissenschaft*
8/11/00	ILF	Flavour chemistry	Paul Clancy/*Australian Viticulture and Australian New Zealand Wine Industry Journal*
10/11/00	PBH	Silver nitrate in wine	Jeni Port/*The Age*
12/12/00	PBH	Pesticide spray and residues	Philip Satchel/ABC 5AN
22/2/01	CSS	Blood products in wine	Natalie Larkin/ ABC Radio National
22/2/01	CSS	Blood products in wine	ABC TV News
18/1/01	PWG	Closure trial and AWRI	Mark Stean/*The Advertiser*
27/1/01	SJB	Conventional versus organic viticulture	Nick Galvin/ ABC's *Organic Gardener*
1/2/01	PBH	Impact odorants in wine	Max Allen/*The Australian*
15/2/01	PWG	Closure trial	Eric Cummins/ *National Grapegrower*
2/3/01	PWG	Closure trial	Laura Lee Madonna/ www.WorldWineTrade.com
9/3/01	ILF	Mouthfeel wheel	Mark Steene/*The Advertiser*
12/3/01	PBH/MG	Near Infrared Spectroscopy project	Natasha Mitchell/ABC
16/3/01	ILF	Mouthfeel wheel	Cindie Lange/ *National GrapeGrowers*
20/3/01	PBH	Genetically modified organisms in vines/yeasts.	Florence Kennell/*La Revue du Vine de France* and www.magnumvinum.fr
16/4/01	PBH	AWRI	Channel 9 *Directions*
16/4/01	PWG	Closure trial	Channel 9 *Directions*
31/5/01	PBH	Chemicals used in vineyards	Ken Dickin 5DN
31/5/01	PWG	Closure trial	Rose Murray Brown

Table 9. Staff interviews by media 2000/2001

Eleventh Australian Wine Industry Technical Conference

Full details of the Eleventh Australian Wine Industry Technical Conference will be provided in the 2002 Annual Report. However, during the year, Rae Blair, in her role as Conference Manager and Treasurer of Australian Wine Industry Technical Conference Inc.; spent much time liaising with international speakers, venue operators, sponsors, graphic designers and exhibition management. She is ably assisted by the Secretariat of the Conference, Carolyn Grant, who is responsible for the registration process, and who had processed a record number of 'early registrations' to the event by 8 June 2001.

The Institute's Director, Peter Høj is Chair of the Conference Planning Committee; Peter Godden, Trudy Goode and Ella Robinson are coordinating 74 workshops and Jeremy Hack is coordinating the poster display. Excellent backup is provided by our Accountant and Company Secretary.

11AWITC website

During the year, the Conference Manager purchased server space and coordinated the production and uploading of information for the website of the Australian Wine Industry Technical Conference. The website address is www.awitc.com.au.

26th World Congress and 81st General Assembly of the Office International de la Vigne et du Vin

The Communication and Publicity Manager also manages the Secretariat of the OIV Congress, Marcia Burnett. The Institute Director and the Communication and Publicity Manager are both members of the Australian OIV Planning Committee. The Communication and Publicity Manager secured accommodation for delegates; produced the logo, letterhead, first announcement and poster announcing the event in Australia, the preliminary programme (in three languages) and established a skeleton budget for the event. Further details of the Institute's contribution to the OIV Congress and General Assembly held in Australia will be shown in the 2002 Annual Report.

Analytical Service

Staff: Don Buick, John Hughes, Matthew Holdstock, Greg Ruediger, Gayle Baldock, Amanda Cook, Matthew Cream, Jeremy Hack, Radka Kalouch, Andrea Kemp, Adam Loveys, Kevin Pardon, Randall Taylor, Sandra Lloyd-Davies, Bao Tran and David Boehm

The Analytical Service is a commercial facility operating independently on a fully-costed basis within the Institute with the Manager, Mr Don Buick, reporting to the Director. It is funded by the income generated from fee earning work and contributes significantly to the financial well being of the Institute. The Analytical Service provides services to the Australian wine industry including use of the practical outcomes of the Institute's published research. Due to the nature of some of its work, the Analytical Service, under appropriate cross-charging arrangements, makes use of the expertise existing within the Industry Service team and within the Research groups to interpret or advise on results or project design.

Don Buick and John Hughes have visited clients in the McLaren Vale region and in the Margaret River region during the year as well as attending Wine Australia in Melbourne in November and the Margaret River Field Day in May. These visits plus direct mail and the placement of advertisements in industry journals and publications have assisted in the development of improved client relationships and the generation of several new clients. As a consequence, a significant increase in workload particularly from the McLaren Vale region has occurred during the 2001 vintage period. This is evident from the increased numbers of tests for malic acid performed during the year. The personal contact with winemakers, technical staff and export staff has been successful and will be continued in the new year. For the first time, the Analytical Service sponsored a trophy for 'the best tawny port' at the Adelaide Wine show in September 2000.

In October 2000, a new fee schedule incorporating details and pricing for the services offered was mailed to all clients. The terms and conditions of our services have been brought to the attention of clients through the fee schedule, the web site and in formal quotations. Formal quotations are issued for larger or non-routine jobs including clear identification of the services to be provided. Most incoming work of a routine nature has client requirements identified by letter or sample labels.

The services provided include analyses consistent with the requirements of export certification and support for the quality control activities of winemaking and viticulture often requiring more specialised skills than those commonly found in production laboratories. The Analytical Service conducted over 46,000 individual analyses on wine and grapes in 2000/2001 and covering simple analyses such as malic acid to more complex analyses such as the multi-residue screen for agricultural chemicals. The aim is to provide a range of quality analytical services that are important to industry and which can be provided in a reliable, price competitive, accurate and timely manner.

The number of tests performed by the Analytical Service increased by 3% from the previous year with significant increases in some areas of testing being offset by a lower number of tests performed as part of the Institute led closure trial during the year. Significant increases occurred in AWBC certificates, EU certificates, malic acid, sensory assessments, multi-residue assays, colour in red grapes, glycosyl-glucose in grapes and 4-ethyl phenol analyses. The number of data points produced increased by 7% providing a better measure of the increase in workload.

Analytical methods for organic acids, ascorbic acid, and methanol have been re-validated following the purchase of a new high pressure liquid chromatograph and the refurbishment of a gas chromatograph.

The sensory assessment service has been a significant growth area with large numbers of samples assessed for taints, faults or general quality parameters. New software purchased by the Institute has streamlined the recording of comments, the scoring and the reporting of sensory work. Much of the sensory work has been conducted in conjunction with chemical testing and used for dispute resolution or insurance purposes.

Staff have worked closely with the Industry Services' team to analyse the 18 month and 24 month samples from the closure trial (see report under Industry Services activities). The analysis of large numbers of free and total sulphur dioxide, dissolved oxygen and OD_{420} were performed with staff working in shifts to complete the work within two to three days of commencement.

John Hughes successfully completed the Advanced Wine Assessment Course conducted in July 2000 and has contributed to the planning of the July 2001 course to be conducted by Industry Services. Other Analytical Service staff contributed to the course including pouring of wine, cleaning up and recording of the trainee's results for evaluation.

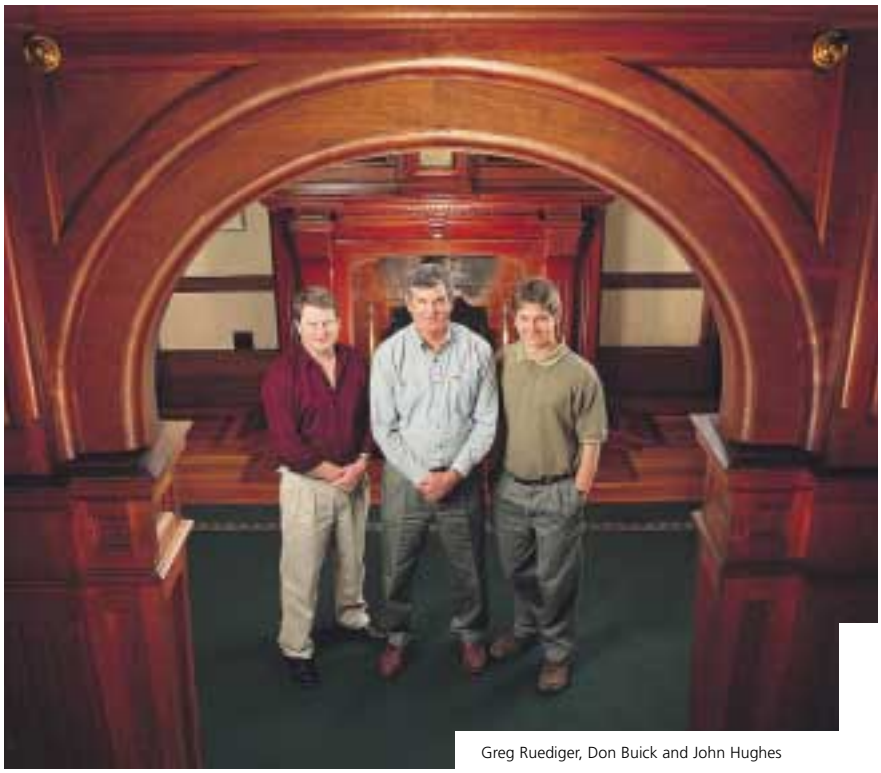
The Analytical Service was re-assessed by NATA in September of 2000 and complimented on the quality of its documentation and procedures. The method for ochratoxin A was approved and the inclusion of malathion and fenitrothion in the multiresidue assay is under review. Don Buick and Randell Taylor were approved as NATA signatories. NATA accreditation previously required compliance with ISO Guide 25. This international standard has been replaced by ISO17025 with laboratories required to meet the new standard by 2002. Compliance with ISO17025 ensures the laboratory operates in accordance with ISO9000. The assessment provided a check against the new standard and confirmed good progress towards compliance with the new standard.

Trace Analysis Laboratory

The Trace Analysis Laboratory, operating within the Analytical Service, performs more specialised testing including a multi-residue screen using gas chromatography/mass spectrometry including 30 residues, covering over 50 brand name agrochemicals. Several pesticides given special approval for use in controlling plague locusts have been included in the multi-residue screen including chlorpyrifos, carbaryl, dimethoate, malathion and fenitrothion. A multi-residue screen for several compounds unable to be

	97/98	98/99	99/00	00/01
Total number of tests	35852	39087	44846	46037

Table 10: Comparison of selected tests performed by the Analytical service by period.



Greg Ruediger, Don Buick and John Hughes

analysed by gas chromatography has been developed using high pressure liquid chromatography and includes cyprodinil, fludioxinil, pyrimethanil, fenhexamid and tebufenozide. Other methods have been developed and validated for spiroxamine, trifloxystrobin, axoystrobin and indoxacarb.

Several extended residue trials have been performed for chemical companies during the year to establish the levels of residue present or absent in grapes or wine following the application to vines of newly developed agrochemicals. These trials involve testing of grapes, small scale winemaking and analysis following development and validation of methods of analysis. The data produced is used by the chemical companies for further development of agrochemicals and for application to the National Registration Authority for registration approval. From 2003 this work will require Good Laboratory Practice (GLP) accreditation from NATA. The Trace Analysis Laboratory has begun to prepare for accreditation and Greg Ruediger is to attend a training course on GLP in the near future.

Large numbers of samples have been analysed during the year for chloroanisoles, the principle contributors to cork taint in wine and for 4-ethylphenol which is an indicator of *Brettanoyces/Dekkera* yeast spoilage activity. 4-ethylphenol is included in the oak flavour compound assay but is now offered separately when only 4-ethylphenol is required. Eugenol is now included in the oak flavour compounds assay. The relatively high concentrations of 4-ethylphenol in many samples indicates that industry practitioners seriously should consider performing this relatively cheap test on a more routine basis.

The validation of a method for the determination of histamine in wine using high pressure liquid chromatography has commenced following limited success using an ELISA kit method for this purpose. The measurement of histamine is necessary to meet the requirements for importation into Switzerland and will allow a survey of Australian wine to be performed in the new year. Little change in staffing occurred during the year. Adam Loveys formerly employed as a casual Laboratory Technician graduated from the University of Adelaide with an Honours Degree in Agricultural Science and subsequently resigned to take up a viticultural position on Kangaroo Island. Adam has worked at the Institute for several years and made a valuable contribution to the Analytical Service whilst continuing his studies. John Hughes took long service leave during the year including travelling in the winemaking regions of Germany and France. Matt Holdstock, Analytical Service Supervisor, has commenced part-time studies to complete the Graduate Diploma in Oenology. This will mean that the Analytical Service will have two degree qualified oenologists able to service industry's needs. Several other staff have attended specialised instrument training courses to ensure new equipment is well maintained and used to its maximum capability. The Analytical Service aims to support the continual upgrading of staff skills as an important element in expanding our portfolio of services.

Statement of Financial Performance

For the year ended 30 June 2001

	2001 $	2000 $
Revenue from operating activities		
Grape and Wine Research and Development Corporation		
Project funds	3,569,186	3,191,013
Capital grants		
Building	0	250,000
Equipment	426,000	722,514
Other project funds	545,348	409,742
Analytical Service	1,167,825	1,130,203
Sundry income	193,853	161,180
Expenses from operating activities		
Employee benefit expense	3,437,532	2,961,477
Consumables used	514,417	418,478
Depreciation and amortisation expense	551,179	338,130
Repairs and maintenance	99,282	72,429
Travel expenses	96,512	98,800
Borrowing cost expense	12,406	24,447
Other expenses from ordinary activities	959,196	1,092,163
Profit from operating activities	231,688	883,175
Net gain (loss) on disposal of assets		
Motor vehicles	14,935	0
Equipment	397	(495)
Profit from ordinary activities	**247,020**	**882,680**
Write back of asset revaluation reserve on reversion to measuring buildings at cost	(603,067)	0
Transfer of accumulated amortisation on buildings	125,536	0
Total changes in equity	(230,511)	882,680

Hans Muhlack

Statement of Financial Position

As at 30 June 2001

	2001 $	2000 $
Current assets		
Cash assets	920,798	120,020
Receivables	307,584	346,413
Commercial bills	670,000	648,000
Other current assets	52,326	25,621
Total current assets	**1,950,708**	**1,140,054**
Non current assets		
Leasehold buildings	1,596,247	2,102,021
Plant and equipment	1,918,127	1,960,983
Australian Wine Industry Chair of Oenology	840,000	840,000
Total non current assets	**4,354,374**	**4,903,004**
Total Assets	**6,305,082**	**6,043,058**
Current liabilities		
Payables and other accruals	924,288	436,328
Project funds not expended		
GWRDC	37,150	51,877
CRCV	13,936	0
Interest bearing liabilities	120,000	170,000
Provisions	391,490	362,822
Total current liabilities	**1,486,864**	**1,021,027**
Non current liabilities		
Provisions	90,970	64,272
Total non current liabilities	**90,970**	**64,272**
Total Liabilities	**1,577,834**	**1,085,299**
Net Assets	**4,727,248**	**4,957,759**
Equity		
Reserves	966,750	1,569,817
Retained profits	3,760,498	3,387,942
Total Equity	**4,727,248**	**4,957,759**

Statement of Cash Flows

For the year ended 30 June 2001

	2001 $	2000 $
Cash flows from operating activities		
Grants and other income	5,874,426	5,929,599
Interest received	68,845	67,825
Payments to suppliers and employees	(4,605,745)	(4,701,320)
Net cash provided by operating activities	1,337,526	1,296,104
Cash flows from investing activities		
Payment for commercial bills	(22,000)	(27,000)
Payments for building improvements	(8,858)	(684,620)
Payments for plant and equipment	(507,390)	(911,819)
Proceeds from sale of plant and equipment	51,500	49,283
Net cash used in investing activities	(486748)	(1,574,156)
Cash flows from financing activities		
Repayment of loans	(50,000)	0
Proceeds from loans	0	170,000
Net cash provided by financing activities	(50,000)	170,000
Net increase (decrease) in cash held	800,778	(108,052)
Cash at 1 July	120,020	228,072
Cash at 30 June	**920,798**	**120,020**
Reconciliation of net cash provided by ordinary activities with ordinary profit		
Profit from ordinary activities	247,020	882,680
Non cash flows in operating profit		
Amortisation and depreciation	551,179	338,130
(Profit) loss on the sale of plant and equipment	(15,332)	495
Charges to (reduction in) provisions	55,366	45,351
Changes in assets and liabilities		
(Increase) decrease in inventories	(29,332)	(12,000)
(Increase) decrease in receivables and prepayments	41,456	(68,043)
Increase (decrease) in sundry creditors and accruals	487,169	109,491
Net cash provided by ordinary activities		1,337,526

Appendix

Appendix 1. External seminars, talks and poster papers presented by institute staff during 2000/01

Author	Title	Organization/Location	Date
External seminars and talks			
K.F. Pocock, D. Tattersall[2], Y. Hayasaka, I. Dupin[1,2], B. McKinnon[1,2], R. van Heeswijck[2], P. Høj, E.J. Waters	Effects of winemaking and viticultural practices on protein instability in wine	Lincoln University Grape and Wine School, Christchurch, New Zealand	24 July 2000
Z.K. Peng, P.J. Iland[2], R. Ristic[2], R. Gawel[2,5], A. Oberholster[1,2], I.L. Francis, E.J. Waters	The influence of winemaking and viticultural practices on colour and tannins in red wine	Lincoln University Grape and Wine School, Christchurch, New Zealand	24 July 2000
P.W. Godden	Bunch rots: understanding the winemaker's dilemma	ASVO *Managing Bunch Rots* seminar Mildura Vic	28 July 2000
P.W. Godden	Persistent wine instability issues, and, Towards better barrel management – a review of the AWRI oak research	New Horizons for Wine Quality, Victorian Wine Industry Association's Seminar. Melbourne Vic	10 August 2000
P.B. Høj	What happened in the wine industry in the last 10 years that has transformed it from a shadow of Europe to the vibrant player in the global area it is today?	Committee for Economic Development of Australia's seminar 'Building of the knowledge economy' Hyatt Regency Adelaide	6 September 2000
G.M. Elsey	C13-Norisoprenoids: their generation and contribution to aroma	Flinders University weekly seminar program, Flinders University, SA	8 September 2000
P.A. Henschke	Fermentation management and importance of yeast nutrition	E. & J. Gallo Winery, Sonoma, California, USA	10 September 2000
P.B. Høj	Genetic engineering, the environment and the wine industry - a personal perspective	First National Environmental Conference Adelaide SA	1 November 2000
P.W. Godden, P.J. Costello, S.J. Bell, E.J. Bartowsky, A.D. Coulter	Roadshow seminars	McLaren Vale, SA	8 November 2000
P.B. Høj	Current trends in Australian oenological research: the quest to produce grapes and wine to a predetermined specification	South African Society for Enology and Viticulture's 2nd International Viticulture and Enology Congress, Cape Town South Africa	8-10 November 2000
P.W. Godden, S.J. Bell, M. Gishen, I.L. Francis, G.A. Ruediger, A.D. Coulter	Roadshow seminars	Adelaide Hills, SA	9 November 2000
P.B. Høj, P.W. Godden, S.J. Bell, M.A. Sefton, E.J. Bartowsky, P.A. Henschke, I.L. Francis, M. Gishen	Roadshow seminars	Barossa Valley, SA	13 November 2000

Appendix

Appendix 1. External seminars, talks and poster papers presented by institute staff during 2000/01

Author	Title	Organization/Location	Date
P.B. Høj, P.W. Godden, S.J. Bell, M.A. Sefton, E.J. Bartowsky, P.A. Henschke, I.L. Francis, M. Gishen	Roadshow seminars	Clare Valley SA	16 November 2000
P.B. Høj	An update of the use of Near Infrared Spectroscopy for measurement in grape quality parameters	Grape and Wine Industry Outreach Seminar, Adelaide University, Plant Research Centre, Urrbrae SA	17 November 2000
P.J. Costello, P.A. Henschke, A.J. Markides	Towards understanding the interactions between wine yeast and lactic acid bacteria	Grape and Wine Industry Outreach Seminar, Adelaide University, Plant Research Centre, Urrbrae SA	17 November 2000
P.A. Henschke	Wine microbiology and biotechnology projects overview	Lallemand meeting, AWRI Urrbrae, SA	20 November 2000
J.M. Eglinton	The search for alternative wine yeasts. Flavour and aroma diversity using *Saccharomyces bayanus*	Lallemand meeting, AWRI Urrbrae, SA	20 November 2000
E.J. Bartowsky	Microbial spoilage of bottled red wine by acetic acid bacteria, and, Diacetyl 'buttery' attribute of wine and MLF?	Lallemand meeting, AWRI Urrbrae, SA	20 November 2000
P.J. Costello	Mousy off-flavour spoilage of wine, and, Interaction of wine yeast and lactic acid bacteria	Lallemand meeting, AWRI Urrbrae, SA	20 November 2000
M. Esler	New technologies for rapid measurement (NIRS and FT-IR)	Interwinery Analysis Group meeting, Renmark, SA	24 November 2000
P.B. Høj, P.W. Godden, S.J. Bell, P.A. Henschke, I.L. Francis, M. Gishen	Roadshow seminars	Berri, SA	4 December 2000
M.A. Sefton, M. Raunkjaer, G.K. Skouroumounis, G.M. Elsey	Precursors to oak lactone: synthesis of gallate ester derivates of 3-methyl-4-hydroxyoctanoic acid	Annual Adelaide Organic Chemistry Symposium, Flinders University, SA	4 December 2000
P.W. Godden, S.J. Bell, P.A. Henschke, M. Gishen, A.D. Coulter	Roadshow seminars	Mildura, Vic	6 December 2000
I.L. Francis	Tannins and mouthfeel	Southcorp Winemakers' Forum Mildura, Vic	7 December 2000
P.B. Høj, P.W. Godden, A.D. Coulter	Roadshow seminars	Geelong, Vic	15 December 2000
P.A. Henschke	*Brettanomyces* - winemaking significance and prevention	Southern Pinot Noir Workshop, Methven, Canterbury, New Zealand	18 January 2001
A.P. Pollnitz, G.K. Skouroumounis, K.H. Pardon, D. Capone, M.A. Sefton	The analysis of wine volatiles derived from oak	Australian and New Zealand Society for Mass Spectrometry, 18th Conference, Coolangatta, Qld	5 February 2001

Appendix 1. External seminars, talks and poster papers presented by institute staff during 2000/01

Author	Title	Organization/Location	Date
Y. Hayasaka, K.S. Adams[2], K.F. Pocock, G.A. Baldock, E.J. Waters, P.B. Høj	Use of electrospray mass spectrometry for mass determination of grape (*Vitis vinifera*) juice pathogenesis related proteins: potential tool for varietal differentiation	Australian and New Zealand Society for Mass Spectrometry, 18th Conference, Coolangatta, Qld	6 February 2001
E.J. Bartowsky	Wine microbiology	Non-clinical meeting of the Australian Society for Microbiology, Plant Research Centre, Urrbrae, SA	28 March 2001
I.L. Francis	Relating grape composition and wine quality, and, Key grape-derived wine flavour compounds: can we define flavour specifications?	Grape Expectations: University of California, Davis, California, USA	11 April 2001
C.S. Stockley	A role for wine in the diet	Department of Clinical Dietetics' continuing education program, The Royal Adelaide Hospital	11 April 2001
I.L. Francis	Near infrared for rapid grape analysis	Grape Expectations: University of California, Davis, California, USA	12 April 2001
E.J. Waters	Program 1: Vineyard management to meet grape quality specifications	Cooperative Research Centre Board, Plant Research Centre, Urrbrae, SA	9 May 2001
M.J. Herderich	Winegrape tannin and colour specification	CRCV Second Year Review, Plant Research Centre, Urrbrae, SA	22 May 2001
P.B. Høj	Advice to young scientists, a personal perspective	Adelaide University Science Scholars	29 May 2001
I.L. Francis	Wine flavour and sensory analysis	Australian Institute of Food Science and Technology (Victoria) Sensory Evaluation Group, Werribee, Vic	30 May 2001
P.B. Høj	Use of research outcomes for the management of problems and opportunities	BRL Hardy Vintage Conference, Lake Crackenback, NSW	4 June 2001
E.J. Waters, K.F. Pocock, D. Tattersall[2], Y. Hayasaka, I. Dupin[1,2], B. McKinnon[1,2], R. van Heeswijck[2], P.B. Høj[1,2]	The unstable proteins of wine: a summary of their properties and of factors influencing the levels of these proteins in wine	Unité Mixte de Recherche Sciences pour l'Oenologie, INRA, Montpellier, France	13 June 2001
P.B. Høj	A general introduction to The Australian Wine Research Institute and an outline of selected research activities	Unité Mixte de Recherche Sciences pour l'Oenologie, INRA, Montpellier, France	13 June 2001
S. Vidal	Sensory features of grape tannins	In Vino Analytica Scientia, Bordeaux, France	15 June 2001
E.J. Waters, K.F. Pocock, D. Tattersall[2], Y. Hayasaka, I. Dupin[1,2], B. McKinnon[1,2], R. van Heeswijck[2], P.B. Høj[1,2]	The unstable proteins of wine: a summary of their properties and of factors influencing the levels of these proteins in wine	In Vino Analytica Scientia, Bordeaux, France	15 June 2001
M.J. Herderich	Red wine colour and mouthfeel: analysis of phenolic pigments and tannins	American Society of Enology and Viticulture Annual General Meeting, San Diego, USA	29 June 2001

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Appendix 1. External seminars, talks and poster papers presented by institute staff during 2000/01

Author	Title	Organization/Location	Date
Posters			
M.B. Esler, M. Gishen, I.L. Francis, R.G. Dambergs, A. Kambouris, W.U. Cynkar, D.R. Boehm	Effects of variety, region and season on near infrared reflectance spectroscopic analysis of quality parameters in red wine grapes	10th International Conference on Near Infrared Spectroscopy, Kyongju, South Korea	10-15 June 2000
R.G. Dambergs, A. Kambouris, N. Schumacher, I.L. Francis, M.B. Esler, M. Gishen	Wine quality grading by near infrared spectroscopy	10th International Conference on Near Infrared Spectroscopy, Kyongju, South Korea	10-15 June 2000
Workshops			
S-J. Bell	Spray application in viticulture	Research to Practice™, McLaren Vale, SA	15 August 2000
S-J. Bell	Spray application in viticulture	Research to Practice™, McLaren Flat, SA	17 August 2000
S-J. Bell	Grapevine nutrition	Research to Practice™, Langhorne Creek, SA	26 September 2000
S-J. Bell	Integrated Pest Management	Research to Practice™, McLaren Vale, SA	7 November 2000
P.W. Godden, A.D. Coulter, P. Valente, E.M. Robinson, M. Gishen	Identification and management of wine instabilities	McLaren Vale, SA	8 November 2000
S-J. Bell	Grapevine Nutrition	Research to Practice™, Barossa Valley, SA	11 November 2000
S-J. Bell	Grapevine Nutrition	Research to Practice™, McLaren Vale, SA	13 November 2000
P.W. Godden, A.D. Coulter, P. Valente, E.M. Robinson	Identification and management of wine instabilities	Barossa Valley, SA	14 November 2000
P.W. Godden, A.D. Coulter, P. Valente, E.M. Robinson	Identification and management of wine instabilities	Clare Valley, SA	17 November 2000
P.W. Godden, A.D. Coulter, P. Valente, E.M. Robinson	Identification and management of wine instabilities	Berri, SA	5 December 2000
P.W. Godden, A.D. Coulter, P. Valente, E.M. Robinson	Identification and management of wine instabilities	Mildura, Vic	7 December 2000
Advanced Wine Assessment Course			
P.W. Godden, E. Robinson J.B. Hughes, M. Gishen, A. Loveys, A.D. Coulter I.L. Francis		Adelaide SA	18-21 July 2000

Appendix 2. Teaching responsibilities of Institute staff during 2000/01

Subject	Number of lectures	Institute staff
2000—Semester 2		
Adelaide University		
9685 Advances in Oenology	2	Dr M.A. Sefton
9685 Advances in Oenology	2	Dr I.L. Francis
9685 Advances in Oenology/		
9086 Advances in Oenology (supplemented)	3	Dr P.A. Henschke
9685 Advances in Oenology/		
9086 Advances in Oenology (supplemented)	2	Dr E.J. Bartowsky
9685 Advances in Oenology/		
9086 Advances in Oenology (supplemented)	1	Dr P.J. Costello
9685 Advances in Oenology	1	P.W. Godden
1958 Wine packaging and quality management	2	C.S. Stockley
1958 Wine packaging and quality management	1	M. Gishen
The Flinders University of South Australia		
MMED 3921 Industrial and Pharmaceutical Microbiology	1	Dr P.A. Henschke
2001—Semester 1		
Adelaide University		
2580 Stabilisation and Clarification	3	Dr E.J. Waters
2580 Stabilisation and Clarification	1	P.W. Godden
1005/3113 Winemaking	2	Dr P.A. Henschke
1958 Wine Packaging and Quality Management	1	M. Gishen
2213 Grape Industry Practice Policy and Communication (subject coordination and delivery for five weeks)	~ 40 hours	Professor P.B. Høj and C.S. Stockley
5693 Wine and Marketing in Society	6	C.S. Stockley

Appendix

Appendix 3. Graduate and Honours student supervision responsibilities of Institute staff for 2000/01

Student	Supervisor/s	Source of funds
Honours		
C. Day	P.B. Høj, S.J. Clarke[2]	Adelaide University
A. Matthews	E.J. Bartowsky, I.L. Francis	Adelaide University
M. Mercurio	G.K. Skouroumounis, G.M. Elsey	Flinders University
M.A. Smith	G.M. Elsey	Flinders University
A. Walkenhorst	I.L. Francis, R.G. Dambergs	Adelaide University
Masters		
D. Coates	E.J. Bartowsky, R. Gawel[2,5]	Adelaide University
PhD		
S.L. Brown	M.A. de Barros Lopes, E.J. Waters, P.B. Høj	GWRDC
F. Carrau	P.A. Henschke, E. Dellacassa[8]	University of the Republic of Uruguay staff member
N. D'Incecco	E.J. Bartowsky, P.A. Henschke	Italian PhD Scholarship
J.M. Eglinton	P.A. Henschke, P.R. Langridge[6]	Institute staff
R. Gawel	I.L. Francis, A.J. Markides[2,4]	DHVO staff
W. Greenrod	M. Fenech[9], M.Abbey[9], M.A. Sefton, P. Burcham[10]	GWRDC
S. Hoffmann	P. Winterhalter, C.S. Stockley	University of Braunschweig
K.S. Howell	P.A. Henschke, E.J. Bartowsky, G.H. Fleet[3]	APRA scholarship
A. Janusz	G.M. Elsey, M.A. Sefton, M. Perkins[7]	CRCV
D. Lee	G.P. Jones[2], E.J. Waters	CRCV
A. Oberholster	E.J. Waters, I.L. Francis, G.P. Jones[2], P.G. Iland[2]	GWRDC
C.J. Puglisi	G.M. Elsey, M.A. Sefton, R. Prager[7]	CRCV
R. Ristic	P.J. Iland, I.L. Francis	Adelaide University
P. Sivilotti	S.J. Bell	Italian Government and University of Udine
H. Smyth (nee Pain)	I.L. Francis, M.A. Sefton	GWRDC
K.L. Wilkinson	M.A. Sefton, R. Prager[7], G.M. Elsey	GWRDC

Theses completed

Student	PhD/Honours	Title	Supervisor/s
Robert Asenstorfer	PhD	Isolation, structures and properties of anthocyanins and oligmeric pigments in wine	Dr G.P. Jones[2], Dr P.J. Iland[2], Dr E.J. Waters
Patrick Iland	PhD	A study of glycosides in grapes and wines	Dr B. Coombe[2], Dr P.J. Williams, Professor P.B. Høj
Patrick Jones	PhD	Glucosyl transferases involved in the biosynthesis of cyanogenic glucosides in *sorghum bicolor*	Professor P.B. Høj, Dr B.L. Møller[11]
Alan Pollnitz	PhD	The analysis of volatile wine components derived from oak products during winemaking and storage	Dr M.A. Sefton, Dr G.P. Jones[2]
Christopher Smyl	PhD	L-Proline: a potential nitrogen source for yeast *Saccharomyces cerevisiae* under anaerobic conditions	Dr P.A. Henschke, Professor P. Langridge[6], Dr M.A. de Barros Lopes
Vanessa Stockdale	PhD	Biochemical analysis of mannoproteins associated with haze protection in white wine	Dr E.J. Waters, Dr P.J. Williams, Dr G.B. Fincher[6]
Nicholas Yap	PhD	The sensitivity of yeasts to killer yeast toxin, with focus on the killer yeast *Pichia membranifaciens*	Dr P.A. Henschke, Dr M.A. de Barros Lopes, Professor P. Langridge[6]
Julie Campbell	Honours	The influence of chip size, heating processes and other factors on the impact of oak chips on wine composition	Dr A.P. Pollnitz, Dr M.A. Sefton
David Dowden	Honours	Effect of polysaccharides on Grenache wine instability	Dr G.P. Jones[2], Dr E.J. Waters
Freya Hohnen	Honours	Changes in seed phenolic composition during berry maturation and throughout fermentation/extended maceration of *Vitis vinifera* cv. Cabernet Sauvignon	Dr P.J. Iland[2], Dr Z.K. Peng
Helen McCarthy	Honours	Evaluation of the ability of the chemical sensory nose to determine the aroma profiles of wines which have undergone malolactic fermentation	Dr E.J. Bartowsky, Dr I.L. Francis
Marie Pearce	Honours	Optimising diacetyl levels in wine	Dr E.J. Bartowsky, Dr A.J. Markides[2,4]
Nina Viergutz	Honours	The interaction between wine yeast and lactic acid bacteria	Dr P.J. Costello, Dr A.J. Markides[2,4]

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[3] Department of Food Science, University of New South Wales;
[4] now with Lallemand Australia;
[5] now with Roseworthy Wine Tasting Programs Pty Ltd;
[6] Department of Plant Science, Adelaide University;
[7] Flinders University;
[8] University of the Republic of Uruguay,
[9] CSIRO Health Sciences and Nutrition,
[10] Department of Clinical and Experimental Pharmacology, Adelaide University,
[11] Royal Agricultural University of Copenhagen

Appendix

Appendix 4. Institute staff publications 2000/01

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Appendix 5. Institute Committees

Staff member	Management Advisory	Research Steering	Industry Services Steering	Communication Steering	Analytical Service Steering	Quality System Review	Information Technology	Biosafety	Occupational Health and Safety	Super-annuation	Staff Code Negotiation
Peter Høj	C	C	X	X	X		X				X
Eveline Bartowsky							X	X	X		X
Sally-Jean Bell	X	X		X	X						
Rae Blair	X			C							
Shauna Brown									X		
Don Buick	X		X		X	X					
Dimitra Capone									X		
Adrian Coulter	X		X		X	X					
Catherine Daniel				X							
Miguel de Barros Lopes	X	X						X			
Jeff Eglinton				X			C	C			
Leigh Francis	X	X	X							X	
Mark Gishen	X	X	X		C	C					
Peter Godden	X	X	C	X	X	X					
Jeremy Hack							X				
Yoji Hayasaka	X	X	X								
Paul Henschke	X	X			X				C		
Markus Herderich	X	X									
Matthew Holdstock						X					
John Hughes						X			X		
Hans Muhlack	X									X	X
Ingrid Oats											X
Ken Pocock									X	C	
Alan Pollnitz							X				
Greg Ruediger						X					
Mark Sefton	X	X			X						
Creina Stockley		X		X							
Elizabeth Waters	X	X									

C = denotes holder of Chair

The staff of The Australian Wine Research Institute

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