Australian Government

Australian Grape and Wine Authority

The Australian Wine Research Institute

Agreement for Industry Capability Building Activities and Research and Development Program 2013-2017

Final Report to Wine Australia

Research Organisation:

The Australian Wine Research Institute

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Introduction

This report details the key outcomes resulting from the 2013 – 2017 funding agreement between the Grape and Wine Research and Development Corporation (GWRDC) and the Australian Wine Research Institute (AWRI). On 1 July 2014, the GWRDC and Wine Australia merged to form the Australian Grape and Wine Authority (AGWA), which operates under the name of Wine Australia.

The primary objective of the funding agreement was the delivery of research projects and industry capability building activities for the benefit of the Australian grape and wine industry. The agreement specifically sought to enhance the AWRI's ability to deliver those objectives by fostering a strong partnership between Wine Australia and the AWRI, and through that co-operation to progress the commercialisation of project outcomes and to generate intellectual property. The agreement also recognised the potential for the AWRI to collaborate with other Australian and international research institutions and companies, in order to optimise benefits for the Australian industry.

The agreement, which covered some but not all of the Wine Australia-funded activities at the AWRI during this period, provided net Wine Australia funding to the AWRI of $24,126,126 over four years, with the AWRI and other organisations contributing a further $3,910,759. This Wine Australia funding was pivotal to the conduct of many projects in the AWRI's 2013 – 2018 Research, Development and Extension Plan (RD&E Plan), and Schedule 2 of the funding agreement comprised the project summaries and output targets for each project funded under the agreement. The progress against each target was reported to Wine Australia and discussed by a joint management committee on a quarterly basis, with final reports being presented in this document. Almost all of the Performance Targets in the funding agreement were achieved in their original form, or an agreed modified form, and in many cases were exceeded.

The AWRI's RD&E Plan was developed during thirty-six meetings with grape and wine producers and other stakeholders held around Australia, and was aligned with the GWRDC's 2012 – 2017 Strategic Plan, and other industry and government strategic directions. The Plan focused on five themes: Environment and Sustainability; Consumers, Customers and Markets; Improving Products and Processes; Extension and Adoption; and Service Capabilities and Foundational Datasets.

Individual projects were led by the AWRI, which is an internationally recognised centre of excellence in grape and wine RD&E, and were pursued according to the AWRI's stated values of Excellence, Integrity and Passion. The breadth and depth of the AWRI's RD&E capabilities allowed extensive integration between projects, to more effectively address the priorities identified by industry. However, wherever possible, collaborations were developed with other agencies, and with grape and wine producers, if they were deemed to lead to the faster, higher quality, or more cost-effective delivery of project objectives, in line with the stated objectives of the funding agreement.

Investment provided by Wine Australia under the 2013-2017 agreement, also led to an enhancement of the AWRI's pre-existing and proven extension and information dissemination mechanisms, and resulted in a constant flow of new information and knowledge to industry. AWRI staff members also worked 'hands-on' with grape and wine producers, to ensure that the information and knowledge was used to drive tangible adoption and innovation.

On the formation of AGWA, the investment framework and four-year duration of the funding agreement on which this report is based was reviewed by both parties with reference to the preceding agreement, which had a term of seven years and took a different approach to governance

and the practical conduct of RD&E projects. This review yielded useful insights into the optimal structure for partnerships between Wine Australia and the AWRI.

It was recognised that longer-term agreements and a modified investment framework provided greater longer-term surety and reduced risk when establishing projects, and increased the ability of both parties to adapt to changing industry needs in the delivery of research projects and industry capability building activities.

In addition, the AWRI's and collaborating agencies' ability to attract, retain, and develop world-leading researchers and technical experts was increased, resulting in the enlargement of that pool of talent and capability within Australia, to the benefit of Australian industry and society. The development of longer-term relationships between researchers, industry, and other partners was also enhanced, resulting in more resolved project outcomes, and stronger starting points for future collaborations.

The review also concluded that a longer-term funding agreement and investment framework resulted in greater productivity and the maximisation of investment of industry funds in value-adding activities, as opposed to the increased non-value-adding activity which inevitably results from shorter-term agreements.

The review was instrumental in the formation of a renewed partnership agreement for 2017 – 2025, with a revised RD&E Plan, which took effect on 1 July 2017.

Through the activities reported here, the international reputation of Wine Australia and the AWRI was enhanced and, during the investment period, the AWRI became a regular contributor to the itineraries of Wine Australia's inbound visitors. Feedback strongly indicates that the showcasing of the industry's RD&E achievements was highly valued by those visitors, thereby enhancing the overall reputation of Australian wine in the eyes of influential writers, buyers, importers and retailers.

The key objectives of the investment agreement have been achieved, and the report presented here demonstrates the value of the long-term relationship forged between Wine Australia and the AWRI. Through the joint pursuit of strategic and integrated RD&E by the two organisations, timely, relevant, and value-adding outcomes for industry were achieved, and the new knowledge generated has resulted in improved practices, process efficiencies, greater sector profitability, and an enhanced product offering which is increasingly recognised around the world. Through its partnership with Wine Australia, the AWRI is proud to play its part in the ongoing success of the Australian grape and wine sector.

Cost/Benefit Evaluation

This section aims to provide a high-level assessment of the practical implications of the research results for the Australian wine sector. It also contains insights into the benefits provided more broadly to grapegrowers or winemakers, including impacts of various AWRI extension platforms.

The AWRI did not undertake formal Cost Benefit Analyses (CBAs) for this final reporting period, as Wine Australia is required to undertake a more formalised approach to assessing projects via the agreed Council of Rural Research and Development Corporation Chair's approach using a random selection process across their whole portfolio.

Working with Wine Australia, the AWRI obtained copies of reports commissioned by Wine Australia on a range of different CBAs. The first report was titled 'Wine Australia: Ex post cost benefit analysis of selected R&D projects supported by Wine Australia/GWRDC/AGWA investment' reported by IDA Economics Pty Ltd in June 2016. Wine Australia commissioned IDA Economics Pty Ltd to undertake the ex post cost benefit study of a range of selected projects funded by the Grape and Wine R&D Corporation (GWRDC)/Australian Grape and Wine Authority (AGWA)/Wine Australia. Seventeen projects were selected by Wine Australia, covering flavour, refrigeration, trunk disease and nutrition, of which the only AWRI projects assessed were in the flavour and refrigeration areas.

Wine Australia identified the following five projects on flavour for CBA analysis.

- **AWRI 06-01 stream 1.1: Defining and controlling important volatile compounds in wine and their impact on wine aroma and flavour (AWRI-led)**
- **CRV 6AWR: Viticultural control of flavour compounds in wine (AWRI-led)**
- CSP 05/04: Vines to wine - linking fruit quality to wine flavour and aroma (CSIRO-led)
- CSP 0905: Understanding and managing the timing of berry ripening and the flavour-ripe/sugar-ripe nexus (CSIRO-led)
- UWA 04/01: Environment, site and selected management influences on the composition and sensory characteristics of Chardonnay growing in selected sites within a coastal, cool Mediterranean climatic region (University of Western Australia-led)

The 'flavour' projects covered selected flavour and aroma R&D undertaken by CSIRO, the AWRI the University of Western Australia and the CRC for Viticulture between 1999 and 2010. Taken together the investment was broadly described as being both of an applied (direct) benefit and of strategic benefit. A prime focus was on developing an analytical base to better assess flavours and aromas and enabling growers and winemakers to address specific taint issues. The R&D investment in this flavour area was considered to have delivered:

- capability in research and analysis
- analytical tools
- targeted future research directions (i.e. a focus for future R&D)
- some, but limited, specific management recommendations for grapegrowers/winemakers.

Through this analysis the potential industry benefits arising from the better understanding of flavours and aromas included both cost savings (for example avoiding processing wine that has a significant adverse taint) and potential revenue increases for producers. The estimated benefits were a minimum, since a range of other benefits were not included in this analysis.

The investment returns calculated are summarised in Table 1.

Table 1. Investment in flavour R&D: Investment analysis (2015 NPV, 5% discount rate)

Benefits ($m)	$370
Costs ($m)	$38
Net Benefits ($m)	$332
B/C	10
IRR	33%

Given that the 2013-2018 GWRDC/Wine Australia investment agreement contained a similar project, (Project 3.1.1 Identification and origin of volatile compounds responsible for important wine sensory attributes), a similar benefit to cost ratio of 10:1 may be expected in the future for this project, with some outcomes having significantly shorter lead times where benefits can be realised by industry.

In addition to these Wine Australia CBAs, the AWRI helpdesk (Project 4.1.2– Specialised technical troubleshooting and responsive helpdesk services for the Australian wine sector) conducted some evaluative activities to understand its broader industry impact, including a form of CBA. As part of the investigation service offered through the helpdesk, each client was surveyed once their investigation had been completed. The survey response rate was approximately 9% of investigations.

The survey results indicated:

- 86% of respondents rated the access to the helpdesk as easy, or very easy
- 88% of respondents were satisfied or very satisfied that their problem had been resolved
- 83% of respondents indicated that it was easy to understand the solution to their problem
- 70% of respondents were able to avoid product loss through contacting the AWRI helpdesk
- the potential value of wine involved in the 860 investigations conducted was estimated to be $69m
- 97% of respondents indicated the suggestions provided by the helpdesk team were helpful
- 72% of respondents indicated that they would change their practice based on recommendations provided by the helpdesk team to prevent a repeat of the problem.

Further to this survey a basic economic return on investment was calculated, with each client asked to indicate how much wine was involved in the investigation conducted and the quality grade of the wine involved. Using these two figures an approximate cost of the wine involved in the investigation could be estimated, where bulk wine was assumed to be $0.98 per litre, premium wine was assumed to be $5.94 per litre and ultra-premium wine $14.81 per litre based on export approval data (sourced from Wine Australia). The value of wine involved in the investigations (potential direct savings) ranged from $0 to $594,000 with a mean value of $81,029. Extrapolation of the data to the total number of investigations conducted (860) suggests the potential direct savings over the project was $69,685,083. This equates to a benefit to cost ratio of 25:1 on a $2,750,418 investment from Wine Australia over the investment period. It should be noted, however, that while this figure of 25:1 is based on the work of the helpdesk as a discreet project, in reality the success of the project is highly reliant on the rest of the work at the AWRI and elsewhere which informs the investigations conducted and advice given.

While every effort has been made to communicate and facilitate uptake of R&D in a timely manner over the life of this agreement, the full value of a project's outputs and the associated tangible benefits will be realised over an extended period ahead. Hence the AWRI has focused on qualitative evaluation approaches of project benefits, seeking to map the investment agreement projects to Wine Australia strategies and collecting key outputs, outcomes and, where possible, evaluation metrics. Table 2 captures the key outputs and outcomes from each project classified according to the original GWRDC theme/area and the new Wine Australia strategy.

Table 2. table of major project outputs, outcomes and benefits from 2013-2018 Wine Australia/GWRDC – AWRI investment agreement

AWRI Project	GWRDC theme /stream	Wine Australia strategy	Key project outputs, outcomes and benefits
Project 2.2.1 – Collecting and disseminating information regarding agrochemicals registered for use and maximum residue limits in Australian viticulture	1.4 Pest and disease management	4 Biosecurity, pest and disease management	• 850 Sanitary and Phytosanitary (SPS) notifications and 110 APVMA gazettes were reviewed for viticulture-relevant information and the information was used to update the 'Dog book' and associated online resources. • More than 30,000 'Dog books' were distributed to the Australian grape and wine community. • As part of ongoing efforts to have an MRL established for phosphorous acid, a major dossier was submitted to the Joint Meeting of Pesticide Residues. • Residues in wine were averted after changes to the residue definition for captan in the EU were identified and acted upon. • A 'Dog book' app was developed for Apple and Android devices. Since the launch of the app there have been more than 5,412 downloads. • The following active constituents were reviewed and evaluated: amisulbrom, boscalid, captan, clothianidin, cyflufenamid, diuron, fenamifos, fenpyrazamine, fluazinam, flumioxazin, fluopyram, phosphorous acid, proquinazid.
Project 2.2.3 – Informing Australia's wine consumers through understanding issues of wine consumption, health and nutrition	2.2 Market Access	2 Regulatory Services and 7 Market Access	• Nine peer-reviewed papers, two book chapters and six articles were published on a range of wine and health/nutrition related issues. • In May 2016, a paper authored by the AWRI and collaborators in Italy entitled 'The case for anthocyanin consumption to promote human health: a review' won a Tanner Award as the most-cited paper published in *Comprehensive Reviews in Food Science and Food Safety* in 2013. • Five industry fact sheets were produced on wine and cardiovascular diseases, cancers, cognitive decline/dementia, diabetes and obesity; twelve complementary AWRI wine and health fact sheets and frequently asked questions were revised and disseminated via the AWRI website.

Project 2.2.4 – Increasing Australia's influence in market access, safety, regulatory and technical trade issues	2.2 Market Access	2 Regulatory Services and 7 Market Access	• Tentative provisions for additives metatartaric acid and yeast mannoproteins have been developed by the WHO/FAO Joint Expert Committee on Food Additives (JECFA) based on submissions prepared by the project team and submitted on behalf of WFA, WA, DAWR and OIV. Together with the toxicological evaluation, they will be presented next to the Codex Committee on Food Additives for its consideration and adoption. • Following an AWRI submission, Food Standards Australia New Zealand provided permission for the use of specific enzyme processing aids, Aspergillopepsin I and II, in Australian winemaking. • Potassium carbonate has been included in the *International Code of Oenological Practices of the OIV*, authorised for the reduction of titratable acidity and actual acidity in winemaking • Processing aids dimethylpolysiloxane and Aspergillopepsin I and II (Proctase) have advanced to Step 5 in the 8-step resolution process of the OIV prior to inclusion in the *International Code of Oenological Practices*. • Analytical and other supporting scientific data were provided which prevented the potential for establishment of an OIV maximum limit for phthalates in wine. • Analytical and other supporting scientific data were provided which promoted the revoking of a maximum limit for manganese in wine internationally. • The AWRI's online databases were expanded to contain information on analytical requirements for the export of Australian wine specific to 44 individual countries and trading blocs, and on permitted additives and processing aids for winemaking and wine importing countries for 28 individual countries.
Project 3.1.1 – Identification and origin of volatile compounds responsible for important sensory attributes	3.1 Objective measures of quality and assessment systems	3 - Wine provenance and measures of quality	• The main compound known to be responsible for 'green' flavour in Cabernet Sauvignon, isobutyl methoxypyrazine, which was previously thought not to be biosynthesised in Shiraz grapevines. was found to contribute 'green' aroma in Shiraz wine made with stems, notably in whole bunch fermentations. • Nearby windbreak trees were found to contribute 'green' flavours to harvested grapes and thus wines. • The monoterpenes geraniol, linalool and nerol were found to give Viognier wines their distinctive 'apricot-like' flavour. • Thiols were confirmed as major contributors to Chardonnay flavour, with 3-mercaptohexanol and 3-mercaptohexyl acetate contributing, at moderate levels, 'citrus fruit' character and at higher levels 'tropical' aromas, and benzyl mercaptan adding 'flint'/'struck match' aromas.

Project 3.1.3 – Flavour precursors: contribution to wine aroma, in-mouth sensory properties and flavour release	3.1 Objective measures of quality and assessment systems	3 - Wine provenance and measures of quality	• Glycosides in wine, previously considered to be flavourless, were shown to release flavour during consumption, enhancing fruit characters and aftertaste without giving any bitterness. • The flavour release was highly variable across individuals, providing further insight into the possible reasons for individual differences in perception and wine style preferences. • Increasing glycoside concentration in wines, by addition of glycosides isolated from grape skins from a floral variety high in monoterpene glycosides using a simple procedure, gave increased 'fruit'/ 'floral' aroma and flavour with no bitterness and has potential as a practical means of enhancing flavour in some wine types.
Project 3.1.4 – Factors affecting wine texture, taste, clarity, stability and production efficiency	3.1 Objective measures of quality and assessment systems	3 - Wine provenance and measures of quality and 6 - Efficient winery production	• Compositional drivers for texture, hotness and bitterness were identified. • Improved tests for haze and potential alternatives to bentonite were identified. • New understanding was gained that winery filtration processes do not affect macromolecules important for texture. • Ways of lowering alcohol without losing tannin and texture in wine were identified. • A new tannin extraction method for grapes was developed that provides prediction of wine tannin. • New methods were applied to understand wine texture and stability.
Project 3.2.5 – Safeguarding and realising the potential of the Australian wine microbial germplasm collection	3.2 Germplasm (yeast and bacterial)	6 - Enhanced yeast and bacterial performance	• The AWRI wine microorganism culture collection (AWMCC) now contains more than 10,000 wine-related microorganisms with some isolates dating back almost 80 years (more than 3,000 natural yeast isolates and laboratory-modified yeast strains for research; a wine yeast genome deletion library of more than 1,700 strains; a laboratory yeast genome deletion library of around 4,800 strains; and more than 1,100 bacterial strains, the majority of which are malolactic bacteria). • During the investment period, the AWMCC received 1,301 microorganisms from industry and researchers (1,012 yeast and 289 bacterial strains) and 2,423 microorganisms were supplied to industry and researchers (1,804 yeast and 619 bacterial strains). • A back-up collection was established at a secure offsite facility (TechinSA) to ensure the ongoing integrity of the collection in the event of a catastrophic failure of the storage facilities at the AWRI. • A total of 1,763 deletion strains from the wine yeast deletion library were provided to researchers at the University of Adelaide for a Wine Australia-funded project. • A total of 83 previously difficult-to-classify yeast strains were identified, and more than 400 microbial isolates were provided for genome sequencing projects.

Project 3.3.2 – Influencing wine style through management of oxygen during winemaking	3.1 Objective measures of quality and assessment systems and 3.3 Process efficiency	3 - Wine provenance and measures of quality and 6 Efficient winery production	• Practical methods of measuring oxygen in the winery were evaluated and selected sensors were recommended for different situations. • Practical tools to aerate ferments were developed and there was good evidence of early industry adoption of these methods following the 16th AWITC. • New understanding was gained that the impacts of oxygen in white wine production can be positive. • New understanding was gained that oxygen in red wine production can provide beneficial tannin softening and curtail the production of reductive aromas.
Project 3.5.3 - Formation and fate of positive and negative sulfur compounds	3.1 Objective measures of quality and assessment systems and 3.3 Process efficiency	3 - Wine provenance and measures of quality and 6 Efficient winery production	• Key precursors to several key low molecular weight sulfur compounds (LMWSCs) were identified. • The addition of copper was found to significantly influence the evolution of LMWSCs. Oxygen concentration played a significant role in copper's effect on the formation of LMWSCs in wine. • New knowledge was gained about managing copper and its impacts through the timing of its addition. • The formation of LMWSCs from their precursors in wine was found to be influenced by the presence of not only copper, but also other metal ions that naturally occur in wine, when they are present in high concentrations. • New understanding was gained of the differences among wine yeast in their ability to release both positive and negative LMWSCs.

Project 4.1.1 – The staging and conduct of extension programs	4.1 Adoption	10 - Extension and adoption	• 118 roadshow events were delivered over the four years reaching 3,212 attendees, averaging 27 attendees per event.
			• 90 webinars were delivered to 1,463 attendees, averaging 16 attendees per event.
			• 237 articles and webpages were prepared.
			• Three new workshops were prepared and delivered:
			  ○ *Adapting to difficult vintages* – providing solutions to growing grapes and making wine in a warmer climate
			  ○ *Addressing regional challenges* – providing solutions to regional issues in each region highlighted by AWRI helpdesk trends
			  ○ *Pinot Noir winemaking trial tastings* – showcasing wines prepared using different winemaking practices.

Project 4.1.2 – Specialised technical troubleshooting and responsive helpdesk services for the Australian wine sector	4.1 Adoption	10 - Extension and adoption	• Helpdesk staff responded to 7,647 requests for technical advice, covering grapegrowing, wine production and scientific and regulatory-related matters. • More than 80% of enquiries were answered within 24 hours. • Helpdesk staff performed 860 winemaking investigations. • A rapid response was provided to regions affected by smoke from significant fire events in 2014, 2015 and 2016. This included face to face Q&A sessions, practical management techniques post-harvesting and detailed discussions and interpretation of analytical results. • To assist with the interpretation of smoke taint analytical data, a database of background levels of smoke-related phenols and phenolic glycosides was established, initially with five common grape varieties, and later expanded to include a total of ten varieties, namely: Semillon, Chardonnay, Sauvignon Blanc, Riesling, Pinot Gris, Pinot Noir, Merlot, Cabernet Sauvignon, Shiraz, and Grenache. • Assistance was provided to help wine exporters understand and adapt to regulatory changes regarding metal content (particularly manganese) in wine exported to China. • The helpdesk addressed a number of enquiries regarding sooty mould in vintage 2017. This has been identified as a priority issue for some regions, and has led to the commencement of new research activities.
Project 4.1.3 – Library service	4.1 Adoption	10 - Extension and adoption	• More than 16,000 items were added to the library collection and many of the total 87,800 items are now in digital formats. • More than 5,900 requests were responded to, with 9,569 articles delivered in that period. Over 80% of requests were completed within one business day. • A version of the library catalogue optimised for viewing on mobile phones and tablets was launched, enabling users to search and order items using mobile devices. • An eBook collection was launched and then expanded to two eBook platforms. • Online information packs were first added to the AWRI website in 2013 and usage has steadily increased in the last three years.
Project 4.1.4 – Communication with stakeholders	4.1 Adoption	10 - Extension and adoption	• The AWRI website underwent a major update in January 2016. This, along with the removal of password protection from some areas of the site, greatly improved usability and resulted in increased web traffic, with annual page views growing from ~255,000 to ~336,000 during the investment period. • More than double the target number of industry articles were delivered over the term of the agreement– ensuring AWRI research was regularly being translated into formats tailored to an industry audience.

			• A special issue of the Australian Journal of Grape and Wine Research commemorated the AWRI's 60th birthday, featuring 18 review articles authored by AWRI staff. • The *eBulletin* format was used to respond quickly to industry issues such as floods, frosts, heatwaves, bushfires, pest/disease outbreaks and changes to agrochemical recommendations. • Webinar recordings were added to the AWRI YouTube channel – greatly increasing the reach of this important communication mechanism. • Close cooperation continued with the helpdesk and extension projects to ensure consistent messaging and relevant up-to-date content.
Project 5.1.3 – Efficient management and administration	?	12 - Corporate Services	• Progress was made towards RDE reform, including, for example, the AWRI's more active role in Wine Australia's market development activities. • The AWRI achieved charitable registration and concessional tax status. • Support was provided to other wine industry organisations, including Australian Wine Industry Technical Conference Incorporated, Wine Innovation Cluster (WIC), WIC Winemaking Services, the Interwinery Analysis Group, and Bioplatforms Australia. • A diversified investment strategy of AWRI reserves was developed to invest in future Australian wine industry activities. • An IT Strategic Plan was developed and implemented. • An electronic Director election process was developed. • Workplace health and safety systems were strengthened. • The AWRI's 'Employer of Choice' program remained an important focus. • The AWRI's risk management framework was strengthened.

References

Wine Australia – Performance Evaluation Report 2015-16 - https://www.wineaustralia.com/getmedia/1fcc5b0b-1e26-42a4-8f39-de5a6c88d4c9/AGWA-Performance-Evaluation-Report-2015-16.pdf

Australian Grape and Wine Authority – Evaluation Framework 2016-17 - https://www.wineaustralia.com/getmedia/bb43377a-09c4-4261-8fce-2818ffb72e1d/Wine-Australia-Evaluation-Framework.pdf

Wine Australia: Ex post cost benefit analysis of selected R&D projects supported by Wine Australia/GWRDC/AGWA investment – Final Report IDA Economics Pty Ltd -June 2016, 79 pages.

Benefit Cost Analysis of Wine Australia R&D Investments 2015-16 - Report: 8 June 2017 – prepared by Gillespie Economics and AgEconPlus Pty Ltd, 70 pages.

Intellectual Property

This section of the final report provides a summary of the intellectual property (IP) developed and third-party IP Rights used during the investment period. The AWRI sought to comply with Clause 22 of the Investment Agreement, which required the AWRI to comply with the GWRDC IP Policy and Procedures, including the IP register outlined in Schedule 5 of the Agreement. The AWRI prepared and maintained an IP register throughout this investment period and managed all IP in accordance with the IP policies and procedures in the Agreement.

The Australian grape and wine sector's position on intellectual property management previously articulated in both the Winemakers' Federation of Australia (WFA) Innovation Policy Committee (now evolving to a Research Advisory Committee), R&D policy states that priority must be given to the timely dissemination of research results and uptake of research. Also, under clause 11 of the Primary Industries Research and Development Act 1989 (PIERD Act - amended in 2014) – subsection (e), the AWRI should look to disseminate and commercialise, and facilitate the dissemination, adoption and commercialisation of, the results of research and development in relation to the primary industry or class of primary industries in respect of which the Corporation (Wine Australia) was established. As such, throughout the investment period 2013 – 2018, the AWRI favoured the extension of research outcomes into the Australian wine sector through a range of publication and industry outreach and extension programs.

Intellectual property that was developed or enhanced throughout the investment agreement includes:

- Policies and procedures

- Subject matter and technical know-how across all technical aspects of viticulture and oenology, including chemistry, microbiology, molecular biology, genomics, bioinformatics, wine processing, engineering, sensory science, mathematics, spectroscopy and the practical application of information, methods and technologies

- Know-how in experimental design, data generation, analysis, interpretation and reporting including:
    - establishment of replicated viticulture and oenology field and winery trials
    - technical expertise in sensory evaluation methodology and statistical analysis
    - genomic and systems biology experimentation, including bioinformatic analysis
    - use of chemometrics and metabolomic approaches to link to compositional data to winery grade allocations
    - methods for establishing the identity of target compounds, producing robust and reliable analytical methods, and determining their sensory significance and importance to wine flavour.

- Publications, reports, presentations, data and their interpretation, expertise, knowledge, methods, training materials, website content, web and mobile-enabled tools, smart phone apps and industry-relevant fact sheets.

- Systems and processes involved in collating and packaging of information for dissemination and the development and staging of seminars, courses and workshops including:

    - AWRI roadshow seminar, workshop and webinar platforms
    - The AWRI's John Fornachon Memorial Library
    - *eNews* and *eBulletin*s
    - Upgrades to the Query Investigation System database
    - New events management systems
    - Internal information management systems.

- Collections of training manuals and images, the collection and management of the Australian wine industry's bacterial and yeast strains, many of which were isolated prior to the investment agreement or secured under materials transfer agreements (MTAs) for research purposes from universities and commercial suppliers, and a wine yeast gene deletion library.

- Tables of information, raw and assembled genome sequences and databases including those for *eNews* and *eBulletin* contacts, the AWRI helpdesk and library enquiries, permitted additives and processing aids for winemaking and wine-importing countries.

- Custom-made materials, synthesised templates/target molecules and chemical compounds, analytical standards, enzymes, substrates for enzymes and their application.

- Experimental protocols, measurement and detection methods and techniques, the ability to develop new methods and techniques, and the expertise and technical skills to undertake those methods and interpret the results including:
    - methodology for high throughput screening, high throughput assays and phenotyping of yeast technology
    - methods for tannin and phenolics extraction and characterisation
    - assessment of grape and wine matrices using metabolomic approaches.

- New yeast or bacterial strains:
    - The development of new yeast and bacterial strains was reported in separate projects, outside this investment agreement (Project 3.2.2 - Enhanced winemaking outcomes and wine style diversification through provision of fit for purpose yeast starter cultures; Project 3.2.3 - Defining the nutritional drivers of yeast performance and matching yeast to must; and Project 3.2.4 - Efficient and reliable malolactic fermentation to achieve specification wine style). Please see copies of the final reports for these specific projects for more details about yeast and bacteria strain development.

- Indicia, patents, trademarks and associated domain names including:
    - The AWRI 'Dog book' app - *Agrochemicals registered for use in Australian viticulture*,
    - 'AWRI Roadshow Seminar and Workshop Program' Trademark (application number 1571793 via IP Australia – 31/7/2013
    - 'The Agrochemicals Dog Book' Trademark (application number 1571797) via IP Australia – 31/3/2013

- Use of third-party IP rights:
  A range of bacterial and yeast strains were secured under MTAs from universities and commercial suppliers for research purposes.

Project 3.1.1 – Identification and origin of volatile compounds responsible for important sensory attributes

Abstract

Knowledge of the flavour compounds that are responsible for the sensory characteristics of wines is of great importance to be able to control and adjust wine aroma properties in production. Compounds not previously recognised as active flavorants in specific varieties have been identified in this project for the first time, including several compounds that give Viognier wines their distinctive 'apricot' flavour, and a 'green' flavour compound in Shiraz. In addition, numerous compounds not previously understood adequately have been revealed as significant in wine flavour, such as sulfur compounds in Chardonnay being found to confer fruit attributes. Ways of adjusting the contribution of various compounds have also been examined. For example, absorption onto food grade plastic film was shown to be effective in removing 'green' character from a red ferment, while avoidance of material from specific wind-break vegetation planted near vineyards reduced 'green' flavour in wine. Another outcome of this research has been the development of analytical tools for measurement of key wine flavour compounds, and improved understanding of winemakers' concept of 'green' tannin and flavour with reference to consumer preferences. Studies of Chinese consumer red wine preferences and behaviour have helped shed light on this rapidly growing and important market for Australian wine.

Executive summary

A good understanding of the relationship between wine composition and wine flavour is important to being able to control grape and wine quality. This research project was carried out to improve knowledge of:

- compounds responsible for key flavours, including the identity of previously unrecognised compounds, especially for the varieties Shiraz and Chardonnay
- the relationships between wine composition and wine sensory properties;
- the effect of viticultural and oenological techniques on the formation of compounds, such as 'green' flavour

and to develop routine analytical methods for flavour compounds that can be applied in research and industry trials.

Using sensory-guided chemical methods, gas chromatography-mass spectrometry, in combination with olfactometry (use of the human nose as a detector), liquid chromatography –mass spectrometry, and formal sensory studies, compounds responsible for flavour attributes in wine were identified. Analytical methods using isotopically labelled standards were adopted where possible, to ensure accurate, precise and sensitive analyses of compounds at or below ppb levels. Surveys of commercial wines were used to confirm the importance of compounds, while detailed chemical studies shed light on the formation reactions occurring in winemaking. Use of replicated viticultural and winemaking studies enabled the effect of different production practices to be determined, and formal sensory and consumer preference data were obtained.

Analytical methods were established for a number of key flavour compounds, providing enhanced ability to measure levels of compounds with excellent context regarding their sensory significance, including detection thresholds and flavour contributions. The compounds included 3-mercaptohexanol, 3-mercaptohexyl acetate, 4-methyl-4-mercaptopentan-2-one, benzyl mercaptan, furfuryl thiol and numerous aldehydes and other compounds related to oxidative effects. Being able to measure such compounds allowed greatly improved understanding of the effect of yeast and fermentation, oxygen, nutrients and many other variables through various AWRI research projects, and the methods were used by numerous wine industry personnel, through collaborative studies or in resolution of issues between third parties.

The compounds responsible for the 'apricot' flavour in Viognier wine were identified as the monoterpenes, geraniol, linalool and nerol. These are compounds not previously associated with this flavour, and their sensory interactions with other aroma compounds were established. While a series of lactone compounds were found to be generally unimportant to wine flavour, their contribution in combination with monoterpenes to 'stone fruit' flavour was indicated. Several esters were shown to contribute to 'peach-like' aroma in Chardonnay.

Several potent thiol compounds, previously known in Sauvignon Blanc wines, were determined to be major contributors to Chardonnay fruit flavour, including at high concentrations a Sauvignon Blanc-like 'tropical fruit' character. This opens a new area of control of flavour of this important variety. Similarly, the volatile thiol, benzyl mercaptan, was found to be related to 'flint'/'struck match' aroma in Chardonnay, providing a basis of control of this 'Burgundian' character in Australian wines.

Regarding 'green' flavour in red wines, grapevine proximity to Monterey Cypress trees was shown to be a cause, with grape rachis included in Shiraz ferments also a major influence. 'Green tannin' was elucidated to be related to both 'green' volatiles and elevated bitterness, probably related to specific tannins.

Studies investigating consumer preference and behaviour of Chinese consumers of red wines gave insight into Chinese-based language to describe wines, appropriate for marketing and communication purposes, with recognition of sensory attributes that relate to preference.

Overall, the results of this research project have increased knowledge of the main volatile compounds involved in wine flavour attributes, so that the causative compounds for many of the most important sensory attributes of wines are now established and many of the influences on their formation understood. While there are several key sensory attributes of wines where the cause is still not known, this research stream has given producers new knowledge to avoid negative flavours and enhance positive flavours.

Background

While many sensory attributes of wines have a known cause, there are major flavour characteristics where the source compounds are not known. The measurement of characteristics of grapes and wines that determine quality from a consumer perspective relies on knowledge of the constituents that give rise to these characteristics. Measurement of key flavour compounds to assess viticultural effects and outcomes from winemaking or processing options is of great importance; knowledge developed in this project will be applied in associated projects.

For attributes such as 'green' flavour in Shiraz and 'stone fruit' flavour in Chardonnay there are no clear target compounds known. For 'green' flavour, it is not clear if this attribute, which is used rather loosely across the wine sector, involves: volatile compounds such as methoxypyrazines; C6 compounds such as cis-3 hexanol; dimethyl sulfide; a low level of fruit flavour compounds; any relationship with tannin ('green tannin'); or links to higher acidity/lower alcohol matrix effects. There is also the possibility of an in-mouth effect of a compound such as isobutyl methoxypyrazine (IBMP) suppressing desirable mouth-feel characteristics.

For 'stone fruit' flavour, several γ-lactones important to peach/apricot fruits have been found in wines, however at levels well below aroma threshold (Cooke et al. 2009). There is some evidence that these lactones can act additively (Jarauta et al. 2006). While 'tropical' thiols are well-known key flavour compounds for Sauvignon Blanc, there is little knowledge of their importance to Chardonnay. However, unpublished work at the AWRI has provided evidence that they can contribute to the

flavour of this variety, and wines from some cooler regions (Margaret River and Great Southern in WA, and from Tasmania) can have a distinct 'tropical fruit'/'passionfruit' flavour.

For 'green' aroma, while methoxypyrazines are important to Bordeaux varieties, they are not found in Shiraz or Pinot Noir except potentially through extraction from green stalks. C6 alcohols, notably cis-3 hexanol, give 'grassy' aromas and have been implicated in greener wine flavour, but the evidence is not strong. There is very little evidence regarding 'green' tannins,

For volatile aroma and flavours from extraneous sources, the effect of cover crops on wine flavour has been studied only to a very limited extent (Xi et al. 2011), while a small quantum of work has been conducted on the effect of grapevine leaves, mainly on 'green' C6 compounds (Wildenradt et al. 1975). A recent paper outlined more than 600 volatile compounds in grapevine leaves (Weingart et al. 2012), while glycoconjugates of volatiles are known to be much more abundant in grapevine leaves than berries (Wirth et al. 2001). Recent work on eucalyptol at the AWRI showed that stalks and leaves mixed in the crusher with grapes can be a source of this compound in the final wine, greater than that of direct transfer from nearby eucalypt trees (Capone et al. 2012; Black et al. 2015).

Many Australian wine producers seek to achieve specific wine styles to suit specific markets, a task reliant on sourcing a suitable supply of grapes and selecting production methods to achieve the target style. Markers for measuring style have long been sought to inform production. However, relatively few objective measures of grape and wine compounds which can be directly related to style are currently available. Aroma compounds are of great importance in determining style, and building understanding of those compounds which influence consumers' perception of wine quality is an industry priority. Measures that can be applied in viticultural and winemaking studies are badly needed for evaluating industry trials and research studies, and potentially for streaming fruit.

Highlights

- The main compound known to be responsible for 'green' flavour in Cabernet Sauvignon, isobutyl methoxypyrazine, which was previously thought not to be biosynthesised in Shiraz grapevines. was found to contribute 'green' aroma in Shiraz wine made with stems, notably in whole bunch fermentations
- Nearby windbreak trees were found to contribute 'green' flavours to harvested grapes and thus wines
- The monoterpenes geraniol, linalool and nerol were found to give Viognier wines their distinctive 'apricot-like' flavour
- Thiols were confirmed as major contributors to Chardonnay flavour, with 3-mercaptohexanol and 3-mercaptohexyl acetate contributing, at moderate levels, 'citrus fruit' character and at higher levels 'tropical' aromas, and benzyl mercaptan adding 'flint'/'struck match' aromas.

Objectives

The project has an overall objective of identifying, and developing analytical methods for volatile compounds causing key flavour attributes of wines, and acquiring sufficient knowledge regarding their sensory significance, consumer response and origin to allow industry-relevant specifications to be developed.

There are three main objectives:

- To identify compounds responsible for specific important flavour characteristics in wine, and acquiring information about levels required for desirable flavour:

    a. Stone fruit in white wine
    b. ‘Green’ in red wine
    c. ‘Tropical fruit’ in Chardonnay
- To develop analytical methods for key volatile aroma compounds.
- To determine factors that affect the concentration of specific aroma compounds, including work on *Botrytis cinerea* and rotundone, leaves and stems and their effect on ‘green’ aroma, and volatiles from the local environment.

In addition, the interaction of wine composition, information and wine sensory properties on Chinese consumers’ preference, purchase intent and choice was studied.

Method

The project used several procedures to meet the objectives. For identification of key compounds, sensory-guided chemical analysis was used, with gas chromatography-mass spectrometry (GC-MS)-olfactometry, sensory-based aroma detection threshold measures and bench-style sensory assessments applied to identify probable compounds responsible for key sensory attributes of wines (Mayr et al. 2014). The identity of the volatile compounds responsible for aroma attributes in wine was established through synthesis of compounds for use as analytical standards, and through GC-MS studies and addition sensory studies, to confirm the identity and the importance of the compounds (Mayr et al 2014).

Targeted, specific analytical methods using GC-MS or LC-MS were developed for a range of compounds using stable isotope dilution analysis, with synthesised standards as required. The compounds included (E)-2-alkenals ((E)-2-hexenal, (E)-2-heptenal, (E)-2-octenal and (E)-2-nonenal), several Strecker aldehydes (methional, 2-phenylacetaldehyde, 3-methylbutanal and 2-methylpropanal), aldehydes (furfural, 5-methylfurfural, hexanal, and benzaldehyde), furans (sotolon, furaneol, and homofuraneol), as well as alcohols (methionol, eugenol, and maltol), in the same analysis, with the aldehydes derivitised using O-(2,3,4,5,6-pentafluorobenzyl)hydroxylamine hydrochloride. These compounds were analysed by GC-MS/MS (Mayr et al. 2015). The thiol compounds 3-mercaptohexan-1-ol, 3-mercaptohexyl acetate, 4-mercapto-4-methylpentan-2-one, 2-furfurylthiol, and benzyl mercaptan were quantified using 4,4'-dithiodipyridine (DTDP) as a derivatising agent with HPLC-MS/MS (Capone et al. 2015). A new GC-MS/MS method for analysis of dairy lactone ((Z)-6-dodeceno-γ-lactone) was also developed (Siebert et al. submitted) using SPME, together with a chiral analysis olfactometry method using a chiral capillary column.

The methods were validated and simplified where possible to allow routine, relatively rapid analysis for high throughput as well as transfer of methods to commercial laboratories, with appropriate limits of quantification. Quantitative analytical methods for the major known aroma compounds in wines were applied to support other research programs and for analysis of industry samples (Mayr et al. 2015; Capone et al. 2015).

These quantitative analysis methods were used to survey commercial wines (e.g. Capone et al. 2017; Siebert et al. submitted), with the numbers of wines where a compound was above sensory detection threshold providing evidence that the compound was of importance to wine.

The formation of compounds was established through kinetic and mechanistic studies and ways of adjusting their levels through viticultural and winemaking practices were assessed (Capone et al. 2016). The project team worked closely with multiple industry partners to assess commercial options for adjusting levels of aroma compounds.

For many compounds, the sensory significance was further determined through collection of sensory descriptive and consumer preference data with consumers rating liking on a nine-point hedonic scale (e.g. Capone et al. 2017), with rapid sensory methods also applied (Pearson et al. 2015). Partial least squares regression and principal component analysis were commonly applied to assess the multivariate datasets obtained. Consumer preference information in China was approached using a discrete choice experiment methodology (Williamson et al. 2016), with preference mapping of identified clusters (Williamson et al. in press 2017)

Results and discussion

The research project concentrated on identifying and studying volatile compounds responsible for several important sensory attributes, attributes that were identified from previous consumer studies as important to wine quality. Knowledge of Shiraz and Chardonnay flavour was considered high priority as these varieties are of greatest commercial importance to the Australian industry.

'Stone fruit' flavour in white wine

The compounds that cause 'stone fruit' flavour in Chardonnay and Viognier have been identified. Over the initial period of the project, by studying commercial wines showing high and negligible 'stone fruit' aroma, a set of n-alkyl lactones was indicated as important, including a little understood lactone (gamma-dodecalactone, known as 'dairy lactone'), together with the monoterpenes linalool, geraniol and nerol (Siebert et al. 2016). From further extensive investigations, it was shown that for Chardonnay, the relatively subtle 'peach-like' aroma of wines of this variety was closely linked to several specific esters, notably 3-methylbutyl acetate, hexyl acetate and ethyl hexadecanoate. For Viognier, which was investigated as this variety is little studied and commonly shows a clear 'apricot-like' aroma, linalool, geraniol and nerol were notably important to the 'apricot' character (Figure 1, Siebert et al. submitted). There remains a possible sensory contribution of the lactones, but they are substantially less important than the other compounds identified, except for *Botrytis*-affected sweet wines, where gamma-nonalactone was identified as a major aroma contributor. This compound may also be a contributor to the 'apricot' character in other white wines. Consumers did not overall prefer wines with high 'apricot' flavour, although this was likely to be related to mouth-feel/taste attributes such as bitterness which correlated with the aroma character. The recognition of the monoterpenes linalool, geraniol and nerol as key compounds for the 'apricot' flavour attribute is novel, opening up viticultural and winemaking options to enhance this character in finished wines.

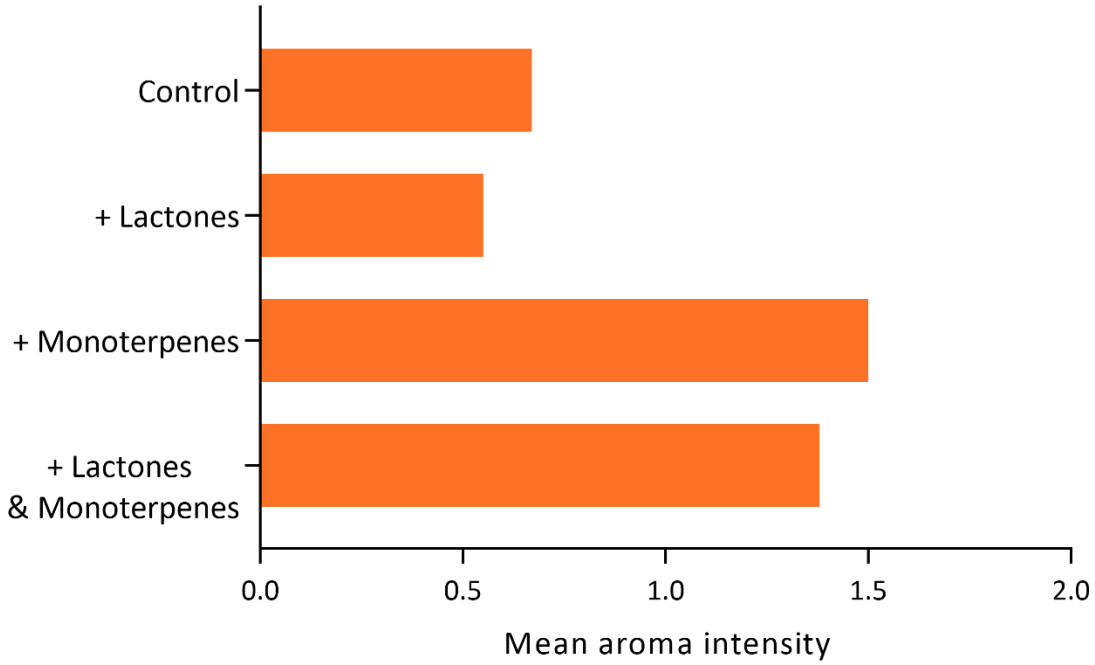

Figure 1. Mean 'apricot' aroma rating from a sensory panel for a model wine control, with added wine aroma compounds such as esters, acids and alcohols, compared to the model wine with aliphatic lactones and monoterpenes, added at the concentrations found in a white wine with distinct 'apricot' flavour.

Overall, this aspect of the project was completed largely as planned, although the publication of refereed manuscripts was not achieved to the final timeline. Three manuscripts describing the work have been drafted at the time of writing, with one submitted. One paper was published with collaborators from the University of Bordeaux describing work conducted on 'cooked orange' aroma in sweet white wines. A poster was presented at the 16th Australian Wine Industry Technical Conference in July 2016, and was awarded a best poster prize.

'Green' flavour in red wine

Studying commercially available Shiraz and Cabernet Sauvignon wines, the strong relationship of isobutyl methoxypyrazine to 'capsicum'/'bell pepper'/'stalky' character was confirmed. A major advance in knowledge from this project was the identification of this compound at a sensorially important concentration in 'green' Shiraz wines, a variety that previously was not considered to not be able to biosynthesise this compound. Working with CSIRO scientists, the compound was demonstrated to be present at high concentrations in research-scale wines made from warm inland grapes from vigorous rootstocks. From a winemaking study, it was clearly shown that the inclusion of stems in a Shiraz ferment gave an elevated concentration of the compound (8 ng/L), together with the closely related isopropyl methoxypyrazine (10 ng/L), and a clear sensory effect (Figure 2). Interestingly, grape leaves present in the ferment did not give any 'green' flavour but enhanced 'red berry' fruit, most likely due to conversion of leaf alcohols to hexyl acetate (Capone et al. 2016). This study shone new light into the practice of whole bunch fermentation, where compounds from the rachis will be extracted.

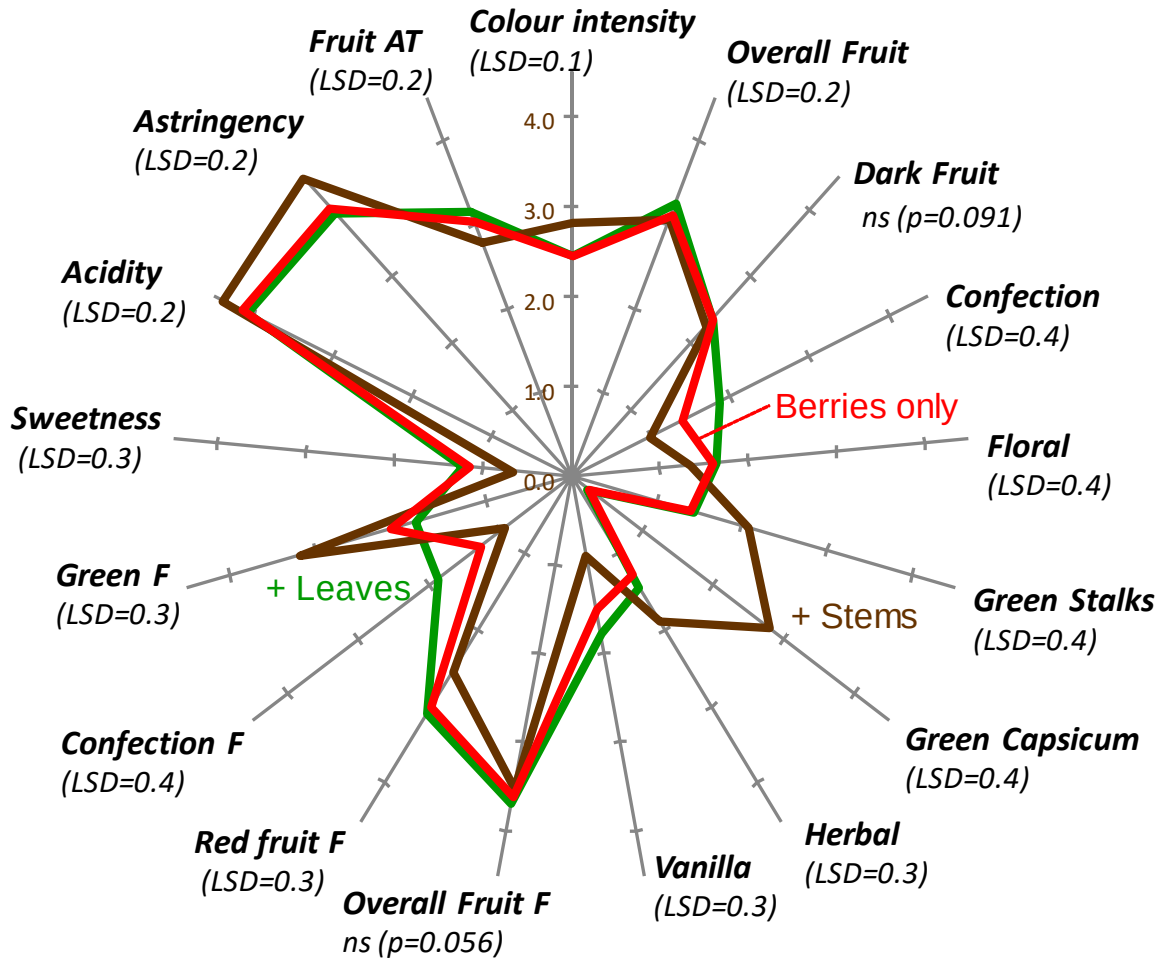

Figure 2. Mean sensory panel ratings for a Shiraz wine made from grape berries only (Berries only), berries plus stems (+ Stems), and berries plus grape leaves (+Leaves). The least significant difference (LSD, P=0.05) is also shown. Duplicate or triplicate ferments were assessed in triplicate by nine trained judges.

From a sensory point of view, improved understanding of winemaker concepts of 'green' character was achieved by using the technique of projective mapping with winemaker groups and comparing the data to that from trained sensory descriptive analysis panels and to compositional data (Pearson et al. 2015). For the commercially available wines studied, a winemaker description of 'green' or 'green tannin', through a projective mapping sensory task, related to the trained sensory panel attributes of bitterness and 'green stalks'/'vegetal' aroma and flavour attributes (see Figure 3 for the Cabernet Sauvignon sample set). Importantly, the proportion of catechin and percentage galloylation in the tannin was associated with bitterness, and the 'green stalks' attribute was linked to IBMP, as well as dimethyl sulfide concentration for both the Cabernet Sauvignon and Shiraz datasets. Consumer preference was also assessed and those wines with 'green' characters were found not to be liked by most consumers. Thus, this study has shown for the first time that the common, but hitherto poorly defined character of 'green tannin', can be related to chemical markers.

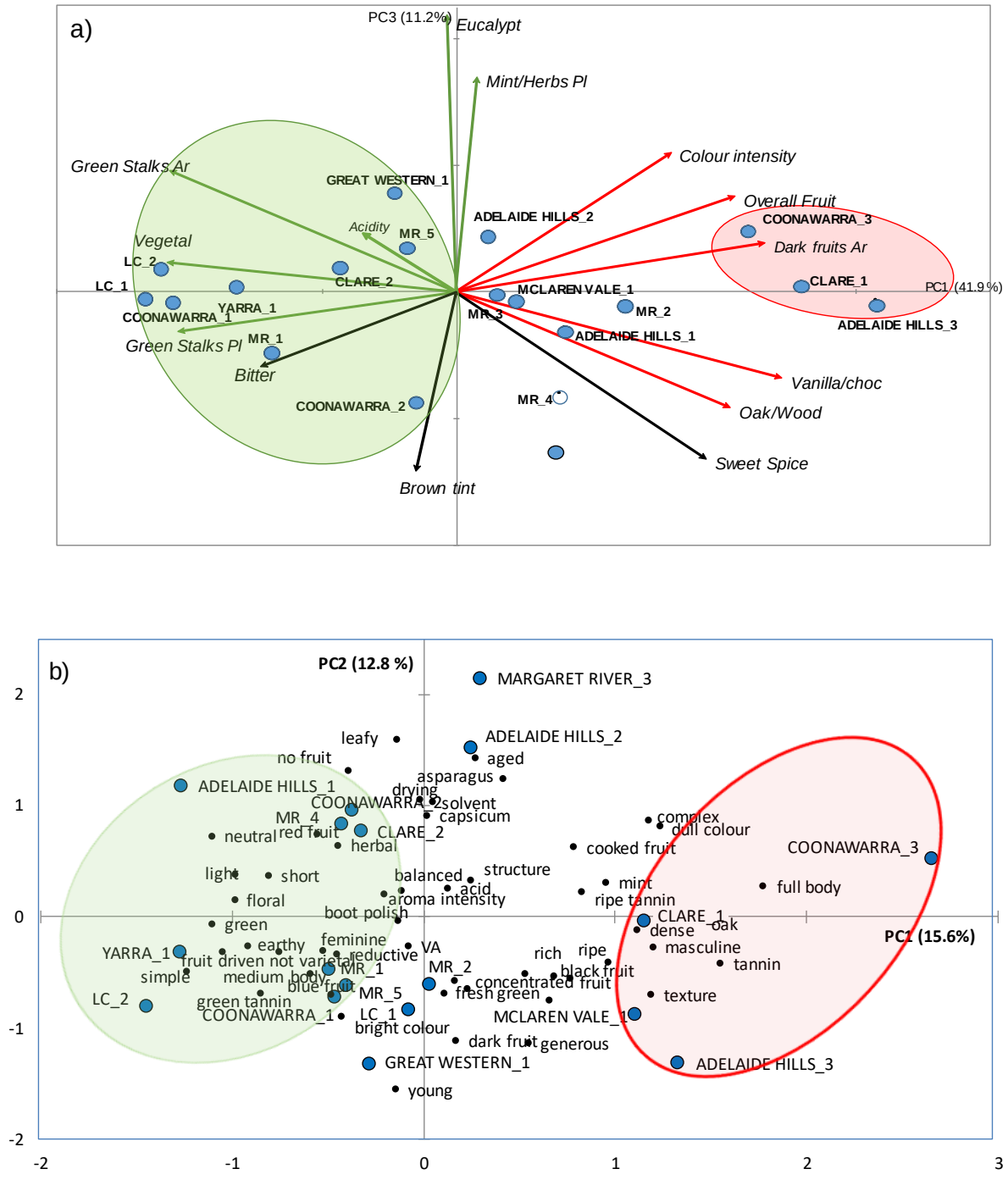

Figure 3. a) Principal component biplot of trained sensory panel descriptive analysis data and b) Mutiple factor analysis biplot from projective mapping data from a group of winemakers; for a set of commercial Cabernet Sauvignon wines.

A small-scale fermentation study was completed that showed that IBMP concentration was dramatically reduced if a food grade PVC-based cling wrap material was added to ferments, with the effect greater than for previously studied silicone or polyethylene materials (Figure 4). Some fermentation-derived esters and acids were reduced in concentration as a result of the treatment, but generally there was no significant effect of the plastic on most of the positive volatile compounds.

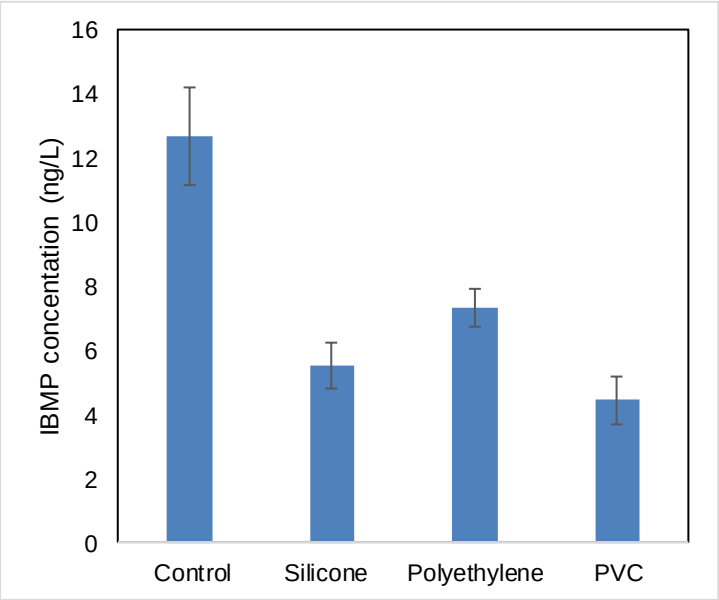

Figure 4. Mean isobutyl methoxypyrazine (IBMP) concentration in a Shiraz wine made from must with added IBMP and treated with three plastic materials, compared to the control, untreated must.

'Tropical fruit' in Chardonnay

The compounds responsible for 'grapefruit'/'tropical fruit' aroma in Chardonnay wines have been shown to be the polyfunctional thiols, 3-mercaptohexanol (3-MH), 3-mercaptohexyl acetate (3-MHA), and 4-methyl-4-mercaptopentan-2-one (4-MMP).

In data from 106 commercially available wines from multiple price points and multiple regions, each of these compounds was well above the reported sensory detection threshold in all wines, and the concentration for some wines approached that of wines from varieties such as Sauvignon Blanc, where these compounds are key varietal contributors. From data reported previously, the concentrations of these thiols in most of the wines examined was in the range where they would enhance fruit freshness but not confer 'tropical fruit'/Sauvignon Blanc-like flavour (Figure 5). Trends were observed that cooler climate regions produced wines with higher concentration of the potent 3-MHA, and younger Chardonnays were also generally higher in this compound.

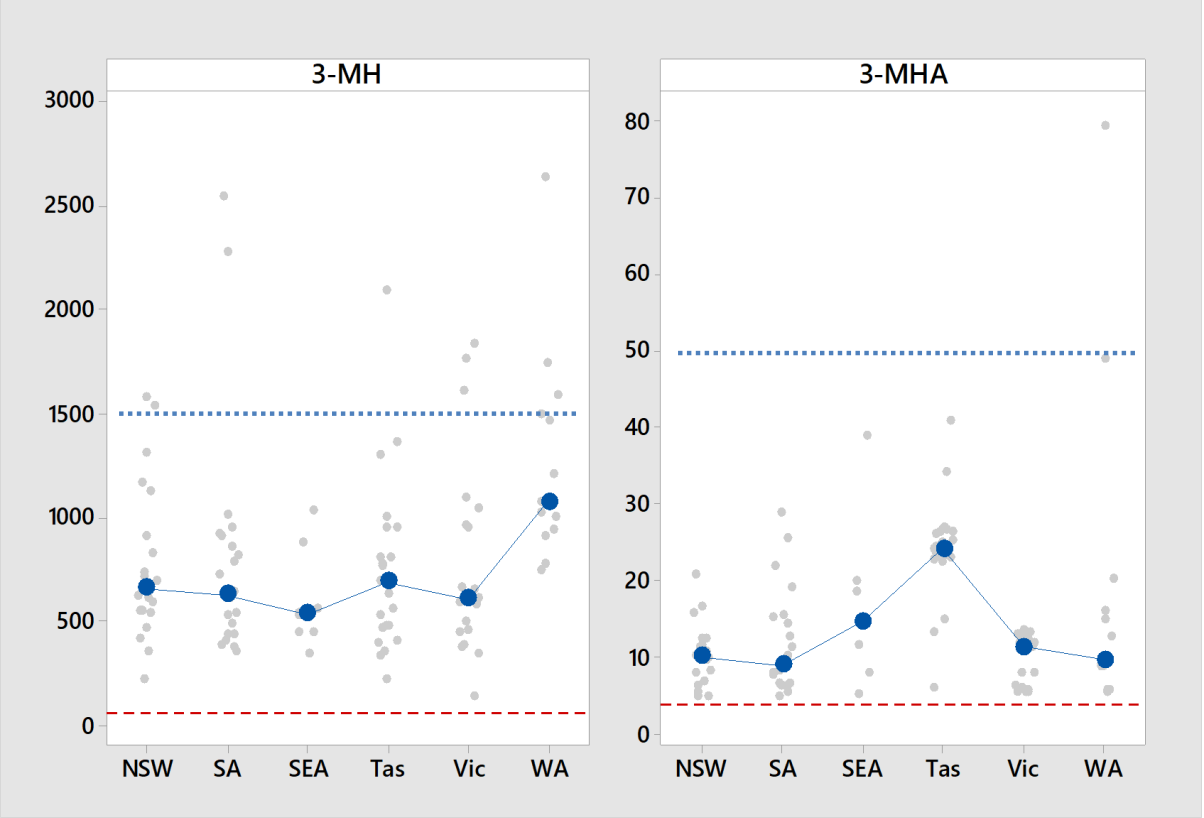

Figure 5. Concentration of two thiols for 106 commercial Chardonnay wines by Australia state, with the blue symbols indicating the median values and the grey symbols the individual data points. The broken red line denotes the aroma detection threshold, while the broken blue line the concentration previously reported to be the level above which the compounds give a clear 'tropical fruit' aroma.

A further sulfur aroma compound quantified was found to be related to 'struck flint'/match'/'smoky' aroma, namely benzyl mercaptan. This compound was also determined to be at higher concentration than the aroma detection threshold for all 106 commercially available Chardonnay wines, and from regression analysis those wines that had a higher retail price, that were from cooler regions and were somewhat older were more likely to have higher concentrations of benzyl mercaptan, most likely reflecting lees contact and barrel fermentation, which are associated with formation of this compound. Wines from Tasmania were also generally higher in this compound.

An experiment was completed where a set of 16 Chardonnay juices were obtained from across multiple viticultural regions, including the main premium-Chardonnay producing regions, and wines were produced using standardised winemaking with no oak influence. The thiol composition, as well as that of other volatile aroma compounds, and sensory properties were related. All of the wines had above threshold concentrations of 3-MH, with many above the level likely to give 'tropical fruit'-like flavour. Similarly, almost all wines had surprisingly high concentrations of 3-MHA, and benzyl mercaptan. 'Tropical fruit' character in the wines was found to be linked to the concentration of 3-MH and 3-MHA (Figure 6), while a 'box tree'/'cat pee' flavour was related to the presence of 4-MMP. The compound benzyl mercaptan was confirmed to be associated with 'struck flint'-like aroma. 'Citrus' flavour was also related to thiol concentration, in a non-linear fashion (Figure 6), indicating that higher concentration of 3-MHA/3-MH gives 'citrus'/'grapefruit' aroma, but at very high concentration the aroma changes to 'tropical fruit', confirming previous reports that fruit intensity is enhanced at moderate concentrations, and 'tropical fruit' is contributed at high concentration. Consumer preference was related to wines with relatively high concentration of the thiols, showing

that these compounds can be desirable and positive for wine quality for a large proportion of consumers. Interestingly, benzyl mercaptan was determined to above its aroma threshold concentration in these unoaked wines. This compound's mechanism of formation is not known but both oxidative and reductive conditions are probably required.

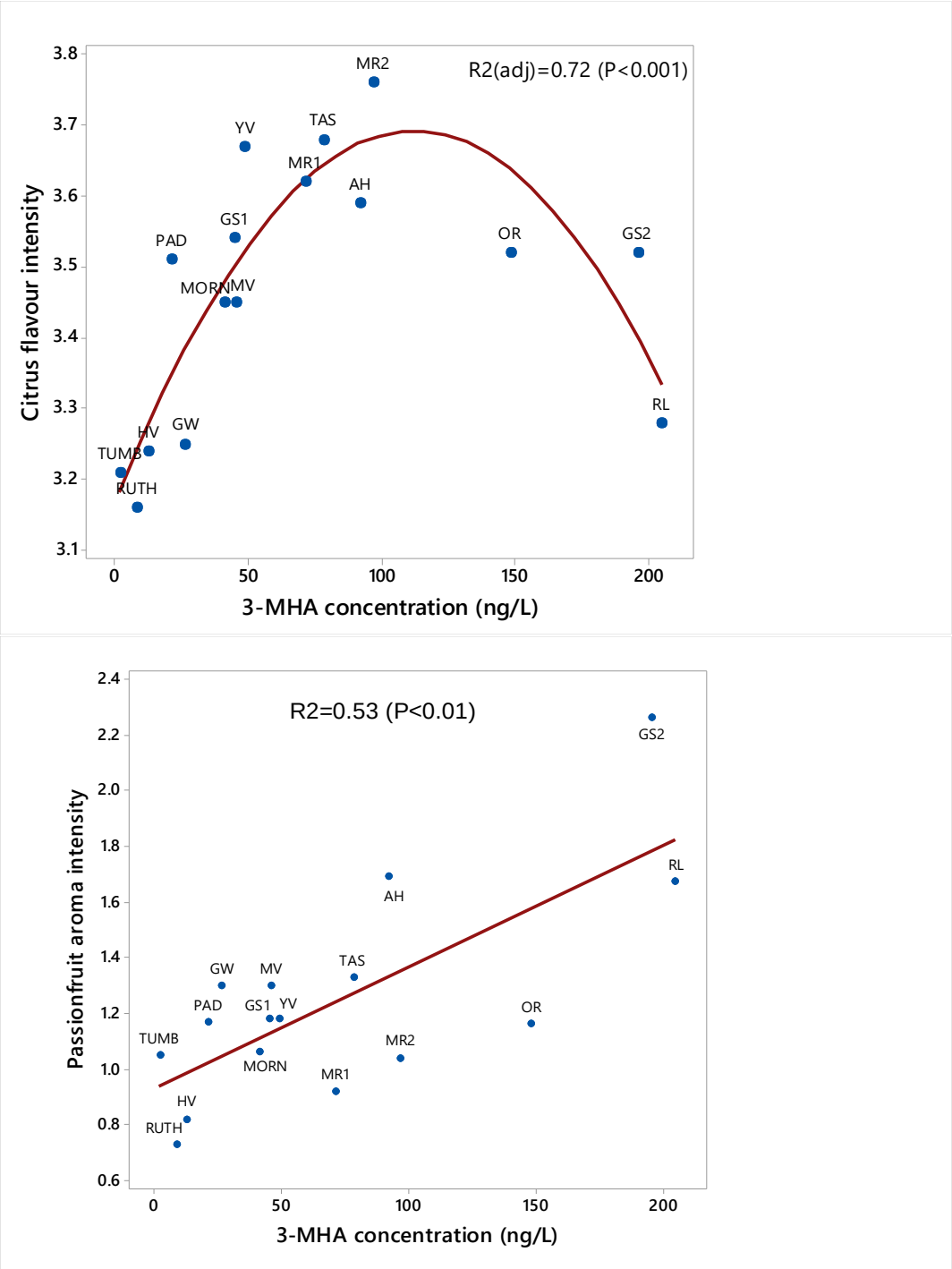

Figure 6. Regression plots showing the relationship for 'citrus' flavour and 'passionfruit' aroma scores from a trained sensory panel, with 3-MHA concentration for 16 Chardonnay wines made from juices sourced from multiple viticultural regions (RUTH: Rutherglen, TUM: Tumbarumba, PAD: Padthaway, GW: Great Western, MORN: Mornington, GS: Great Southern, MV: McLaren Vale, YV: Yarra Valley, MR: Margaret River, TAS: Tasmania, AH: Adelaide Hills, OR: Orange, RL: Riverland). Note that the juices were not necessarily typical of the respective regions.

This study also found that thiol precursors present in wine could act as a reserve to be released in-mouth upon consumption, as indicated by the importance of these non-volatile compounds to the fruit aftertaste sensory attribute.

It was of interest that for both the commercially available wines studied, as well as the wines made using standardised winemaking methods, that some warm inland irrigated regions such as the Riverina and the Riverland produced wines with thiol concentration at the upper range. It is possible that apart from yeast choice, which is well known to be influential in releasing these compounds from their precursors during fermentation, there may be other strong influences on thiol concentration, such as the nitrogen or sulfur fertilisation regime used. This has been previously shown from research done in France, and in discussing with some wine producers, it is a potential variable that warrants further study.

This sub-project was completed to the deadlines originally set, with a paper published (Capone et al. 2017), an industry article (Capone et al. 2016) and several AWITC posters and industry workshops/seminars completed.

Development of analytical methods for key volatile aroma compounds.
A method to quantify the main potent thiols that give 'tropical fruit', 'cat pee' and 'struck flint'-like aromas was developed with colleagues from the University of Adelaide. The method is much simpler than previous methods and is relatively rapid. A research paper describing the method was published (Capone et al. 2015).

A GC/MS method for quantification of a number of aldehydes and related compounds, including those most important to oxidative off-flavour and aged character, and some that are contributors to 'red berry' flavour, was optimised and the method published during the life of this project (Mayr et al. 2015), following from earlier development work.

Analytical methods have also been generated for the compound 'dairy lactone' (Siebert et al., in preparation) implicated in 'stone fruit' aroma, and a modification of the rotundone analytical method has been made using multidimensional GC to allow greater specificity and throughput (Scarlett et al. 2014).

Determining factors that affect the concentration of specific aroma compounds: Botrytis cinerea *and rotundone*
It has been previously shown that rotundone concentrations in grapes are elevated on the shaded back sides of bunches (Zhang et al. 2015). It had been speculated that this observation could be related to a relatively higher probability of fungal infections under shaded conditions, leading in turn to oxidation of sesquiterpene precursors to yield rotundone. After several experiments, it was determined that *Botrytis cinerea* infection of Shiraz berries had no obvious effect on production of the 'spicy'/'pepper' compound rotundone. However, in collaborative work with a French research group, powdery mildew infection of grapes was associated with the rotundone concentration of the subsequent wines (Geoffroy et al. 2015).

Substantial advances in knowledge of the influences on rotundone formation in the vineyard and winery were made over the life of this project. A study conducted with CSIRO and Mount Langi Ghiran wines, designed to confirm within-vineyard variability of rotundone in a premium Shiraz vineyard in the Grampians (Scarlett et al. 2014, Bramley et al. 2017), showed that independent of seasonal factors that alter rotundone in berries, spatial variation was clearly and consistently evident across seasons for a single site. While there were major differences in concentration of rotundone between the three seasons studied (2012, 2013 and 2015), the areas of high or low rotundone

concentration in the vineyard were highly consistent (Figure 7). This major study, the first of its kind to target a key grape-derived flavour compound, confirmed that rotundone concentration was related to temperature of the growing season, notably highlighting the veraison to harvest period. Within the vineyard, topographic variables were indicated as important. The work provided evidence that selective harvesting of vineyards, especially in cooler seasons, can give producers the ability to adjust wine style.

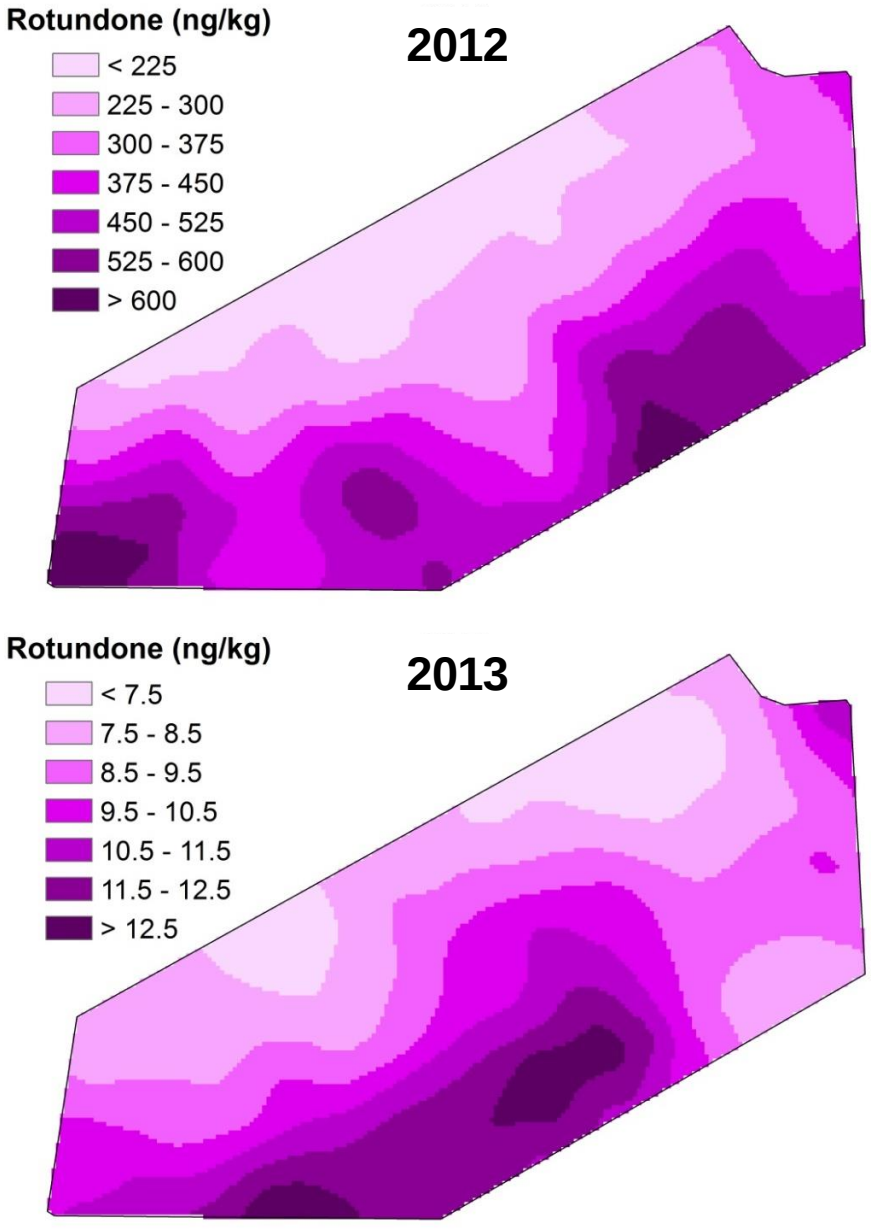

Figure 7. Spatial distribution of rotundone in Shiraz grape berries sampled, close to commercial harvest, across a vineyard from two seasons.

Investigations into the influence of sunlight on formation of rotundone showed that low sunlight conditions, mimicking 50% shade in the vineyard, resulted in a greater formation of rotundone than under full sunlight. This experiment was conducted in a model system using the major precursor of rotundone, α-guaiene, in a plant growth chamber or greenhouse environment. Similarly, under no light conditions, maximal concentration of rotundone was produced. This work showed that low light conditions in shaded bunches are likely to be favourable to the accumulation of rotundone and

might lead to being able to manipulate rotundone levels in the vineyard. Further research is required to include a number of vineyards comparing vine row aspect and shading or trellising of vines or application of physical sunscreen agents to confirm these results. The work was presented as a poster at the International Cool Climate Wine Symposium in 2016 and was awarded a best poster prize.

Studies have been completed into the effect of several viticultural and winemaking factors on rotundone concentration in grapes and wines. These projects have been carried out with international collaborators to increase data collection during both the northern hemisphere and southern hemisphere growing seasons. The ripeness of grapes was found to be a major influence (Geffroy et al. 2014), with grapes harvested 44 days post-veraison, that is at late stage ripening, having the highest concentration of rotundone. Leaf removal around the bunch zone had a large effect, resulting in strongly reduced rotundone concentration. An irrigation treatment was also found to increase rotundone, with veraison to harvest water status being a key factor (Geffroy et al. 2014). In a subsequent study, measurements of bunch surface temperature suggested that the effect of irrigation may be due to a cooler bunch microclimate through an increase in leaf area (Geffroy et al. 2016).

In a study on the variety Duras, which consistently has relatively high rotundone concentration, clonal effects were observed, with wines made from specific clones being widely varied in rotundone concentration (Geffroy et al. 2015), pointing to planting material being of importance to rotundone in other varieties. Assessing several winemaking variables, skin removal pre-ferment resulted in lower rotundone concentration, while use of macerating enzymes, increased fermentation temperature or longer skin contact time during fermentation did not result in elevated rotundone concentrations. Post-fermentation extended maceration or semi-carbonic fermentation resulted in lower rotundone concentrations.

Volatiles from the local environment.
The effect on wine flavour of vine proximity to windbreak trees was investigated. Wine made from Cabernet Sauvignon vines close to Monterey cypress trees had a notable eucalypt/pine-like aroma and flavour (Figure 8), while there was no clear sensory influence of she-oak trees or radiata pine trees on wines made from fruit from other vineyards studied (Capone et al. 2017). Wine producers can consider techniques to remove ‘matter other than grapes’ (MOG) from harvest bins or separate hand-harvesting of grapes from vines close to Monterey cypress trees if they wish to avoid their flavour influence.

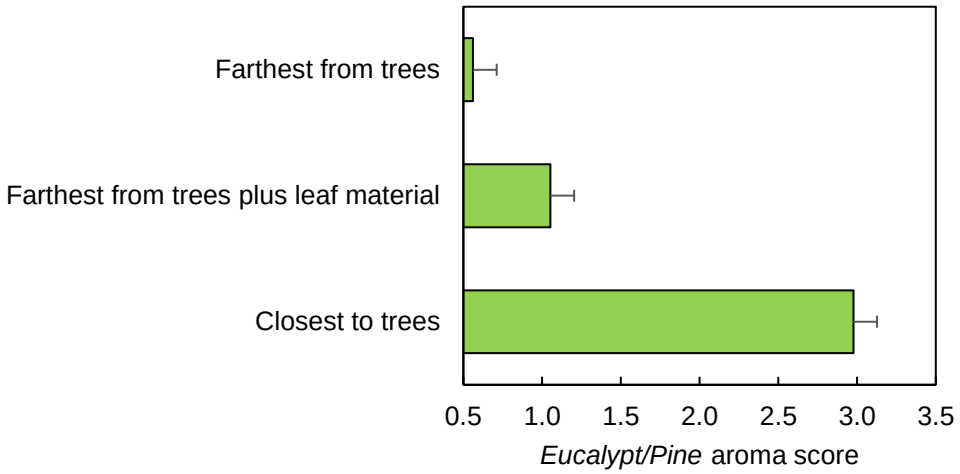

Figure 8. Mean sensory rating for the 'eucalypt'/'pine' aroma attribute for a Yarra Valley Cabernet Sauvignon wine made from grapes picked furthest from Monterey cypress trees, wine made from grapes picked furthest from the trees with the addition of Monterey cypress leaf material and wine made from grapes picked closest to the trees.

The interaction of wine composition, information and wine sensory properties on Chinese consumers' preference, purchase intent and choice

This study used sensory evaluation techniques to test the relative influence of country of origin, price and sensory attributes on consumer preferences in China, an example of a new wine market, and a key market for Australian producers.

From a methodological point of view, a study assessing a choice procedure with Chinese consumers provided the conclusion that in an emerging market with consumers less familiar with a product category, a discrete choice experiment approach with actual products is not sufficiently discriminating (Williamson et al. 2017).

Red wines from France, Australia and China were tasted by Chinese consumers under blind or informed conditions (Williamson et al. 2017, Figure 9). Sensory descriptive data were collected from a Chinese-trained panel using Chinese language attributes. A higher price and being from France were the strongest predictors of liking under informed conditions, while being from China had a negative influence for most of the consumers. Some consistency was found in sensory preferences between blind and informed tastings, indicating that sensory aspects of the wine are important, with one consumer cluster not influenced by price or country information. Australian wines were well-liked under both blind and informed conditions. Well-liked wines were generally high in 'fermented bean curd' (related to oak flavour), 'alcohol' flavour, 'hawthorn' ('ripe blackberry-like') and 'woody' characters, and low in 'dried longan' ('prune', 'raisin') attributes. French wines in contrast were not well-liked under blind conditions. Price and country of origin generally were more important than the sensory aspects. A significant proportion of consumers, however, were less concerned about origin and price and were more influenced by the sensory characteristics.

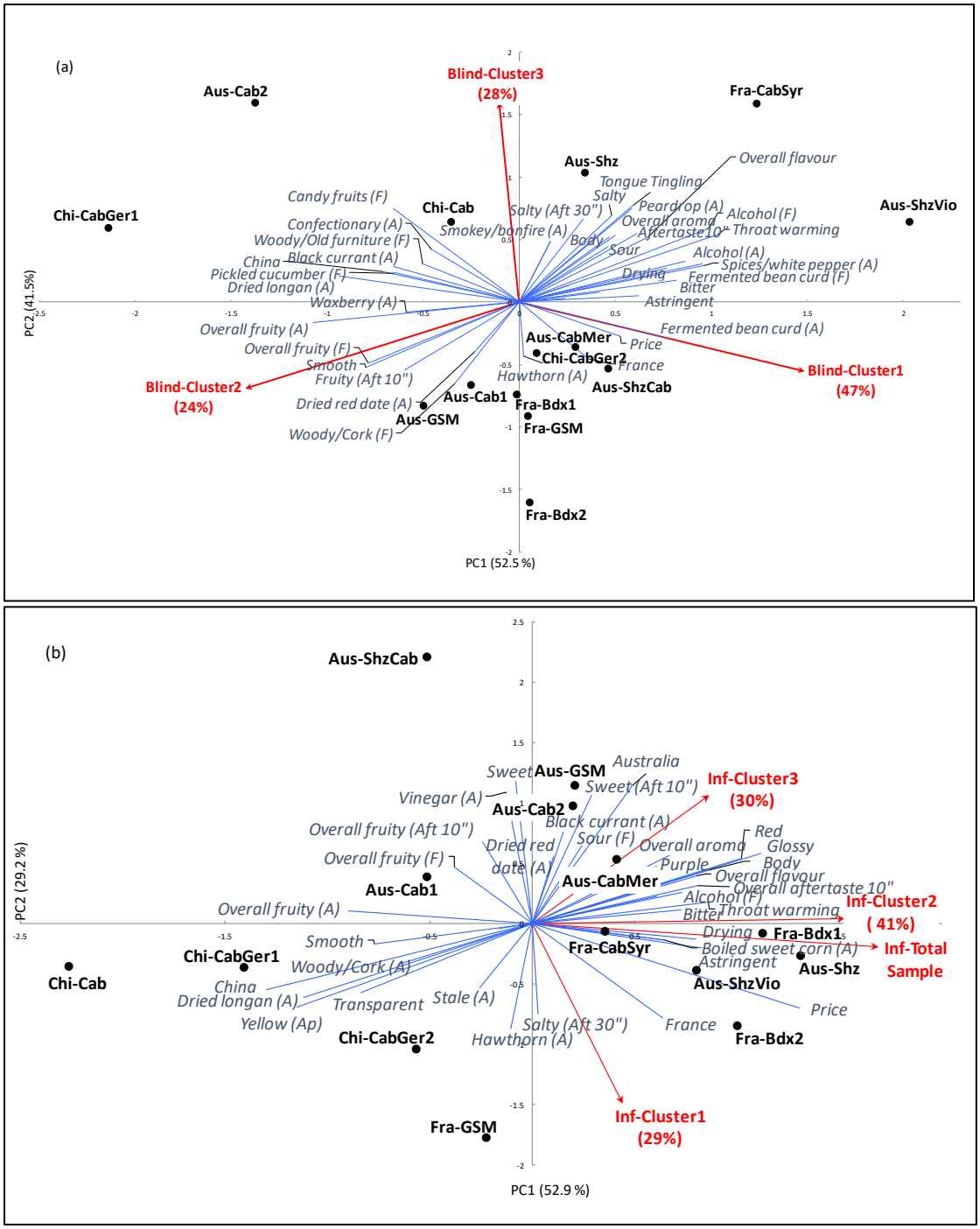

Figure 9. Principal component analysis biplot of the mean liking scores of three Chinese consumer clusters for (a) blind liking and (b) informed liking shown as vectors, together with the liking scores for the total Informed sample. The proportion of consumers in each cluster is shown in parentheses, and the 14 wines are shown as solid circles. The sensory attributes from a Chinese sensory descriptive analysis panel are indicated in italics.

In a further study, the effect of information about Australia on initial choice of Australian wines compared to that of other countries was investigated, and the results showed that information about Australia increased likelihood of choice of Australian wines, even after more than ten days, with specific messages such as Australia being a clean environment; Australian wines being great tasting to Chinese consumers, and Australia having strong regulation being strongest, while Australia being an

attractive tourism destination or with a long tradition of prestigious wines being less strong (Williamson et al. 2014; Williamson et al. 2016; Lockshin et al. 2017).

Outcome and conclusion

The project has advanced knowledge of volatile compounds that give rise to important flavour in varieties of commercial importance to the Australian wine industry. The recognition of isobutyl methoxypyrazine as a key contributor to 'green' aroma in Shiraz, especially when whole bunch fermentation is practiced, is a step forward in controlling 'green' characters in this variety. The defining of 'green tannin' as related to both 'leafy'/'stalky' flavour and bitterness, will also help to focus efforts to avoid these specific characteristics. Simple treatments such as food grade plastic material included in a red ferment were shown to have significant potential in removing this flavour.

In related work, 'green' aroma was shown to be able to be transferred to a wine from wind-break trees planted near a vineyard. Avoiding MOG in grapes near to the trees or harvesting separately are straightforward steps producers can take to minimise this character. A producer in the Yarra Valley has already taken advantage of this information to control this character.

As demonstrated by this project, the role of monoterpenes in 'apricot' flavour of white wines; specific esters in 'peach' aroma; thiols in both 'fruity'/'citrus' flavour and more overt 'tropical fruit' flavour in Chardonnay opens up avenues for producers to be able to control and adjust these flavours in their products. For the thiol compounds in Chardonnay, the effect of production variables such as skin contact post-harvesting, or choice of yeast strain, are well studied and allow straightforward optimisation of these flavours by winemakers in their processes. The importance of monoterpenes in Viognier has not been previously shown, and as these compounds are relatively easy to measure in grapes, this new knowledge gives the ability to understand the impact of different planting material and viticultural options on the attractive varietal flavour of this variety. A major Viognier producer has taken steps to use this information in their production systems.

Thiol compounds that contribute to general 'fruity' flavour in Chardonnay, as well as distinct 'grapefruit' or 'tropical fruit' flavour, can be adjusted in concentration through various options previously identified. The confirmation of benzyl mercaptan as having a role in 'struck flint'/'match' character has generated interest among winemakers at seminars as this is considered a positive 'funky' element for ultra-premium Chardonnay.

The studies on behaviour and sensory preferences of Chinese wine purchasers and consumers have clearly shown that messages about Australia will increase initial purchase of Australian wine compared to French or local Chinese wines, with information about the clean environment of Australia and the attractive flavour of Australian red wines to Chinese consumers being the most influential. This work allows Australian wine companies and Wine Australia to focus their efforts in specific approaches to improve sales. Studying the sensory preferences of Chinese consumers when tasting a range of wines informed with knowledge of country of origin, price and brand/packaging, provided insight into sensory properties and showed that there are specific Chinese language sensory attributes related to preference, which can be used not only to consider the most appropriate wine styles for the market but also with communication strategies alongside the complementary Chinese wine lexicon outcomes.

Recommendations

Further investigations into the contribution of thiols to the positive flavour of other wine types should be conducted. The enhancement of fruit intensity of Chardonnay wines may be a feature in other varieties, notably red wines, where these compounds have been little studied. While they can be susceptible to hydrolysis and oxidative degradation, their role may be significant. The unusually high levels of these thiols in a few warm climate wines may be related to viticultural factors such as fertiliser use, as previously indicated, and this would be a fruitful area to assess. The mechanism of formation of benzyl mercaptan should be identified, as it is likely formed from a yeast-derived compound with further transformation during barrel fermentation and ageing in oak.

Investigating the viticultural factors influencing monoterpene concentration in Viognier would also be an avenue for further work, with little known about aroma biosynthesis, clonal and ripening effects in this variety.

Whole bunch fermentation is widely practised in red wine production, and variables that affect 'green' aroma and flavour from this process should be further examined. The efficacy of food-grade fining materials in selectively reducing green character should be confirmed.

The role of specific volatiles in other key sensory attributes that are not well understood should be determined, with attributes previously shown to be important in influencing consumer response including 'raisin'/ 'jammy' flavour in Shiraz, 'musk' and 'chocolate' aroma and compounds other than rotundone contributing to 'spicy' flavour in red wine.

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Project 3.1.3 – Flavour precursors: contribution to wine aroma, in-mouth sensory properties and flavour release

Abstract

Glycosides were previously thought to be flavourless, needing the action of enzymes during fermentation or slow chemical reactions during wine ageing to release and express their flavour. This project has established that the presence of glycosides in wine and their concomitant breakdown during tasting can boost desirable 'fruity' and 'floral' lingering flavour attributes. As a persistent aftertaste is a hallmark of quality wines, flavour release from glycosides may be a key factor differentiating between good and excellent wines.

In this project, experiments were conducted with sensory panels tasting glycosides from white wines and parallel measurement of flavour compounds in saliva or in the mouth. The results confirmed that enzymes in the saliva act like a key to a locked door, releasing a wave of additional flavour that can be perceived over the time after swallowing, creating a positive long-lasting fruity flavour sensation.

Adding purified glycosides to a juice or wine resulted in increased flavour with no negative characteristics; it was also established that white grape skins are a readily available source for extraction of glycosides which can be used as a natural flavour boost that has potential for an easily controlled new way of enhancing a wine's sensory properties. Finally, knowledge about the profile and/or concentrations of glycosides in grapes or a wine could be used by winemakers as a quality or style indicator.

Executive summary

This project demonstrated the potential of non-volatile glycosides as flavour precursors during wine consumption. The studies on floral varieties such as Riesling and Gewurztraminer showed that there is a surprising ability of in-mouth enzymes, most likely from salivary bacteria, to quickly liberate volatile aroma compounds from their bound form during wine drinking, enhancing flavour and contributing to a lingering aftertaste. The quantity of flavour release and/or retronasal perception of flavour released by this mechanism seems to be fairly variable across individuals, suggesting one reason for variation in people's sensory perception of wine and, potentially, their preferences.

To further corroborate this work, a winemaking experiment was completed to explore various methods winemakers might use to intensify the contribution of these precursors in their wines. Several sets of grape juices were treated to increase their level of glycosides, and following fermentation, sensory and chemical analyses were completed on the finished wines, as well as wines with glycosides added prior to bottling. The addition of glycosides had a major effect on wine aroma and flavour, enhancing 'fruity'/'floral' attributes with no effect on bitterness or astringency. Chemical data showed an increase in key aroma compounds as a result of glycoside addition, as well as higher levels of intact glycosides, acting as flavour precursors and contributing to an enhanced persistence of 'fruity'/'floral' flavour.

The demonstrated benefit of increasing the amount of glycosides present in wines to boost desirable flavour and flavour persistence opens up the option for wine producers to make additions of glycosides isolated from grape skins, or indeed changing vineyard management practices to increase glycoside concentrations. Further studies of the practical application of glycoside preparations from grapes in a production setting should be completed, as well as work on other commercially important grape varieties and investigations of other precursor classes, notably amino acid conjugates of sulfur aroma compounds.

Background

Potent aroma compounds can be present in grapes and wines in a bound form (e.g. glycoconjugates or amino acid conjugates) and may be released during winemaking and storage (Parker et al. 2017a). The pool of precursors can break down through chemical hydrolysis reactions, or through the action of yeast or bacterial enzymes, significantly changing the aroma profile of a wine, a fact that can be exploited by winemakers to influence flavour profiles and persistence in wine. Figure 1 provides a schematic representation of flavour release.

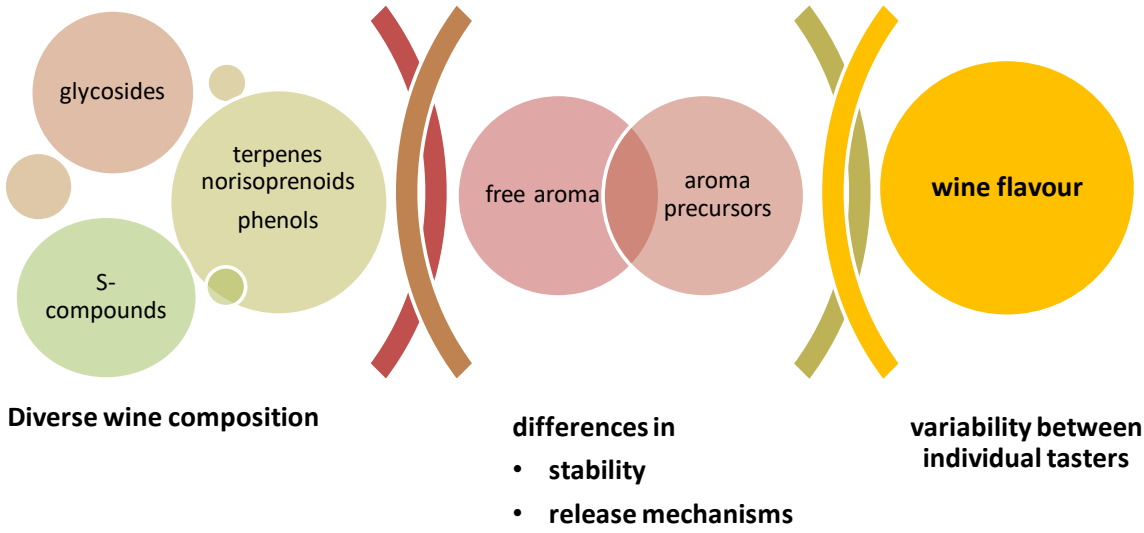

Figure 1. Illustration of free volatiles and precursor compounds involved in wine flavour release

Previous studies conducted at the AWRI from the late 1980s to the late 1990s found that there are numerous glycoconjugates of aroma compounds in grapes and wine and a relatively simple, although broad, analytical quality measure (the 'GG assay') was developed from this work. The important variety Shiraz was not studied to any extent at that time. Since then, analytical tools have progressed significantly, with options now available to characterise key precursors, glycosides and sulfur-conjugates by LC-MS and the ability to measure volatiles in expired air.

An important outcome from recent AWRI smoke taint research was that glycosidically bound aroma compounds can be released in-mouth and add directly to the flavour and aftertaste of a wine (Parker et al. 2012). As some sulfur compounds have very low odour thresholds, they are of particular interest, and an interaction of saliva with precursors to give free 'tropical fruit' thiol aroma compounds has previously been demonstrated in other food areas. The release of volatiles in-mouth after swallowing was described as a delayed perception of retronasal odour, and is of particular interest. A more comprehensive understanding of the role of aroma precursors and of their sensory impact is indispensible to fully understanding wine flavour, flavour complexity and perception.

Model studies so far have shown that some glycosides - including those of non-volatile phenolics - are susceptible to hydrolysis in the mouth, through the activity of human enzymes and/or enzymes derived from oral microflora, although their direct sensory significance was not demonstrated. An early indication of hydrolysis of esters in-mouth was reported. Studies in vegetable flavour showed that a cysteine conjugate precursor to the potent thiol 3-mercaptohexanol was broken down in-mouth by salivary bacteria, with sensory impact of aftertaste for up to three minutes. In recent studies at the AWRI, smoke taint-related volatile phenols were released from their bound

glycoconjugate form to produce important retronasal flavour (Parker et al. 2012), and it was found that this effect occurred in the presence of ethanol and acid. The specificity of release in-mouth is not known, that is, whether all glycoside types will break down as a result of certain salivary bacteria and enzymes or only a small proportion. From preliminary work carried out at the AWRI, non-volatile fractions of glycosides from wines not affected by smoke taint have also been shown to be broken down in-mouth, releasing 'fruity' flavour. The degree of importance of fruit flavour released from glycosides relative to other sources is not clear.

Australian winemakers aim to produce wine styles to suit specific markets, which is reliant on sourcing a suitable supply of grapes and selecting production methods to achieve the target style. Markers for measuring style have long been sought to inform production. However, relatively few objective measures of volatile grape and wine compounds which can be directly related to style are currently available. Precursor compounds have previously been considered a pool of potential flavour rather than as flavorants themselves, and this research will open up the identity of classes of compounds that are highly important to wine flavour intensity and which can act as flavour markers. The slow breakdown of precursors during wine ageing in bottle may be a cause of loss of some flavour in-mouth over time, but degradation of key free volatiles such as thiols is typically much faster and an understanding of these phenomena could open ways of increasing shelf-life or cellar-life of wines.

Despite their relevance in the consumer liking of wines, important phenomena such as the role of retronasal aroma and in-mouth aroma release are scarcely characterised in food analysis, and the wine industry could greatly benefit from the development of this knowledge, as such information will result in helpful decision tools and analytical methods for producing wines with certain desired in-mouth sensory characteristics.

Highlights

- Glycosides in wine, previously considered to be flavourless, were shown to release flavour during consumption, enhancing fruit characters and aftertaste without giving any bitterness.
- The flavour release was highly variable across individuals, providing further insight into possible reasons for individual differences in perception and wine style preferences.
- Increasing glycoside concentration in wines, by addition of glycosides isolated from grape skins from a floral variety high in monoterpene glycosides using a simple procedure, gave increased 'fruit'/ 'floral' aroma and flavour with no bitterness and has potential as a practical means of enhancing flavour in some wine types

Objectives

The project has the overall goal of understanding the flavour of wine in-mouth that is due to precursor compounds as well as retronasal (aroma by mouth) perception of volatiles. The project has the following specific objectives:

- To characterise glycoconjugates and amino acid conjugates of volatile compounds from Shiraz and other varieties, including volatile hydrolysis products.
- To assess the extent and sensory significance of in-mouth breakdown of glycosides, amino acid conjugates and free volatiles such as esters.
- To determine the relationship between levels of key precursors in grapes and the amounts remaining in wines.
- To determine the effect of other wine constituents such as alcohol and non-volatiles on in-mouth release and sensory properties of precursor-derived volatiles.

The project will provide insight into the consumer experience when drinking a wine, including the intensity of flavour and the persistence of aftertaste, rather than on perceptions by orthonasal

evaluation by simple sniffing as often practiced by winemakers and other experts. The project will lead to analytical targets for quality markers for grapes and wines.

Method

Glycoconjugates were isolated from grapes and wines using polymeric adsorbents and analysed using LC-MS methods (Parker et al. 2017b). Importantly, the phenolic glycosides that contribute to bitterness were removed using a high pH wash. The hydrolysis products of the glycoconjugates were determined through enzyme and elevated temperature acid hydrolysis, followed by GC-MS (Parker et al 2017b). Synthetic glycosides were also produced for study.

In assessing in-mouth release, conditions were developed for capturing volatiles in the oral cavity following from preliminary work conducted at the AWRI, using in-mouth solid phase microextraction (Mayr et al. 2014). Volatiles released from isolated and synthetic precursors were determined with in-mouth volatile release *in vivo* studied as well as incubation with saliva *in vitro* (Parker et al 2017b). Matrix conditions were evaluated, notably alcohol and pH. Sensory studies were conducted, using time intensity methods (Parker el al 2017b), expert assessments and a sensory descriptive analysis study. A range of subjects were used, with pre-screening to assess variation in salivary breakdown ability, as previously observed. Application of chlorhexidine gluconate mouthwash was used to investigate whether in-mouth biota elimination is a cause of this variation.

A winemaking study was completed with precursors derived from grapes added to juices and wines, followed by sensory and compositional analysis, using procedures detailed in Mayr et al. (2014).

Results and discussion

In initial studies, extracts isolated from Chardonnay and Shiraz juice gave negligible flavour in contrast to Gewürztraminer or Riesling samples, which showed major flavour effects. Further investigations concentrated on glycosides from Gewürztraminer and Riesling (Parker et al 2017b).

Experiments showed that detectable monoterpenes were released both *in vivo* and *in vitro* through interaction of glycosides with saliva. Up to 80% release of monoterpenes could be achieved during 30-minute incubation of glycosides isolated from Gewürztraminer with saliva. From expired air trapping using a stir bar sorptive extraction technique, it was surprising to be able to detect monoterpenes using GC/MS after subjects tasted glycoside material, as it was expected that any release would be only apparent by GC-olfactometry, as previously demonstrated with smoke glycosides. The ability of subject's saliva to breakdown the glycosides varied in extent across individuals, from 26% to 76% release, in line with previous work on volatile phenol glycosides. Interestingly, even the saliva from those individuals who could not perceive any flavour when tasting precursors could break down glycosides and release monoterpenes, which indicates perceptual or in-mouth retronasal air flow differences among individuals may be more important than differences in saliva microflora. This experiment showed that enzymes or microflora in saliva are capable of liberating free monoterpenes from their glycosides during 30 minutes of incubation, but to be relevant to flavour, this must happen in a shorter time frame in the range of seconds to minutes.

The glycoside profile of 15 Gewürztraminer and 16 Riesling wines was determined. There is little quantitative information in the literature on the concentration of specific glycosides in wine. Using a LC-MS method developed for the purpose, with synthesised internal standards, it was found that geranyl glucoside was the major glucoside, with multiple disaccharide monoterpene glycosides also observed. The Gewürztraminer wines had much higher total monoterpene glycoside concentration than the Riesling wines, from 300-1800 µg/L compared to 20-140 µg/L.

Volatiles released from the Gewürztraminer glycosides upon enzyme hydrolysis included the monoterpenes α-terpineol, nerol and geraniol, as well as the less important octanol, decanol, benzyl alcohol and 2-phenylethanol. The Riesling glycosides gave similar volatiles, with a lower relative abundance of geraniol, and higher octanol and decanol abundance. These results confirmed the presence of enzyme-labile precursors, and provided further supporting evidence that glycosides in wines from these varieties have the potential to give rise to aroma-active compounds during consumption.

The sensory importance of monoterpene glycosides during tasting was assessed, to determine whether odorous aglycones were released in-mouth. Monoterpene glycosides were isolated from Gewürztraminer and Riesling wines and juices, characterised using LC-MS, and studied using a time-intensity technique, together with a synthesised monoterpene glucoside. When assessed in model wine at five times the concentration expected in wine, Gewürztraminer glycosides isolated from either juice or wine, together with geranyl glucoside, gave significant flavour and enhanced aftertaste (Figure 2).

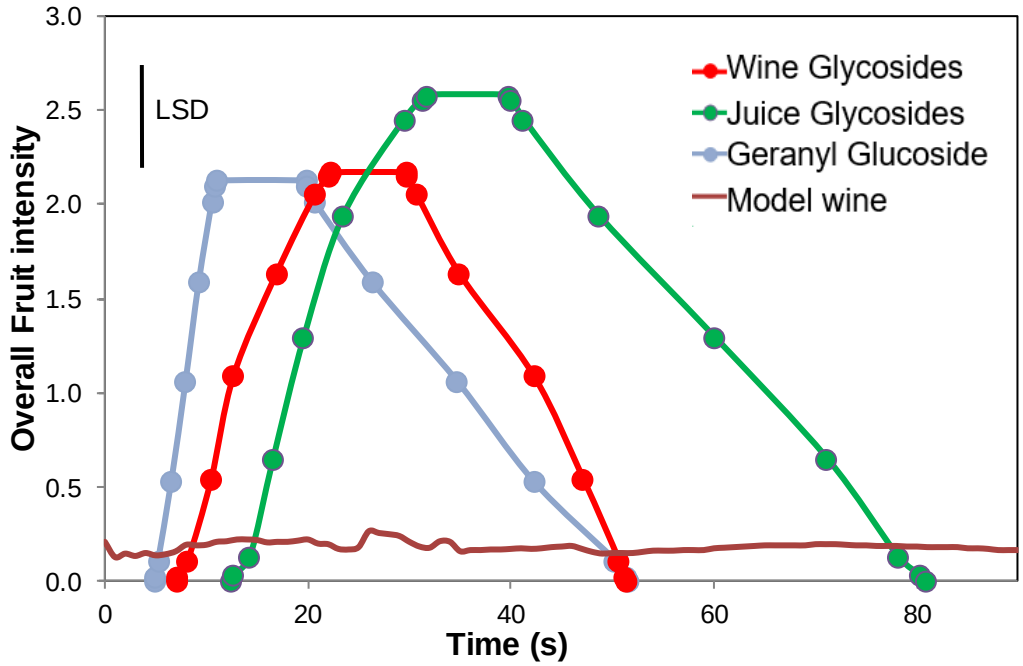

Figure 2. Mean time-intensity sensory rating curves for precursor samples assessed in a model wine. Also shown is data for a model wine control. LSD: least significant difference (P=0.05) for the maximum intensity parameter.

To assess the relative effect of glycoconjugates of Riesling and Gewürztraminer compared to free volatiles, they were assessed in a more challenging, but more realistic, model wine system at single strength using a time intensity methodology. Combinations of the glycoconjugates and volatiles were tested at levels closely comparable to those found in Riesling and Gewürztraminer wines. There was an indication that the monoterpene glycosides enhanced the duration and intensity of the perceived flavour. A third of the judges were most responsive to the flavour from the glycosides, and for this sub-group, the glycosides isolated from Riesling contributed significant flavour, with the combination of Riesling volatile aroma compounds and the precursors giving the longest duration of aftertaste compared to the volatiles alone. An example of one judge's responses is shown in Figure 3. This result means that at wine-like concentrations, with the influence of ethanol and wine pH, release of monoterpenes from glycoside precursors for most people is relatively subtle, but likely to

be an important source of flavour for varieties such as Riesling. It may be that the effect is larger for wines of lower alcohol or higher pH. The results indicate that important flavour may be contributed by the glycosides at wine-like concentrations for a proportion of the population.

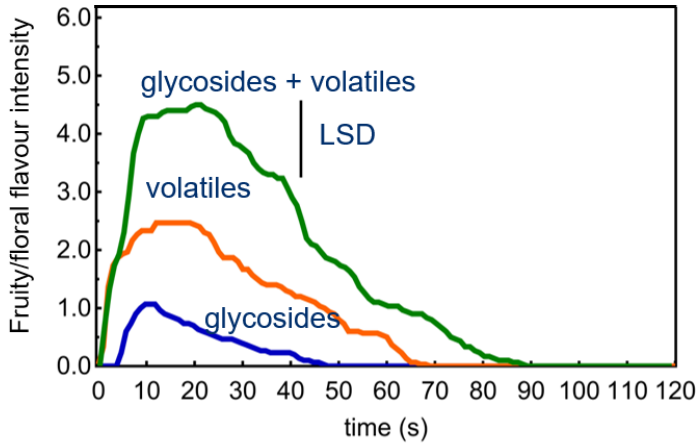

Figure 3. Average time-intensity curves (mean of three presentation replicates) for one judge who responded to the glycosides: for glycosides (blue), free volatiles (orange) and glycosides plus free volatiles (green) isolated from a Riesling, and assessed at wine-like concentration. The solid vertical bar is the least significant difference at the 5% level of significance for the maximum intensity parameter.

Gewürztraminer glycosides, geranyl glucoside and guaiacyl glucoside were investigated using a larger sensory panel (*n*=39). Results confirmed that there was large inter-individual variability, with 77% of panellists responding to at least one glycoside, and 28% responded to all three (Figure 4). The variation in responses is likely due to differences in ability of saliva enzymes to break down the glycosides, due to differing populations of microflora in the mouth cavity, and may also be related to differing ability to perceive the volatile compounds released.

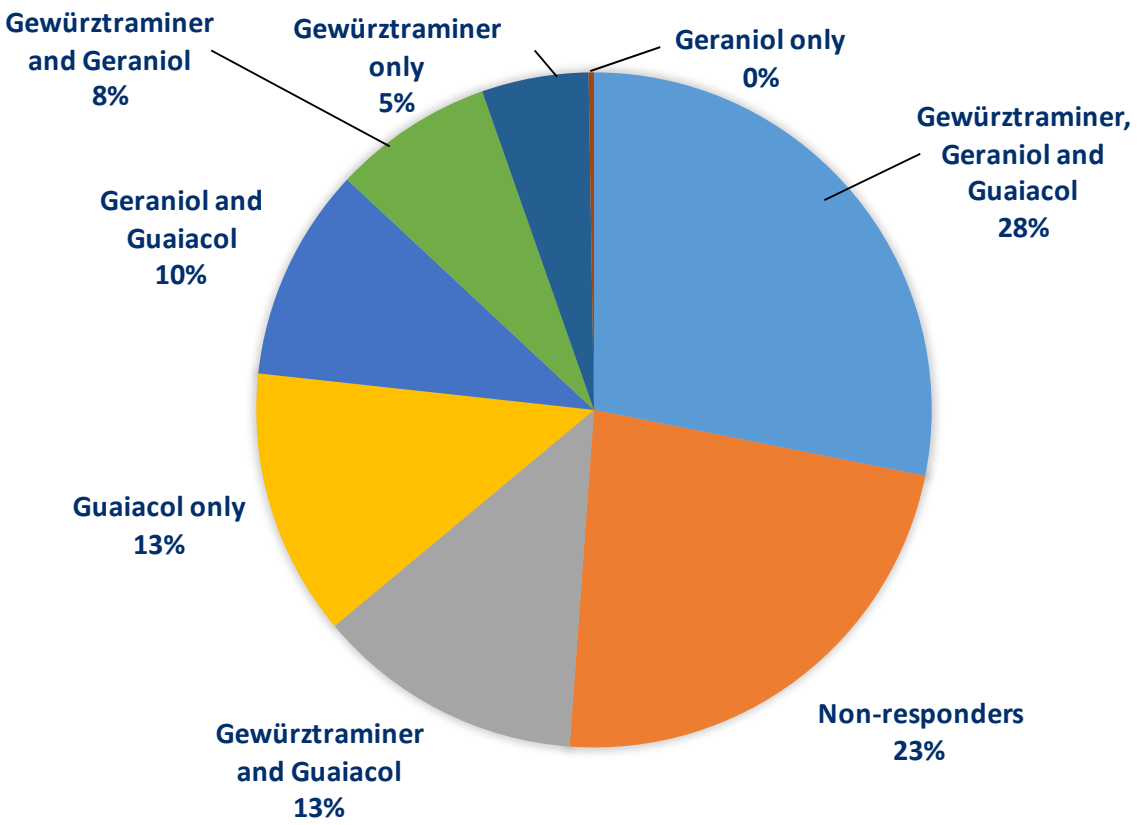

Figure 4. Variability in response to three glycosides: Gewürztraminer glycosides assessed at double strength; and two synthesised glycosides, geranyl glucoside and guaiacol glucoside.

Overall, these studies have demonstrated for the first time that non-volatile glycosides of monoterpenes can contribute to retronasal olfaction and after-odour through hydrolysis in-mouth. The effect can be eliminated through use of antibacterial mouthwash, and the extent of hydrolysis is almost certain to be related to microbiota populations in the oral cavity.

For wine producers, enhancing glycosides remaining in wines could be a feasible way of improving intensity and persistence of desirable flavour. As glycosides are present in other fruits and foods, breakdown in-mouth could be of importance to the consumption experience for other products, and glycosides could be valuable ingredients to enhance flavour. The study showed that grape-derived glycosides can give 'fruity' flavour, providing a means of enhancing flavour in wines, and confirms that the effect is very different across people, adding to evidence that sensory experiences are highly variable within a population.

In a follow-up experiment, glycosides were extracted from a large quantity of Gewürztraminer marc and purified using a polymeric resin column to remove phenolic compounds that contribute bitterness. The glycosides were added to Riesling and Chardonnay juices at two concentrations, and fermentation was conducted using standardised conditions. A treatment involving addition of glycosides when the wines were bottled was also included in the study. The sensory properties of the wines were quantified using sensory descriptive analysis, and chemical determination of monoterpenes and residual glycosides was also completed. The addition of glycosides significantly increased the concentrations of free monoterpenes and monoterpene glycosides in all treatments, resulting in significant increases in 'fruity'/'floral' aroma, flavour and aftertaste, while not significantly altering the bitterness or astringency. The timing of the addition was not as important as the overall amount of glycosides added. Interestingly, the sensory panellists who were confirmed in separate testing to be able to perceive flavour from glycosides (46% of the panel), rated 'floral aftertaste' in the wines with enhanced glycoside concentration substantially higher than those who could not, providing evidence that the residual monoterpene glycosides were a significant factor contributing to the flavour enhancement for these assessors. The concentration of geranyl glucoside, together with free beta-damascenone and linalool, was found to be strongly associated with the intensity rating of 'fruity'/'floral' attributes.

Consumer preference testing was conducted using a subset of the wines from the winemaking study, and showed that the Riesling wine made with an addition of glycosides to the juice was well liked for a sizeable cluster of consumers, while the larger additions were not appreciated. Moscato and Riesling drinkers showed a trend for increased liking of the Riesling addition wines compared to drinkers of other wine types.

The project was completed as planned, with an agreed shift early in the project from the varieties Chardonnay and Shiraz to Riesling and Gewürztraminer. A scientific paper was published describing the main outcomes of the project (Parker et al 2017b), with a further manuscript submitted. The work was communicated as an oral presentation at the 16th Australian Wine Industry Technical Conference in July 2016, and was awarded a best poster prize (Parker et al. 2017c), as well as presented at a flavour workshop at the conference. An *AWRI Technical Review* article was published (Parker et al. 2015), and outcomes have been presented at AWRI roadshows.

Outcome and conclusion

These studies have shown that non-volatile glycosides, previously considered a reserve of flavour that may be slowly broken down over time in-bottle, releasing flavour, can in fact contribute directly to wine flavour and aftertaste through in-mouth breakdown, most likely by microbiota in the saliva. Wines made from juices with added glycosides had more intense aroma and flavour, including a more intense and prolongated aftertaste. As aftertaste is one of the hallmarks of a high quality wine, the recognition of this hitherto unrecognised source of flavour is an important step in better understanding the factors required for an attractive, enjoyable and complex wine.

The project established a technique of isolating non-phenolic glycosides, eliminating any potential for bitterness. As glycosides can be quite easily isolated from white grape skins, there is potential to make use of a resource previously considered mainly as a waste material in the industry. The extraction procedure used in the project to generate a glycoside fraction suitable for winemaking was relatively simple and straightforward, and could be applied either by wineries using equipment available through winery engineering firms, or potentially by a company interested in supplying such material. There could be major benefits economically from improving flavour of lower value wines through use of a waste product.

Recommendations

Further studies are warranted into the cause of individual differences in perception and release of glycosides in-mouth. Studies involving other varieties, as well as investigations into the role of other precursor classes, notably cysteine and glutathione conjugates of sulfur compounds, would likely prove highly informative.

Regarding the effect of increasing glycoside concentration through isolation of non-phenolic glycosides from grape skins, several approaches could be taken. There is a need to consult with wine producers regarding their potential use of this technology, notably the relevance and potential for enhancing products, suitability for particular styles and also whether cost or certain processing constraints might be serious obstacles for uptake.

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Project 3.1.4 – Factors affecting wine texture, taste, clarity, stability and production efficiency

Abstract

Wine texture is considered a major product differentiator both for wine style and value in the marketplace. In addition, clarity and colour stability (absence of haze development and the retention of colour) are generally considered to be pre-requisites to market success. Achieving the optimum levels for each of these parameters is often done at significant cost using current technology and does not always ensure the wine will meet its full potential. The ability to modulate these characteristics of wine while retaining the ability to economically process the wine to ensure microbial stability and stylistic integrity is a significant challenge for the wine industry. This project focused on the key compositional drivers behind texture, bitterness, clarity, stability (protein and colour) and wine filterability and developed strategies to modulate them in a production-based environment. Specifically, it covered molecular drivers of taste and texture, 'smart' surfaces for efficient production, predicting haze formation, understanding and preventing wine haze, effects of filtering red wines, solids management effects on white wine style and composition, impact of winemaking methods on wine macromolecules and texture, colour development/management and a practical method to determine extractable grape colour and tannin.

Executive summary

Wine texture is considered a major product differentiator both for wine style and value in the marketplace. In addition, clarity and colour stability (absence of haze development and the retention of colour) are generally considered to be pre-requisites to market success. Achieving the optimum levels for each of these parameters is often done at significant cost using current technology and does not always ensure the wine will meet its full potential. The ability to modulate these characteristics of wine while retaining the ability to economically process the wine to ensure microbial stability and stylistic integrity is a significant challenge for the wine industry. This project focused on the key compositional drivers behind texture, bitterness, clarity, stability (protein and colour) and wine filterability and developed strategies to modulate them in a production-based environment.

The compositional drivers for texture, hotness and bitterness were investigated in both red and white wines. Different white wine phenolic classes were shown to have different effects on mouth-feel (such as oiliness and viscosity) and bitterness of white wine, and could be manipulated by managing extraction from skins during white winemaking. This is to be contrasted with the limited effect of white wine phenolics on astringency demonstrated previously.

A significant discovery was made in relation to the presence of an indole conjugate which was found to statistically correlate with bitterness of white wine fractions. Sensory assessment showed that the indole derivative might be a new bitterant in white wine; this is a significant development as currently there are only few molecular targets known which cause bitterness in wine.

The perceived bitterness in white wine was significantly and consistently reduced by higher dissolved CO_2 levels. Perceived sweetness increased significantly with increased dissolved CO_2 in a Chardonnay wine, which was consistent with a trend seen in a Viognier wine. Dissolved CO_2 did not significantly influence perceived viscosity or astringency. The perception of 'spritz' increased significantly with increasing dissolved CO_2 levels in both wines as expected, but in the case of the Viognier higher pH and higher ethanol content accentuated the perception of 'spritz' when dissolved CO_2 was high. The reasons for increased perceived sweetness and reduction in bitterness in the presence of increasing dissolved CO_2 levels are unclear, but warrant further investigation.

Tannin concentration drives most of the astringency perception in red wine, while pH and alcohol modulate it (as lower pH and lower alcohol increase astringency), but the mechanisms responsible remain unclear. Using red wine tannin, experimental results showed that across a wine-like range (10-15%), ethanol can influence the mechanisms of wine tannin-protein interactions and that the previously reported decrease in wine astringency with increasing alcohol may, in part, relate to a decrease in tannin-protein interaction strength. This is significant as it highlights a key element likely to influence the astringency perception mechanism that has not previously been highlighted.

The role of polysaccharides in wine sensory characteristics remains debated and efforts were directed towards improving the understanding of red wine polysaccharides. Sensory assessment of isolated red wine polysaccharide and three sub-fractions and their interactions with alcohol and pH showed a range of impacts. Astringency suppression was greater for low molecular weight rhamnogalacturonan-rich polysaccharides; low to medium molecular weight polysaccharides increased perceived viscosity; and the bitterness of the higher alcohol/higher pH wine was significantly reduced in the presence of medium molecular weight polysaccharides. Furthermore, perceived hotness from alcohol was reduced in the low alcohol (11.5% v/v) wines by medium molecular weight polysaccharides, consistent with work on white wines. Alcohol and pH most significantly impacted hotness and astringency respectively.

In a production environment, knowledge of macromolecular adsorption onto surfaces is critical for better understanding and control of processes such as filter fouling, binding to tanks and fittings and interactions with processing aids such as bentonite. To increase this knowledge, model surfaces with tailored surface properties (e.g. charge, polarity, chemical functionality, wettability) were developed to explore how wine constituents interact with them. The effect of surface chemical functionalities on the adsorption of white, rosé and red wine constituents was evaluated. The results may aid in the development of the next generation of low fouling membranes, tank materials, wine production surfaces and/or new sensing platforms that will reduce cost and improve productivity in wine and related industries.

Heat stability is an ongoing issue in the wine industry with cloudy wine having the potential to damage brands and reputations for wine quality. Understanding wine haze has been important to better predict and mitigate protein haze. For this project, the focus was on understanding the components that drive haze formation by investigating interactions between proteins and other wine matrix components in model wines and in real wines. Model wine investigations included analysis of interaction strength with isothermal titration calorimetry (ITC), protein stability with differential scanning fluorimetry (DSF) and particle size with nanoparticle tracking analysis (NTA). The components that were found to have the greatest impact on haze were analysed in a selection of real wines to assess the real-world impacts of matrix components on haze formation. However, no single factor had a statistically significant impact on haze formation in real wine, indicating a complex interdependency between matrix components and the haze formation process.

Predicting protein haze potential accurately is essential for determining the amount of bentonite required to prevent wines from developing a haze. Ideally, haze could be predicted easily from measuring different components of the heat test; however, trials undertaken to asses this possibility did not establish any obvious leads. The most widely used method in industry is a heat test method developed in the 1970s. This empirical method was revisited to improve reproducibility in industry and the new method has decreased the turnaround time for results from 24 hours to 5 hours without compromising accuracy.

The most widely used method for preventing wine protein haze is the addition of bentonite. However, issues with poor settling and selectivity have prompted much research into alternative

methods for removing proteins. These methods include new proteases for cleaving proteins, new adsorbents that act like bentonite but with better settling and selectivity properties and treatments such as heating grape must. The trials undertaken for this project further explored some potential natural proteases isolated from *Botrytis cinerea* and sunflowers, investigated a range of new protein-adsorbing material including coated magnetic nanoparticles, surface-engineered silica and macrosponges. Alternative treatments for grape must were explored including vortex fluidic device and flash pasteurisation parameters. Pasteurisation of juice (with or without aspergillopepsin enzymes) remains a viable option in many cases and magnetic nanoparticles show promise but require some further development.

Filtering red wines has long been a concern in industry, with the perception that the action of filtering removes some important colour and texture molecules. Laboratory-scale investigations have suggested that filter membranes can remove polysaccharides, tannins and anthocyanins; however, the impact of commercial-scale filtration on red wines was unknown. The results of experiments using industry-scale equipment showed that the particles removed during filtration had minimal impact on wine composition or texture. Further experiments with high solid juices demonstrated that typically white wine produced from high solids juices contained significantly higher concentrations of polysaccharides. In terms of sensory effects, fermentation of high solids juice generally increased 'fruity' aromas, viscosity and oiliness, with the magnitude of the effects varying somewhat between treatments and varieties. The results suggest that both textural and aromatic characters can be diversified through modified settling processes and the associated compositional changes. From a practical perspective they also suggest that the method of clarification will most likely not influence the total phenolic concentration of a wine.

A significant body of work was produced in relation to the use of winemaking methods to diversify wine macromolecules and texture. The use of yeast strain, enzymes and maceration techniques individually or in combination can have a marked effect on wine tannin and polysaccharide. The magnitude of the effects can be large, but varies depending on the maturity of the grapes and the effect of the yeast, enzyme or maceration protocol on the mechanisms driving extraction and retention processes. The research also identified a potential new mechanism by which extracted grape tannin may be lost from red wine during vinification. Several experiments continued to demonstrate how various approaches to lowering alcohol (pre-ferment) affect tannin, polysaccharide and colour outcomes in red wine. Results indicate that to effectively lower alcohol while maximising wine macromolecule extraction and wine texture, must dilution treatments are likely to lead to more favourable outcomes than harvesting earlier.

The key finding from studies on colour development highlighted the important role that higher molecular weight tannins play in colour stabilisation. A simple extraction method was developed to determine 'wine extractable' tannin and anthocyanin in grapes. The 'wine-like' extraction method uses gently-crushed grapes, adjusted to 15% v/v ethanol, pH 3.4, in their own juice and provides a useful prediction of wine tannin and colour. A protocol is available on the AWRI website and a predictive spectral method to determine 'wine-like' extraction is available on the AWRI WineCloud analysis platform.

In conclusion, the project has successfully elucidated key compositional drivers of texture, bitterness, clarity, stability (protein and colour) and wine filterability and developed strategies to modulate them in a production-based environment.

Background

Wine texture is considered a major product differentiator both for wine style and value in the marketplace. In addition, clarity and colour stability (absence from haze development and the retention of colour) are generally considered to be pre-requisites to market success. Achieving the optimum levels for each of these parameters is often done at significant cost using current technology and does not always ensure the wine will meet its full potential. The ability to modulate these characteristics of wine while retaining the ability to economically process the wine to ensure microbial stability and stylistic integrity is a significant challenge for the wine industry. This project focused on elucidating some of the key compositional drivers behind texture, bitterness, clarity, stability (protein and colour) and wine filterability and developing strategies to modulate them in a production-based environment. Key areas that were addressed in the research are:

- determining molecular drivers of texture and tastes to allow approaches to modulating these molecules that are practical and commercially viable
- characterising and understanding the roles of macromolecules and colloids in wine and their contribution to clarity, stability (protein and colour) and filterability
- the path from grapes to wine for macromolecules and the impacts of industry standard processing technologies such as flotation and filtration on their retention and form
- leveraging the increased understanding of influence of macromolecules to develop more consistent and reliable testing protocols for protein stability and to develop alternative processes to attain this stability.

Highlights

Compositional drivers for texture, hotness and bitterness

A non-targeted metabolomic study led to discovery of an indole conjugate that was found to correlate with the bitterness of white wine fractions. Sensory analysis suggests that this compound might be a new bitterant in white wine. This is a significant development as currently there are very few molecular targets for bitterness in wines.

In still wines, perceived bitterness was significantly and consistently reduced by higher dissolved CO_2 levels. Perceived sweetness increased significantly with increased dissolved CO_2 in a Chardonnay wine, which was consistent with a trend seen in a Viognier wine. As such, it is recommended that dissolved CO_2 levels in still wines should be monitored by producers for their effect on the sensory characteristics of wines they produce.

Sensory assessment of isolated red wine polysaccharide and three sub-fractions and their interactions with alcohol and pH showed a range of impacts including suppression of astringency, hotness and bitterness but increased perceived viscosity. Low and medium molecular weight grape-derived polysaccharides contributed the most, more so than high molecular weight yeast-derived polysaccharides. This result was consistent with earlier work on white wine polysaccharides.

Improved tests for haze and alternatives to bentonite

The heat test remains the most useful method for predicting haze and determining bentonite addition rates. A shorter heat test has been developed that provides results with a turnaround time of only 5 hours instead of 24 hours. This research has demonstrated that controlling both the heating and cooling times for the heat test is critical for obtaining accurate and reproducible results.

A novel technology has been developed based on the use of coated magnetic nanoparticles to adsorb proteins. The method is very effective in removing pathogenesis-related proteins and the

nanoparticles can be simply separated from juice or wine with the use of an external magnet. This new technology has potential to become an alternative to bentonite treatment.

Understanding the effect of filtration on macromolecules that are important for wine texture
Filtering Cabernet Sauvignon and Shiraz wines using cross-flow filtration, followed by lenticular filters and then 0.65 µm and 0.45 µm membrane filters did not alter the concentration or composition of wine macromolecules (polysaccharides and tannins). Sensory analysis of samples from the trial suggested that filtration is unlikely to affect wine texture.

Lowering alcohol without losing tannin and texture
One of the ways of reducing alcohol in wine is to harvest grapes earlier but this can lead to less texture and colour in the wine. Through use of enzymes, a lower alcohol Shiraz wine was produced from earlier harvested grapes that had the same tannin concentration as a wine made from later harvested grapes. The use of yeast strain, enzymes and maceration techniques individually or in combination can have a marked effect on wine tannin, polysaccharide and mouth-feel.

New tannin extraction method for grapes provides prediction of wine tannin
A new 'wine-like' extraction method for grape analysis of tannin and colour was evaluated against the standard extraction method. The 'wine-like' method was shown to better predict tannin content in the final wine than the standard method and allows producers to assess the likely extractability of their fruit.

New methods applied to understanding wine texture and stability
New methods were established to study colloids in wine; for example, aggregates of tannins, proteins and polysaccharides. Isothermal titration calorimetry (ITC), small angle x-ray scattering (SAXS) and dynamic light scattering (DLS) techniques are all now in use.

Objectives

This project focused on providing the required knowledge and tools to allow winemakers to more objectively manage texture, stability, clarity and filterability during winemaking. It achieved this through the improved understanding of precursor grape and wine compositional drivers and a clear understanding of the impact of winemaking processes on the macromolecules and colloids that are linked to these wine parameters.

Specifically, the project investigated:

- the compositional drivers for texture, hotness and bitterness;
- the role of macromolecules such as tannins, polysaccharides, proteins and their aggregate colloids in the expression of texture, stability, clarity and filterability;
- the impact of other wine matrix components on macromolecule function and expression;
- the source of these molecules or their precursors in grapes and yeast and the impact of winemaking processes such as clarification, flotation, vinification and filtration on their retention and/or transformation;
- the impact of filtration on macromolecules;
- strategies for modulation of specific compositional drivers through the use of grape-based fining agents;
- alternative strategies for achieving protein stability;
- practical methods for wineries to determine likely extractability of macromolecules during winemaking; and
- strategies for the stabilisation of colour independent of vintage effects.

The knowledge generated by the project provides a framework for the development of winemaking strategies and practical recommendations for managing colour (and colour stability), astringency, viscosity, hotness, bitterness, filtration processes and protein hazes.

Methods

Interactions between phenolics, alcohol and acidity in mouth-feel and bitterness perception in white wine

White wines made from the 2011 white wine phenolics project previously deemed to be bitter by a trained sensory panel were combined and their phenolics extracted using Amberlite FPX resin. The phenolics were then separated into fractions based on their hydrophobicity using high speed counter-current chromatography using a hexane: ethyl acetate: methanol: water system (described in Gawel et al. 2013). These fractions were analysed for phenolic content by HPLC (Gawel et al. 2014a) and assessed for mouth-feel by a trained sensory panel that had been screened for bitterness sensitivity in model wine at two pH (3.3 and 3.6) and two alcohol levels (11.5% and 13.5%).

Identifying compounds responsible for bitterness in white wine

The hydrophobic isolate from the study described above was sub-fractionated using preparative-scale C18 chromatography to obtain 27 fractions of varying hydrophobicity. These were then assessed for bitterness by a screened sensory panel by applying 1 mL aliquots to the back of the tongue using accepted sensory protocols.

The fractions were assessed by HPLC-MS using a Bruker micrOTOFII high resolution mass spectrometer (Metabolomics Australia) and bitterness ratings were correlated with compound features identified in the fractions to identify candidate compounds responsible for bitterness.

One of those identified was commercially available, but needed to be purified using preparative scale HPLC before being sensorially assessed for bitterness. As only small quantities of the purified compound were obtained, alternative sensory approaches to classical threshold testing were required. Therefore, a recently developed sensory method called 'napping' was used, whereby experienced wine tasters mapped their overall perception of the target compound presented at 100 mg/L and 200 mg/L in a sensory space defined by other compounds with accepted mouth-feel and taste properties, with an overall map being produced using a multi-dimensional scaling method.

Assessing the effect of dissolved carbon dioxide on the taste and texture of white wine

Commercially bottled Chardonnay and Viognier wines, each from the same bottling run, were directly sourced from the winery. The uniformity of dissolved CO_2 across bottles in each batch was assessed by in-bottle measurement using Orbisphere (Hach).

Wines with different levels of carbonation, pH and ethanol concentration required for sensory assessment were obtained as follows. A portion of wine was carbonated under pressure at 23^{o}C, and another was extensively sparged with nitrogen gas. These components and the original wine were mixed in-bottle to achieve four target carbonation levels ranging from low to very high by commercial standards (0.5, 1.0, 1.5 and 2.5 g/L). Wines were adjusted to pH 3.4 and 3.2 and to 12 and 14% v/v. The wines were immediately recapped and the sealed prior to immediate sensory assessment.

Samples of 150 mL of wine were poured into glasses typical of restaurant and domestic wine consumption (100 mm height, 80 mm bowl, 60 mm opening). The wines were poured in groups of four at 10-11^{o}C, and tasted within 10 minutes of pouring. Nine trained tasters rated the intensity of mouth-feel characteristics and overall aroma and flavour using standard descriptive protocols.

Dissolved CO_2 concentrations of the wines poured for the tasters were simultaneously sampled and measured directly from the glass using the Orbisphere system modified for *in situ* wine glass

sampling. This approach allowed variations in dissolved CO_2 arising from pouring and losses in dissolved CO_2 in the glass due to the elapsed time between pouring and tasting to be modelled.

Impact of polysaccharides on red wine sensory properties
Whole polysaccharides were extracted from a current vintage Shiraz wine, and fractionated by molecular weight (MW) using preparative scale size exclusion chromatography into high MW (> 93 kDa), medium MW (13-93 kDa) and low MW (5-12 kDa) fractions. These were characterised by their monosaccharide composition (Figure 2). 150 mg/L of each fraction were added to model red wines containing 0.5 g/L tannin (Tanin Galalcool, Laffort, Bordeaux, France) at pH 3.3 and 3.6, and 11.5 and 13.5% v/v alcohol. Their taste and mouth-feel attributes were profiled by a trained sensory panel using standard descriptive sensory methods (Gawel et al. 2016a).

Understanding the effect of juice solids management
In a small-scale scoping study, a Chardonnay juice was left unsettled and settled under gravity to two clarity levels using a pectolytic enzyme, bentonite, and without a clarifying agent. Controls for each solids level/clarification method combination were obtained by centrifugation to differentiate the influence of settling time from the effect of juice clarity.

In a larger scale study, wines from three white varieties were made from full solids juices and the equivalent low solids juices produced by whole bunch pressed, free run and hard pressing juices cold settled using a pectolytic enzyme.

Two further trials were conducted using juices obtained under commercial conditions. A Chardonnay and a Sauvignon Blanc juice were produced by medium-scale wineries using cold settling with pectolytic enzymes, and Chardonnay and Frontignac juices were produced by large-scale wineries using flotation.

With exception of the scoping study the wines were made in duplicate in 20 L fermenters using standard winemaking protocols and were sensorially profiled 12 to 18 months after bottling using standard descriptive techniques (both described in Gawel et al. 2014a,b). The scoping study was conducted using triplicate 500 mL fermenters of wine tank-like dimensions.

Polysaccharide concentrations were determined by either peak area using size exclusion chromatography (>10 kDa), or by phenol-sulfuric assay where proteins may be a significant confounding factor (see Gawel et al. 2016b). Monosaccharide composition was determined by the method described in Ruiz-Garcia et al. (2014). Total phenolics were determined by the Folin assay and specific phenolics by HPLC as previously described (Gawel et al. 2014a).

Surface characterisation
Plasma polymerisation was used to coat magnetic nanoparticles (Mierczynska-Vasilev et al. 2017) and to create surfaces of varied properties, such as polarity and charge (Mierczynska-Vasilev and Smith 2016a, b). Quartz crystal microbalance with dissipation (QCM-D) was used to study wine adsorption on plasma polymer coated surfaces (Mierczynska-Vasilev and Smith 2016a,b). X-ray photoelectron spectroscopy (XPS) analysis was used to determine the surface composition of various plasma polymer coated surfaces before and after wine adsorption (Mierczynska-Vasilev and Smith 2016a,b). Atomic force microscopy (AFM) was used to provide topographical images of wine constituents on polymer-coated surfaces (Mierczynska-Vasilev and Smith 2016a,b) and to determine the amount of hydration water within the wine layers (in combination with QCM-D) (Mierczynska-Vasilev and Smith 2016a). Scanning electron microscopy (SEM) was employed to determine the morphology of the magnetic nanoparticles (Mierczynska-Vasilev et al. 2017). The thickness of the deposited plasma polymers was determined using an ellipsometer (Mierczynska-Vasilev and Smith

2016b). Nanoparticle Tracking Analysis (NTA) was used for the assessment of wine macromolecules size and concentration (Mierczynska-Vasilev and Smith 2016a,b McRae et al. 2017, Mierczynska-Vasilev et al. 2017, Bekker et al. 2016, Bindon et al. 2016a). Wettability of various plasma polymer-coated surfaces was determined by measuring the contact angle (Mierczynska-Vasilev and Smith 2016a). The zeta potentials of the magnetic nanoparticles before and after plasma coating in aqueous suspensions were measured as a function of pH and proteins in wines were analysed by high-performance liquid chromatography (HPLC) (Mierczynska-Vasilev et al. 2017).

Phenolics, tannins, polysaccharides (soluble and insoluble) and cell walls

The methyl cellulose precipitable (MCP) tannin assay was used to measure the concentration of tannins in red wine and colour properties were assessed using the Somers colour measures for wine (Mercurio et al. 2007). Methods for isolating and fractionating tannins from wine included a solid phase extraction (SPE) method which afforded a comparatively uncomplicated technique for the consistent isolation and fractionation of tannin from wine (Jeffery et al. 2008), and a liquid-liquid fractionation, which allowed separation of isolated wine tannins into two fractions with distinctive properties, F2 and F3 (McRae et al. 2013). In addition to these methods, tannins were also isolated from grapes and wine using existing protocols for preparative-scale chromatography with Sephadex LH-20 (Bindon et al. 2010a, 2010b, 2011) or Toyopearl media (McRae et al. 2010). Multi-layer counter current chromatography (MLCCC) was used to isolate polyphenols from white wine (Gawel et al. 2013).

Tannin characterisation was achieved using established methods including gel permeation chromatography (GPC) for elucidating the average molecular mass of tannin (Kennedy and Taylor 2003) as well as acid-catalysed depolymerisation in the presence of phloroglucinol to determine the composition of the tannin subunits (Kennedy and Jones 2001). Also, isothermal titration calorimetry (ITC) was used to measure the protein-binding capacity (McRae et al. 2010).

The research on grape maturity and its impact on extractability of phenolics required isolation and characterisation of polysaccharides and cell wall material (fibre). Isolation of grape skin and flesh cell walls was achieved using a modified extraction with a buffered-phenol solution. Grape and wine soluble polysaccharides were obtained by precipitation in ethanol and dialysis. Semi-preparative fractionation of red wine soluble polysaccharides was based on size exclusion chromatography using a Sephacryl column. A colorimetric galacturonic acid assay was used for quantifying soluble polysaccharides and insoluble cell walls using a 96 well plate format. High-throughput monosaccharide analysis in polysaccharide hydrolysates was accomplished by using derivatisation with 1-phenyl-3-methyl-5-pyrazolone and analysis by HPLC. Cell wall linkage analysis was performed by methylation and analysis as partially methylated alditol acetates by GC-MS. Analysis of white and red wine soluble polysaccharide molecular mass distribution was achieved using size exclusion HPLC with a refractive index detector. Details of these methods can be found in Bindon et al. (2012) and Bindon and Smith (2013).

Best practice representative sampling, handling and processing of grape samples from viticultural trials was undertaken, for example as outlined in Holt et al. (2008). Red and white winemaking was undertaken on varying scales including 10 mL, 1 L, 2 L, 20 L, 80 L and 900 L ferments in rotary tanks, static tanks, coffee plungers, plastic containers, test tubes and other laboratory equipment. Sensory descriptive analysis of wines, and of isolated compounds reconstituted in either model wine solutions or real wines, and consumer preference testing were also performed following standard protocols.

A range of methods for detailed characterisation of phenolic compounds from grapes and wines were used including nuclear magnetic resonance (NMR) spectroscopy, liquid chromatography mass spectrometry (LC-MS), ultra-violet to visible (UV-Vis) spectroscopy, mid infra-red (MIR) and

near infra-red (NIR) spectroscopy, high performance liquid chromatography (HPLC) including gel permeation chromatography (GPC), reverse phase, normal phase and other speciality columns.

Mechanisms of haze formation

To elucidate the mechanism of haze formation and obtain information required to trial new prevention strategies, proteins from wines had to be purified and characterised, and their aggregation behaviour analysed in reconstitution experiments and in combination with other wine components. Isolation of proteins from juice and wine was achieved using the two-step chromatographic method based on strong cation exchange (SCX) and hydrophobic interaction chromatography (HIC) (Van Sluyter et al. 2009). For protein characterisation, the purity of isolated proteins was assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and rapid reverse phased-high performance liquid chromatography (RP-HPLC) (Culbert et al. 2017). Reconstitution experiments were also used, whereby key components likely to be involved in haze formation were purified and added back to wines or model wines. The aggregation behaviour upon heating of samples prepared with different combinations of purified proteins and other wine components was analysed by nephelometry (Pocock and Waters 2006), differential scanning fluorimetry, nanoparticle tracking analysis (McRae et al. 2017) and isothermal titration calorimetry (McRae et al. 2015).

Haze predictions

In order to develop a shorter method for haze prediction without compromising accuracy, wines were subjected to a heat test, bentonite fining trials and storage trials (Pocock and Waters 2006).

Viability of bentonite alternatives

In order to assess the viability of alternatives to bentonite, new processing aids were first trialled at laboratory-scale to define the optimal conditions. If successful, these conditions were scaled up in small-scale winemaking experiments. Experimental wines were prepared with different treatments (such as carrageenan, pectin, zirconia, flash pasteurisation with proctase, bentonite), and characterised through chemical and sensory analysis (Marangon et al. 2012, 2011).

Filtration effects on red wine

In order to assess the impacts of filtration on red wines, wines were first filtered through commercial-scale filters (cross-flow to 0.45 µm membranes) and assessed for particle size, macromolecule composition, chemical composition and sensory profiles (McRae et al. 2017).

Matrix effects on red wine astringency

Isothermal titration calorimetry was used to assess the strength of interactions between tannins and polyproline in different ethanol concentrations (McRae et al. 2015).

Results and discussion

Interactions between phenolics, alcohol and acidity in mouth-feel and bitterness perception in white wine

White wines made from the 2011 white wine phenolics project previously deemed to be bitter by a trained sensory panel were combined and their phenolics extracted using Amberlite FPX resin. The phenolics were then separated into fractions.

The more hydrophobic fraction contained a significantly higher proportion of flavonols (skin phenolics) and contributed to bitterness and perceived acidity of model wine at all pH and alcohol levels, and to hotness in lower alcohol model wines, while the less hydrophobic fraction increased bitterness of only the higher pH model wines (Gawel et al. 2016a). These results suggest that different phenolic classes impact differently on mouth-feel and bitterness of white wine, and therefore can be manipulated by managing extraction from skins during white winemaking.

Identifying compounds responsible for bitterness in white wine

The more hydrophobic isolate from the previous study was sub-fractionated using preparative-scale C18 chromatography to obtain 27 fractions of varying hydrophobicity. These were then assessed for bitterness by a screened sensory panel by applying 1 mL aliquots to the back of the tongue using accepted sensory protocols.

The bitter fraction isolated in the previous study was further fractionated into 24 sub-fractions to isolate the compound(s) responsible for its bitterness. These were found to vary substantially in perceived bitterness despite having the same phenolic concentration (Figure 1).

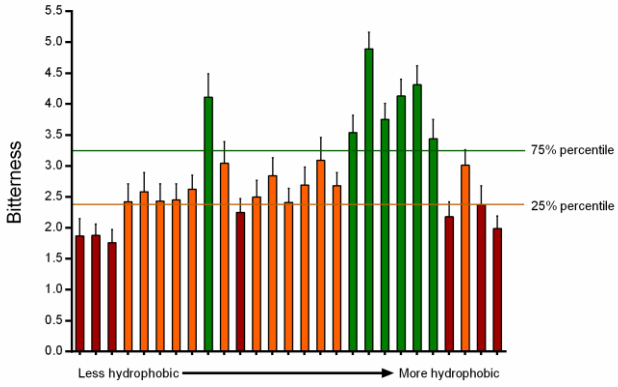

Figure 1. Perceived bitterness of compounds isolated from white wine

The presence of an indole conjugate was found to statistically correlate with the bitterness of the fractions. Sensory assessment showed that the characteristics of the target compound were perceptually more like those of the known bitter compounds epicatechin and quinine sulfate than the astringency of aluminium sulfate and grape skin tannin, hotness from ethanol, or acidity from malic acid. The results suggest that the indole derivative might be a new bitterant in white wine.

Indole compounds similar to that of the target compound have been reported previously. Further work should concentrate on determining the concentration range of this and related compounds in white wine, and establishing whether these bitterant(s) have the potential to work additively like other known bitterants (Keast et al. 2003). It could also explore how the concentration of these

compounds and the associated bitterness in wine are influenced by chemical and biochemical reactions.

The effect of dissolved carbon dioxide on the taste and texture of white wine

Still bottled white wines contain dissolved carbon dioxide (CO_2) as part of their bottling specification, with concentrations typically in the range of 0.5 to 1 g/L. While excessive levels of dissolved CO_2 in still white wines can impart in an obvious 'spritz' sensation which is inconsistent with consumer expectations, some dissolved CO_2 is known to enhance overall mouth-feel. However, its specific effects are unknown.

Commercially bottled Chardonnay and Viognier wines with different levels of carbonation, pH and ethanol required for sensory assessment were modified to four target carbonation levels ranging from low to very high by commercial standards (0.5, 1.0, 1.5, 2.5 g/L). Wines were adjusted to pH 3.4 and 3.2 and to 12 and 14% v/v. The wines were immediately recapped and sealed prior to immediate sensory assessment. The perceived bitterness in both the wines was significantly and consistently reduced by higher dissolved CO_2 levels. Perceived sweetness increased significantly with increased dissolved CO_2 in the Chardonnay wine, a result which was consistent with a trend seen in the Viognier wine. Dissolved CO_2 did not significantly influence perceived viscosity or astringency. The perception of spritz increased significantly with increasing dissolved CO_2 levels in both wines as expected, but in the case of the Viognier wine, higher pH and higher ethanol content accentuated the perception of spritz when dissolved CO_2 was high.

It is believed that no previous studies have evaluated the effect of dissolved CO_2 at still white wine concentrations on mouth-feel perception. In the most analogous situation to white wine, 5 g/L of dissolved CO_2 increased the perceived astringency and sweetness of model apple cider containing polyphenols, but did not affect its bitterness (Symoneaux et al. 2015). These results contradict those of this study. The increased astringency may have resulted from lower pH levels resulting directly from the presence of carbonic acid formed by the dissociation of CO_2 in solution, or indirectly through an increased polyphenol-induced astringency resulting from the lower pH. In this study, the wines were equalised for pH after increasing CO_2 levels which may explain why astringency was unaffected by increased dissolved CO_2. The increase in perceived sweetness is consistent with the reduction in bitterness as they suppress each other, but the reasons for the reduction in bitterness in the presence of increasing dissolved CO_2 levels are unclear, but warrant further investigation.

Matrix effect of ethanol on tannin-protein interactions likely affects red wine astringency

The mechanism by which astringency is elicited within the oral cavity remains unknown, despite wide promulgation of the theory of saliva de-lubrication by tannins. Wines with lower ethanol concentrations are reportedly more astringent (at similar tannin concentrations) although the reasons for this are unclear. Isothermal titration calorimetry (ITC) was used to measure the binding strength between the model salivary protein, poly(L-proline), PLP, and a range of wine tannins (tannin fractions from a three- and a seven-year old Cabernet Sauvignon wine) across different ethanol concentrations (5, 10, 15 and 40% v/v). Tannin-PLP interactions were stronger at 5% ethanol than at 40% ethanol. The mechanism of interaction changed for most tannin samples across the wine-like ethanol range (10-15%) from a combination of hydrophobic and hydrogen-binding at 10% ethanol to only hydrogen binding at 15% ethanol. These results indicate that ethanol concentration can influence the mechanisms of wine tannin-protein interactions and that the previously reported decrease in wine astringency with increasing alcohol may, in part, relate to a decrease in tannin-protein interaction strength (McRae et al. 2015).

Effect of red wine polysaccharides on mouth-feel and taste

Whole polysaccharides (PS) were extracted from a current vintage Shiraz wine, and fractionated by molecular weight (MW) using preparative-scale size exclusion chromatography into high MW (> 93 kDa), medium MW (13-93 kDa) and low MW (5-12 kDa) fractions. These were characterised by their monosaccharide composition (Figure 2). Additions of 150 mg/L of each fraction were made to model red wines containing 0.5 g/L tannin (Tanin Galalcool, Laffort, Bordeaux, France) at pH 3.3 and 3.6, and 11.5 and 13.5% v/v alcohol. Their taste and mouth-feel attributes were profiled by a trained sensory panel using standard descriptive sensory methods (Gawel et al. 2016b).

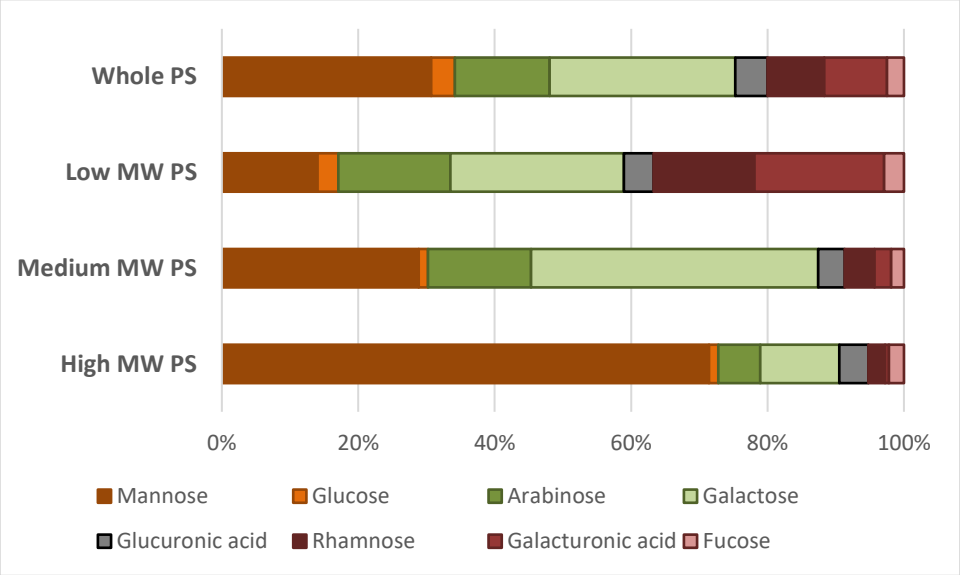

Figure 2. Monosaccharide composition of fractions derived from a red wine polysaccharide. Brown/orange is indicative of mannoproteins, green is indicative of arabinogalactan proteins, and red/pink/grey is indicative of rhamnogalacturonans

Overall astringency and hotness were more influenced by pH and alcohol (respectively) than by PS (Figure 1). However, consistent with work on white wine PS (Gawel et al. 2016), perceived hotness from alcohol was reduced in the low alcohol (11.5% v/v) wines by medium MW PS rich in arabinogalactans (Figure 2). All three PS fractions tended to reduce the astringency of the higher alcohol wines, but the greatest effect was from the low MW fraction consisting mainly of arabinogalactans and rhamnogalacturonans. Vidal et al. (2003) also observed suppression of astringent sub-qualities by purified rhamnogalacturonans. The reduced astringency by PS could be the result of 'shielding' of astringent polyphenols from salivary proteins due to the prior formation of molecular assemblies involving polyphenols and polysaccharides (Soares et al. 2012). The medium and low MW PS increased the perceived viscosity of the model wine most representative of red wine (13.5% v/v alcohol and 3.6 pH) (Vidal et al. 2004). However, in this study, whereby the fractions were also characterised by MW it was notable that a high MW fraction rich in mannoproteins, and typical of those released by yeast autolysis, did not increase perceived viscosity at any pH/alcohol combination. The bitterness of the higher alcohol, higher pH wine was significantly reduced in the presence of medium MW PS but the overall effect of PS on bitterness of the model wines was inconsistent (Figure 3).

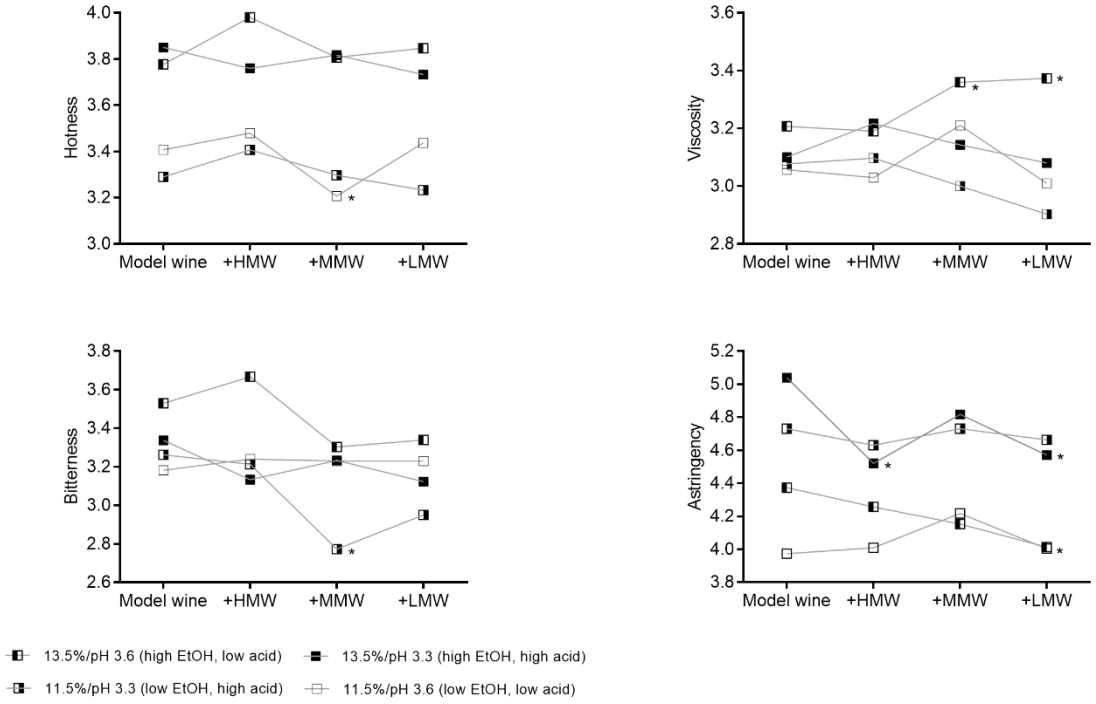

Figure 3. Effect of polysaccharides on the mouth-feel properties of model red wine. * indicates significant difference from respective control model wine ($p<0.1$).

Fundamental methods for understanding texture, taste, clarity, stability and filterability
Knowledge of macromolecular adsorption onto surfaces is critical for a better understanding and control of processes such as filter fouling, binding to tanks and fittings and interactions with processing aids such as bentonite. To improve this knowledge, model surfaces with tailored surface properties (e.g. charge, polarity, chemical functionality, wettability) were developed to explore how wine constituents interact with them. Surface analysis tools not traditionally used in wine science such as QCM-D, XPS and AFM were used to reveal the surface-binding characteristics of different types of colloids in wine without disturbing them through complex isolation and handling.

Surfaces were generated where the presence of surface functional groups such as amines, carboxyls, hydroxyls, formyls, methyls, sulfonate or ammonium could be used to bind wine constituents (Miercszynka-Vasilev and Smith 2016a). The goal was to explore the capacity of functional surfaces to promote or impede red wine constituents' adsorption. To study the adherence and viscoelastic properties of red wine before and after sterile filtration, a QCM-D technology was employed. QCM-D provides label-free measurements of molecular adsorption and/or interactions taking place on various surfaces. In addition to assessing adsorbed mass, measured as changes in oscillating frequency of the quartz crystal, the energy dissipation, which is the reduced energy per oscillation cycle, provides insights regarding structural properties of adsorbed layers. Furthermore, AFM of wine on different modified surfaces and wine morphology were correlated with the wine adherence and viscoelastic properties. The results showed that substrates modified with $-SO_3H$ and –COOH groups can adsorb more of the wine nitrogen-containing compounds whereas $-NH_2$ and $-NR_3$ groups encourage adsorption of carbon-containing compounds. Red wine constituents after filtration were adsorbed to a higher extent on $-NR_3$ and –CHO surfaces. The –OH modified surfaces had the lowest ability to absorb wine components. The results presented above demonstrate that modification of surface chemical states is a strategy to direct the adsorption of red wine constituents such as proteins, polysaccharides and polyphenols.

The effect of surface chemical functionalities on the adsorption of white, rosé and red wine constituents was also evaluated (Miercszynka-Vasilev and Smith 2016b). Allylamine, acrylic acid and ethanol were selected as precursors for plasma polymerization to generate coatings rich in amine, carboxyl and hydroxyl chemical groups, respectively. The results demonstrated that the amine and carboxyl modified surfaces encourage adsorption of constituents from white wine. The hydroxyl modified surfaces can preferentially adsorb rosé wine constituents, whereas red wine adsorbed to the highest extent on acrylic acid surface. Those results are meaningful for the development of the next generation low fouling membranes and/or new sensing platforms that will reduce cost and improve productivity in the wine and other relevant industries.

Improved measurement tools to support understanding of colloidal behaviour in wine
Complex phenomena associated with final macromolecular concentrations are related to colloidal stability and therefore particle size and charge (e.g. protein stability, tannin, polysaccharide extraction and loss). Colloidal systems are generally of a polydispersed nature, that is the molecules or particles in a particular sample vary in size. Typical methods for investigating the formation and properties of colloids in wine (i.e. macromolecular aggregates with particle sizes in the nanometre range) are quite complicated and have mainly been developed for applications in areas other than wine science. They are, however, fundamental to determining how colloids influence the outcomes of many processes in wine production including extraction, settling, clarification, filtration and stability.

To improve existing methods of colloidal characterisation and tailor them to applications in the wine industry, previous research focussed on application of the new techniques of isothermal titration calorimetry (ITC) (McRae et al. 2015), small angle x-ray scattering (SAXS) (McRae et al. 2014) and dynamic light scattering (DLS). Having established their utility, the current project applied a new method, nanoparticle tracking analysis (NTA) to accurately characterise particle size distributions in wine and monitor the zeta-potential of these particles. Zeta potential is a key indicator of the stability of colloidal dispersions and the magnitude of the zeta potential indicates the degree of electrostatic repulsion between adjacent, similarly charged particles in a dispersion.

Many techniques are available for the specific analysis of particle size and particle size distribution such as dynamic light scattering (DLS), electron microscopy (EM) and atomic force microscopy (AFM), analytical ultracentrifugation and the recently developed nanoparticle tracking analysis (NTA) technique. Electron microscopy and AFM both offer direct observation of particle images with high resolution information about the size and morphology of the particles, but both techniques require time-consuming sample preparation. Analytical ultracentrifugation also provides high resolution information about particle size but the technique requires a degree of previous knowledge of the composition of the material and is time consuming. Ensemble methods based on light scattering like DLS are ideally suited for the analysis of monodispersed system but have a limited capability to analyse polydisperse systems. NTA has some clear advantages over DLS.

Nanoparticle tracking analysis (NTA) is an innovative system for sizing particles from about 10-30 nm to 1-2 μm, with the lower detection limit being dependent on the refractive index of the nanoparticles. NTA enables the visualisation of the sample, gives an approximate particle concentration and obtains size information based on the Brownian motion of individual particles. NTA is very accurate for sizing both monodisperse and polydisperse samples and has a substantially better peak resolution than other methods. The presence of a few large particles in a sample has little impact on NTA sizing accuracy. As such, the utility of the NTA was assessed in a range of applications to assess colloidal phenomena important to wine production.

The different macromolecular complexes that occur with different winemaking techniques are known to alter the extraction of tannin, and potentially polysaccharides and protein. While supporting a project on Pinot Noir winemaking techniques (investigating microwave and heating of

musts) led by the Tasmanian Institute of Agriculture (TIA) there was an opportunity to apply this tool. By characterising the complexes formed in the different wines, the aim was to determine the proportion of tannin which became irreversibly bound in complexed forms, and to infer the compositional differences in macromolecules which drove this, if any. The role of altered macromolecule concentration and composition in the formation of complexes or colloids was studied in reconstituted wines using NTA. It was found that particle size increased when the precipitate was recombined with tannin, and was similar to that of the precipitate analysed alone (≅200 nm). Addition of a monomeric fraction increased the incidence and concentration of smaller particles (90 – 200 nm). There were only minor differences in particle size distribution between microwave treatments and standard maceration treatments. However, particle concentration was increased in response to the increased concentration of macromolecules in the microwave treatment. This suggests different colloidal behaviour may occur in these wines, although the functional impact of this remains to be established (Bindon et al. 2016a).

Haze formation in wines is a complex process involving interactions between wine proteins and between proteins and other wine matrix components including phenolics, ionic strength, sulfate ions and polysaccharides. The impact of matrix components on the stability of chitinase M1 protein was investigated using nanoparticle tracking analysis (NTA). The aim of this study was to examine, using reconstruction experiments, the aggregation behaviour of purified chitinase M1 protein and to measure the size and concentration of individual particles formed by this protein before and after heating in the presence or absence of wine phenolics and/or polysaccharides and/or sulfates using NTA. The role played by ionic strength and sulfate in the aggregation of chitinases was also assessed. The study confirmed that protein haze formation in white wine is a multifactorial process where ionic strength, sulfate and also wine molecules such as phenolic compounds and polysaccharides all modulate the white wine protein haze potential.

Macromolecule and colour extraction, stability and retention and influence on wine style and production practices

Tannin and polysaccharide concentration and composition are important in defining the texture of red wines, but can vary due to factors such as cultivar, region, grape ripeness, viticultural practices and winemaking techniques. Colour development in wine is dependent upon the extraction of sufficient quantities of tannin from the grapes together with anthocyanin, to ensure that stable non-bleachable (including polymeric) pigments form. The concentration and composition of these key macromolecules is dependent not only on grape tannin, anthocyanin and polysaccharide concentration and composition, but also their extractability and, in the case of polysaccharides, their formation by yeast.

Previous research at the AWRI has shown that the processes involved in the extraction of these macromolecules from grapes and their retention in wine are very complex. In particular, the isolation and characterisation of polysaccharides and cell wall material (fibre) has shown that grape cell wall material (CWM) can bind tannins and anthocyanins and modify the amount and type of macromolecules retained in wine. The effects of grape composition (including ripeness), macerating enzymes, yeast and certain winemaking treatments on this cell wall material can also profoundly influence the amount of tannin, colour and polysaccharide retained in wine (Holt et al. 2013, Smith et al. 2015). These recent advances present important new factors for consideration in grape selection and processing during winemaking which can allow winemakers to more rigorously control colour and mouth-feel in red wines. The ongoing AWRI research programme has aimed firstly to establish methods to predict tannin and colour extraction from grapes during primary fermentation, and secondly to explore how diversifying grape composition and winemaking technique can result in a divergence from the predicted extractability values.

Establishing a technique to predict tannin and colour extractability from grapes

There are large differences in tannin and colour extractability and these may be due to the interaction between phenolic compounds and other macromolecules during fermentation. Insoluble cell wall material (fibre) can bind and thus trap tannin and anthocyanin during fermentation, and interactions between soluble polysaccharides and proteins may also result in a loss of otherwise soluble phenolics. These may be lost in marc or lees, or be sequestered in colloid complexes in red wines. Cell wall macromolecular composition may therefore influence anthocyanin as well as tannin extractability and retention. The AWRI's research has shown that biochemical changes occurring during grape ripening increase the extractability of grape skin tannins, but may reduce the extractability of seed tannins. Notably, the magnitude of this effect differs markedly between grape varieties.

To further address the role of cell wall composition in tannin release and back binding, a sequential fractionation technique was employed to produce cell wall fractions of defined polysaccharide composition. More than 54% of cell wall-bound tannin was found to be associated with pectic polysaccharides in the cell wall (Ruiz-Garcia et al. 2014). This observation was further corroborated by model adsorption experiments which confirmed that the removal of pectic polysaccharides most significantly reduced the adsorption capacity of cell walls for tannin. Follow-up experiments were designed using enzymes to depolymerise the pectic fraction of grape cell walls. This work showed that the enzyme initially increased polysaccharide extraction from CWM, but depolymerisation to galacturonic acid and arabinose sugars was ongoing, leading to reduced final polysaccharide concentration. Enzyme application reduced the ability of grape cell walls to adsorb tannin, but it was found that a prolonged period in buffer also resulted in a concurrent release of pathogenesis-related proteins from grape mesocarp (pulp) cell walls. This protein, although a minor component of the cell wall, could remove up to 50% of tannin from solution by the formation of unstable complexes. These complexes eventually precipitated, and were too large for an accurate size to be determined by NTA, but could be visualised by microscopy. This research identified a potential mechanism by which extracted grape tannin may be lost from wine during vinification, theoretically proceeding until all protein (or all tannin) is removed from solution. This might explain why enzyme application does not always result in significant increases in tannin concentration, and is dependent on the fruit composition.

These experiments indicated that to accurately predict extractable tannin (and colour) from grapes, a method would need to be developed which factored in potential variations in tannin, pectin and protein concentration in grapes and facilitated the multiple levels at which complexing of macromolecules could occur. Based on successful pilot studies, testing was conducted on the effectiveness of a simple extraction method to determine 'wine extractable' tannin and anthocyanin. Through collaboration with Accolade Wines, triplicate wines were made from 39 Shiraz and Cabernet Sauvignon grape samples sourced from different regions across South Australia. The 'wine-like' (EGT, extractable grape tannin) extraction method used gently crushed grapes, adjusted to 15% v/v ethanol, pH 3.4, in their own juice (Figure 3). To compare the effectiveness of the EGT extraction method against standard methods available to industry, the grapes were also homogenised and extracted in 50% v/v ethanol. Wines and extracts were analysed for tannin and anthocyanin, and colour density (wine only) (Bindon et al. 2014).

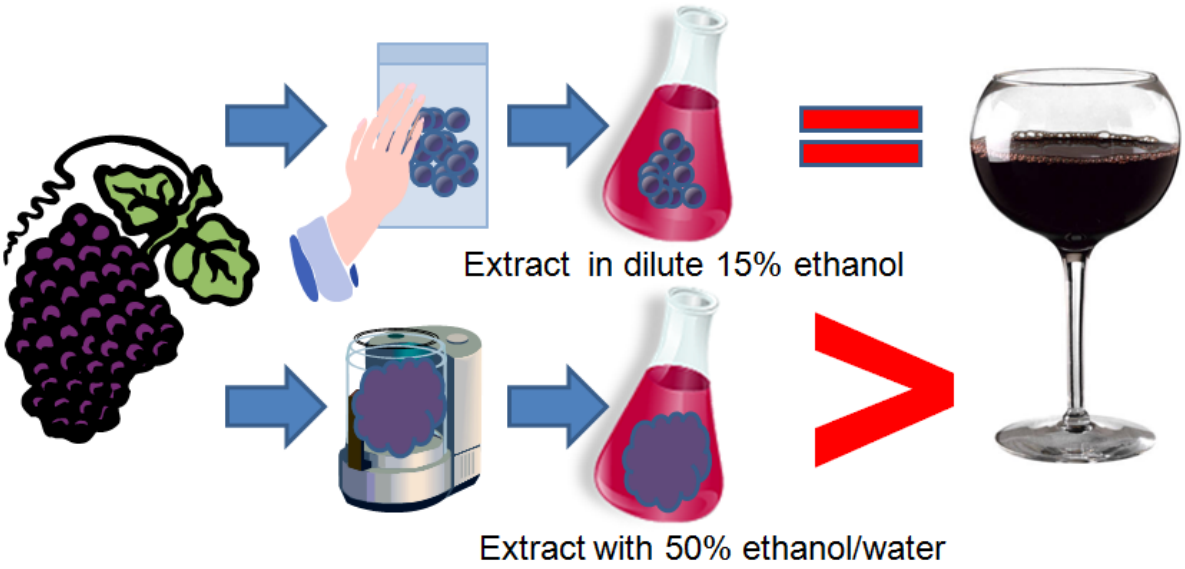

Figure 4. Comparison of 'wine-like' vs standard extraction protocols for tannin and colour analysis. The gentle 'wine-like' extraction in 15% ethanol (top) gives a good prediction of eventual wine tannin, while the standard protocol of grape homogenate extracted in 50% ethanol (bottom) overestimates the eventual wine tannin concentration.

The results were very encouraging, as it was found that the application of the EGT extraction method could provide a good indication of wine tannin (Figure 5) and anthocyanin concentrations extractable during fermentation, as well as predict wine colour density (Bindon et al. 2014). Strong regional differences were found for both grape varieties. When considered on a varietal basis, the 'total' tannin concentrations measured using the grape homogenate method also correlated with those measured in the wines, but were noticeably higher than the actual concentrations achieved through vinification. The overestimation of tannin concentrations in grape homogenate extracts was greater for Cabernet Sauvignon samples than for Shiraz. Correlations between the grape anthocyanin, and wine anthocyanin, as well as wine colour density were strong, and similar for both Cabernet Sauvignon and Shiraz and extraction method. In summary, the work demonstrated that the EGT extraction approach provides a useful prediction of wine tannin and colour.

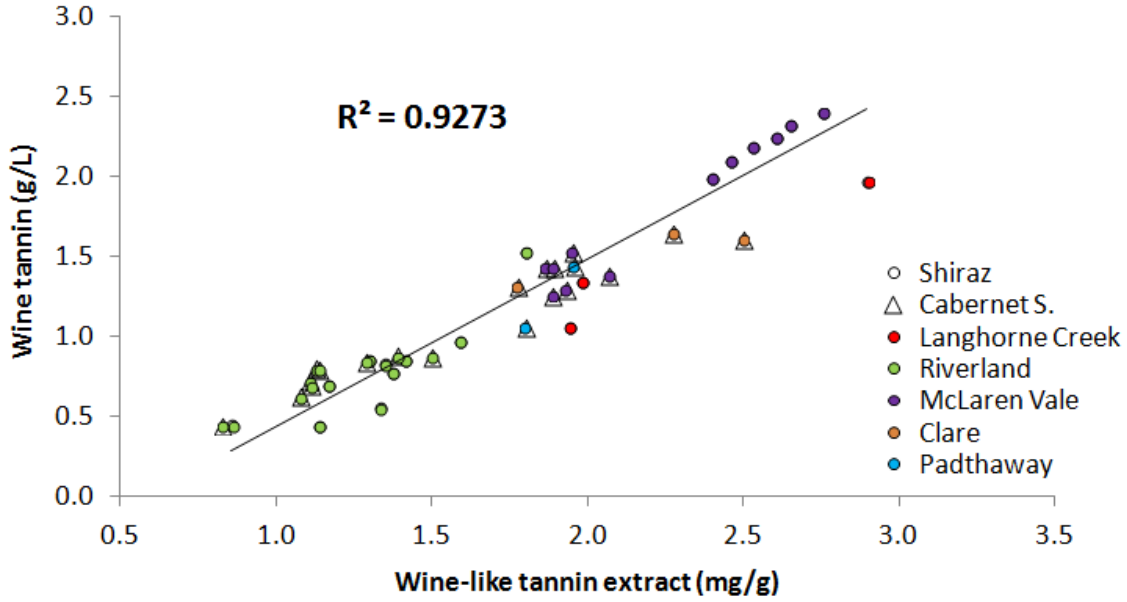

Figure 5. Correlation of grape tannin concentrations measured using the 'wine-like' extraction protocol with resulting wine tannin concentrations

Red winemaking tools to alter wine sensory properties by modulating macromolecule concentration

Once the EGT method was established to determine tannin extractability during primary ferment, ongoing research aimed to establish the extent to which winemaking interventions could influence the final tannin concentration from the predicted value. At a further level, the influence of winemaking treatments on polysaccharide concentration and composition was an area which is poorly understood. The experiments conducted focused on evaluating yeast strains and maceration processes during winemaking as tools to alter wine macromolecule concentration and composition.

In wine made in 2014 it was found that the choice of yeast strain (10 yeast strains were benchmarked) resulted in highly variable polysaccharide and tannin concentrations. At the end of primary fermentation, the two yeasts that yielded the highest wine tannin concentrations (1.5 g/L) resulted in wine with the lowest (0.45 g/L) and highest (0.66 g/L) polysaccharide concentrations respectively. It was found that the wine with the highest polysaccharide and tannin was associated with a transient release of pectic polysaccharides rich in galacturonic acid and arabinose from the grapes, suggesting pectolytic activity in yeast.

Analysis was conducted to compare the influence of yeast strain on wine tannin concentration compared with that predicted by the EGT method. In this instance, it was found that using yeast strain EC1118, that which was employed to establish the EGT method, the predicted value for tannin concentration varied by <5% from the actual value using this strain, confirming the accuracy of the EGT assay. However, the maximum tannin concentration (yeast 2323) was 33% higher than predicted (EC1118), and the range between the lowest (AWRI1503) and highest (2323) tannin concentrations in the wine was 64%, indicating that yeast strain alone can strongly influence the range of tannin outcomes for a given batch of grapes. The results for wine tannin were strongly expressed in wine sensory outcomes with astringency and opacity (colour) of the wines being major drivers of differences among yeasts. Wine opacity was strongly defined by wine colour after 24 months' ageing. Initially, wines which were produced using different yeast strains had very similar colour, however, after a period of 24 months it was observed that the colour density (and non-bleachable pigments) of the wines made from the respective yeast strains was strongly correlated

with wine tannin concentration. Since wine astringency was well correlated with wine tannin, this meant that wine astringency and opacity were also correlated. The strongest differences in wine astringency were shown for the low tannin strain AWRI1503 compared with high-tannin 2323 which also had the highest astringency.

Based on leads from this trial, in 2015 an experiment was performed to investigate the interactive effect of maceration time (7 vs 30 days), macerating enzyme and yeast strains ('high-tannin, 2323' vs 'low-tannin, AWRI1503' yeast) on wine macromolecules in 50 kg Shiraz ferments. A number of experiments on Shiraz grapes showed that macerating enzyme could increase tannin concentration up to 30% with minimal effects on colour. In the 2015 experiment it was found that at 7 days' maceration, enzyme treatment did not affect tannin concentration but yeast strain was a more important contributor. Similar to the preliminary study in 2014, yeast strain significantly affected tannin concentration, with wines made with 2323 having tannin concentrations 35% higher than those made with AWRI1503. Wine polysaccharides, on the other hand, were minimally affected by yeast strain at 7 days' maceration, unlike the earlier 2014 study where 2323 yeast had the highest polysaccharide concentrations. Investigation of wine monosaccharide concentrations showed that both 2323 and AWRI1503 had similar release of galacturonic acid independently of enzyme application, indicating that both yeast strains may in fact have endogenous polygalacturonase activity. As found for other experiments using macerating enzyme, polysaccharides in wines from this treatment were enriched in RGII (high rhamnose:galacturonic acid ratio) and had proportionally higher mannoprotein. The reason for mannoprotein enrichment is not known, but may be due to enhanced release of nutrients (sterols) following CWM breakdown. At 30 days post maceration, no effect of yeast strain on polysaccharide concentration or composition was observed, yet differences in tannin concentration were maintained with 2323 having 31% higher tannin than AWRI1503.

Maceration by itself significantly increased tannin and polysaccharide extraction independently of enzyme or yeast strain. Extending maceration facilitated increased extraction of tannin mainly from the seeds, which resulted in a large colour increase (non-bleachable pigment and colour density). When enzyme treatment was combined with extended maceration, differences between yeast strains were lost. The maximum tannin concentration achieved with the extended maceration and enzyme combination was 52% higher than the predicted value (using the EGT assay). The range in tannin concentration from the AWRI1503 control (lowest) to the extended maceration/enzyme treatment (highest) was 133%. In terms of wine polysaccharides, the type extracted depended upon whether enzyme was applied or not, but final concentrations were similar and independent of either yeast strain or enzyme.

In summary, the combined use of yeast strain and maceration techniques can have a marked effect on wine tannin, but 30 days of maceration achieved the most significant shift in tannin concentration and molecular mass. Interestingly, the effects of these treatments on wine sensory attributes were minimal. In terms of astringency, only wines made with the high-tannin yeast 2323 in combination with extended maceration and enzyme addition had higher astringency than the other treatments. This may point to the fact that polysaccharide increases in conjunction with extended maceration may ameliorate some of the astringency which might be expected with tannin concentration increases, and warrants ongoing study.

Retaining texture and colour in lower alcohol wines

A number of experiments continued to study how various approaches (pre-ferment) to lowering alcohol affect tannin, polysaccharide and colour outcomes in red wine, with the goal of understanding how these impact wine texture. A primary objective was to explore the possibility of harvesting grapes earlier to naturally lower wine alcohol, and to explore whether winemaking techniques could be used to improve the texture of these wines. Changes to regulations in 2017 enabling the pre-fermentation addition of water to high sugar must (to a minimum of 13.5 Baume) warranted the inclusion of must dilution as a further objective of this research. For all the projects

which studied grape maturity to modify wine alcohol, harvesting earlier consistently resulted in wines with reduced wine tannin, polysaccharide and colour. As expected, lowered levels of these key wine components in wines made from earlier harvests reduced astringency, palate fullness and colour, but also importantly, wine alcoholic hotness. To ameliorate the lack of texture and colour in the earlier-harvested wines a number of approaches were attempted, including macerating enzyme treatment, and additions of tannin, polysaccharide and marc.

An experiment compared a standard harvest (Shiraz) at 14% alcohol to a late harvest at 16.7% alcohol (Li et al. 2017). It was found that tannin was increased 28% from the early to late harvest with associated increases in wine colour. Later-harvest wines were found to have greater flavour intensity, 'dark fruit', 'jamminess', sweetness and hotness but importantly also greater wine palate fullness. While enzyme addition to the earlier-harvested fruit increased wine tannin concentration to the same concentration as that in the later-harvest wine, colour was not affected. The principal effect of enzyme addition was to increase tannin to the same concentration as found for the later-harvest wine, without effects on wine colour or polysaccharide. These enzyme-treated wines were found to have higher astringency, with the wines described as having greater surface coarseness. Enzyme-treated wines also lacked palate fullness compared with those made from riper grapes (Li et al. 2017) indicating a possible limitation in the use of macerating enzymes for this purpose. A further study also looked at early (11%, lower alcohol wine) and late-harvest (14.6%) Shiraz but with the addition of both enzyme and marc. Macerating enzyme addition most consistently increased tannin and colour in the wines made from less ripe grapes, but reduced wine polysaccharide. Marc addition (unfermented white grape marc) was also found to contribute additional tannin to early-harvest wines, but not to the same extent as enzyme addition.

As a further element to this work, dilution of high sugar must was explored in conjunction with earlier harvests as a means to lower wine alcohol. To do this, red grapes were harvested at various stages of maturity from early (unripe, low alcohol) to late (commercially ripe, overripe). Various approaches were explored, including water addition and addition of 'green harvest' (5% alcohol) wine. Two dilution approaches were implemented to trial this method of lowering alcohol: dilution and run-off/replace (saignée). Depending upon the condition of the grapes, must dilution produced varying results. A study by PhD student Olaf Schelezki from the University of Adelaide's ITTC Training Centre for Innovative Wine Production in collaboration with the AWRI showed that when grapes were overripe (17 Baume, shrivelled) dilution with either 'green harvest' wine or water had minimal effects on wine tannin, colour or polysaccharide and did not change any wine sensory attributes aside from reducing wine hotness (Schelezki et al. in press). In a study of commercially ripe (15.5 Baume, not shrivelled) grapes by visiting scientist, Dr Bo Teng, it was found that water addition lowered tannin and colour in wines independently of the mode of addition, showing that using a saignée type of approach to add water does not necessarily increase the retention of phenolics in the wine. Nevertheless, wine produced with dilution consistently had higher levels of tannin and colour than wines of the same alcohol concentration produced from earlier harvested grapes. These results indicate that to effectively lower alcohol whilst maximising wine macromolecule extraction and wine texture, dilution treatments are likely to lead to more favourable outcomes than early harvesting.

Understanding the type of tannin that contributes to stable colour formation

Earlier work at the AWRI established techniques to separate wine tannin (polymeric pigments) into small and large fractions, to determine the colour properties of these fractions. The aim of ongoing work in this project was to determine partitioning of colour to polymeric and non-polymeric wine fractions and their relative contributions to wine colour. To do this, two approaches were followed. Firstly, grapes of very different colour properties were sourced and were made into wine using the same winemaking method. In this case Shiraz wines made from unique sites (terroirs) across the Barossa region were sourced and studied over three years of ageing. Secondly, wines produced using techniques with large colour differences (Pinot Noir) were sourced.

For the ageing study, Shiraz wines produced using a standard winemaking protocol from different locations in the Barossa Valley demonstrated large differences in colour properties, and differences were retained during three years of ageing. Strong correlations were observed between wine colour density and the following parameters: tannin concentration, tannin molecular mass, tannin % prodelphinidin (skin tannin) as well as increases in the presence of tannin and colour in two fractions of polymeric pigments F2 (small) and F3 (large). Total anthocyanin in wines was less important in determining colour and polymeric pigment formation (F2, F3 and total) than tannin. The distribution of colour as SO_2-resistant pigments changed only slightly with ageing, reaching a plateau at 18 months. Increases in the colour of F3 were greater than F2. These results showed that compositional differences in wine tannin (when anthocyanin is not limiting such as in Barossa Shiraz) are site-specific and critical to the formation of polymeric pigments and retention of colour in aged wines.

For the second aspect of the work, in collaboration with Dr Anna Carew from TIA, Pinot Noir wines were prepared using standard maceration, microwave treatment (Mwv) followed by standard maceration (+Sk) and, or microwave treatment followed by early press off (-Sk) (Bindon et al. 2016a). Colour properties were analysed at six months and one year. Wine colour density and non-bleachable pigments were higher in Mwv+Sk than in Mwv-Sk and the Control wine, and all underwent similar increases from 6 months to 1 year. Separation of wine pigments by solid phase extraction (using a HLB cartridge) into high (F3) and low (F2) molecular mass fractions was carried out. The contribution of F2 and F3 to wine colour density was similar for the wines, but the proportion of colour and non-bleachable pigments in F2 decreased with age and in F3, increased markedly with age. The change with ageing was similar between treatments. The proportion of colour and non-bleachable pigments in the non-polymeric fraction was initially 30% at 6 months, but was less than 14% at 1 year. This indicated that in Pinot Noir wines, the contribution of small derived pigments and anthocyanins to colour is negligible after one year.

The key finding from both studies on colour development was that the high molecular mass polymeric pigment fraction F3 was more important in colour development during wine ageing than the lower molecular mass fraction F2. Increases in non-bleachable pigment and wine colour density during ageing in F3 were greater than in F2. F3 was also found to form a greater contribution to total colour in aged wine. This points to the importance of the extraction and retention of higher molecular mass tannins in wine. The development of colour in these wines appeared to be somewhat independent of anthocyanin extraction. Rather, tannin, and importantly higher molecular mass tannin, played a greater role in the development of stable, non-bleachable polymeric pigments.

The impact of white juice clarification on wine composition and sensory properties

Fermenting juices with higher levels of solids is an option being taken up by some white winemakers with the view to adding textural complexity or mouth-feel, but this aspect, and what compositional factors may influence it are yet to be explored. As such, this project performed a series of related experiments to improve the understanding of these factors.

Mechanically crushing white grapes to release their fermentable juice always produces insoluble grape debris comprising pulp and skin cell wall fragments collectively known as ‘solids’. By weight, solids are mainly comprised of polysaccharides and lipids essential for yeast cell viability (Alexandre et al. 1994, Casalta et al. 2012). Winemakers typically attempt to remove most solids from juice before fermentation as they are known to promote higher alcohol and reduce ester production by yeast leading to less fruity wines (Konitz et al. 2003). Solids removal is most frequently achieved by settling under gravity with settling agents including pectolytic enzymes and bentonite (Lambri et al. 2012), or by the efficient method of flotation whereby solids ‘hitch a lift’ on introduced gas bubbles to the top of the tank whereby they are removed.

In a small-scale scoping study (study 1), a Chardonnay juice was left unsettled and settled under gravity to two clarity levels using a pectolytic enzyme, bentonite, and without a clarifying agent. Controls for each solids level/clarification method combination were obtained by centrifugation to differentiate the influence of settling time from the effect of juice clarity.

In a larger scale study (study 2), wines from three white varieties were made from full solids juices and the equivalent low solids juices produced by whole bunch pressed, free run and hard pressing juices cold settled using a pectolytic enzyme.

Two further trials were conducted using juices obtained under commercial conditions. A Chardonnay and a Sauvignon Blanc juice were produced by medium-scale wineries using cold settling with pectolytic enzymes (study 3), and Chardonnay and Frontignac juices were produced by large-scale wineries using flotation (study 4).

A general observation was made that wines produced from high solids juices contained significantly higher concentrations of polysaccharides. More detail of results from individual experiments is provided below.

In study 2, high solids ferments produced from wines from all three cultivars were significantly higher in total polysaccharide concentration compared to low solids ferments produced from whole bunch pressed, free run and hard pressings juices following enzyme addition and cold settling. (Figure 6).

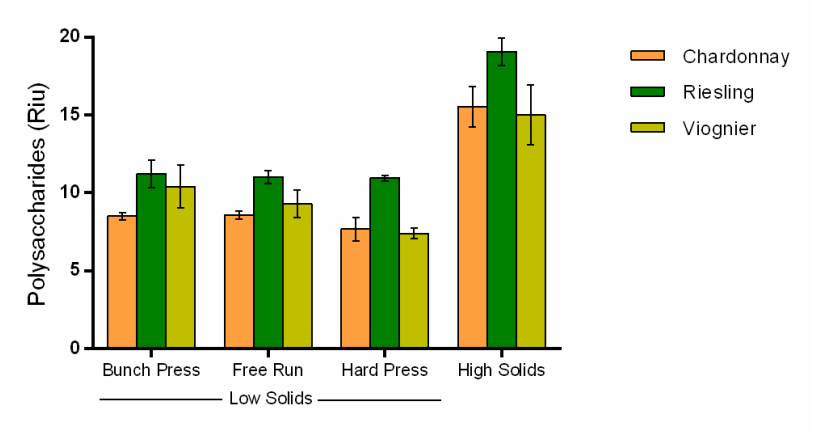

Figure 6. Polysaccharide content of wines made from high solids juice, and low solids juices produced using different extraction methods.

The results of the scoping study (Figure 7), and those of the studies involving commercial juices were consistent with this result.

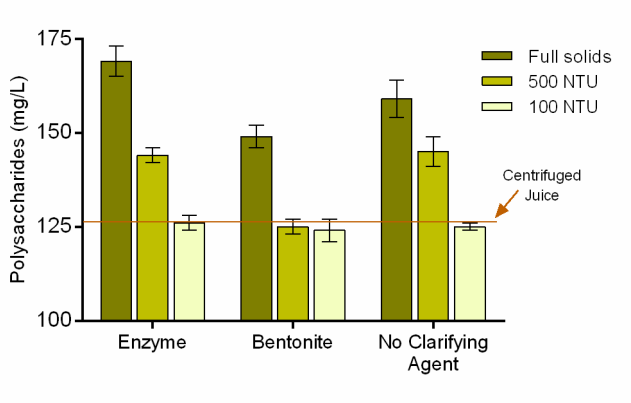

Figure 7. Effects of juice clarification on polysaccharides in white wine in initial scoping study (study 1)

Analysis of the monosaccharide profile in conjunction with the molecular weight distribution of the polysaccharides (study 1) revealed that for all clarification treatments, the polysaccharides in the full solids wines contained a significantly higher proportion of mannose – the diagnostic monosaccharide for yeast activity during fermentation and autolysis post-fermentation. Conversely the mannose content of the polysaccharides in wines produced from centrifuged juices were not consistently different. Full solids wines also had a significantly higher proportion of high molecular weight polysaccharides (>183 kDa), which previous work (Gawel et al. 2016b) has shown to be made up mainly (80%+) of mannoproteins. These results suggest that the increases in total polysaccharides in the full solids treatments were the result of increases in high molecular mannoproteins previously associated with yeast autolysis following fermentation.

The polysaccharides produced from lower solid juices, and therefore those that had experienced longer settling times, tended to have a higher proportion of monosaccharides mostly associated with rhamnoglacturonans (rhamnose, galacutonic and glucuronic acid), and (notably) its diagnostic sugar fucose. There were no consistent significant differences between wines made from high solids juices and those from low solids with respect to these sugars suggesting that rhamnogalacturans are extracted from solids during settling rather than from solids during fermentation.

Effect of juice clarification on white wine phenolics

In study 2, the total phenolics content of the solids wines did not differ from the low solids free run wines in two of the three varieties, with only Chardonnay showing higher phenolic content due to solids (Figure 8). Similarly, in study 1, there were no clear connections between solids level and total phenolics. Comparable studies have found that solids content in juice did not affect the total phenolic concentration of white wine (Singleton et al. 1975, Ollivier et al. 1987). However, HPLC analysis of phenolic composition in these wines showed that wines made from high solids juices possess a different phenolic profile from those made from low solids juices, with high solids wines being significantly higher in flavanol, flavanonol and caftaric acid concentrations, and lower in free hydroxycinnamic acids. Wines in study 1 made from different levels of solids could be clearly differentiated based on their non-polar metabolites, further suggesting that despite producing similar total phenolics, juice solids can influence the phenolic profile of wine.

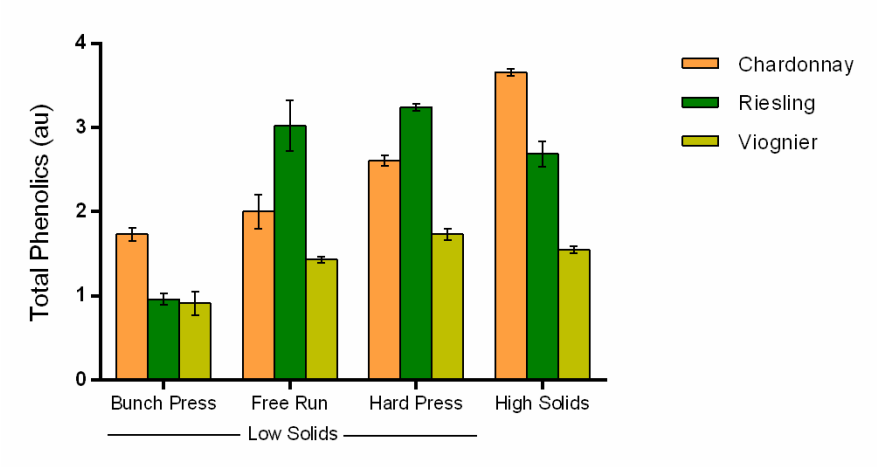

Figure 8. Total phenolic content of wines made from high solids juice, and low solids juices produced using different extraction methods (study 1).

Study 1 also found that the total phenolics levels from full solids ferments were lower with bentonite added than when either enzymes or no clarifying agent was used. This may be the result of low density grape particles distributing bentonite into the ferment via the action of carbon dioxide (Casalta et al. 2012), resulting in phenolic losses by absorption onto bentonite. However, while providing insight into the dynamics of phenolic extraction and loss from grape solids during fermentation, adding a clarifying agent and then simultaneously inoculating the ferment is not a typical commercial practice (Full solids, Bentonite and Enzyme treatments, Figure 7). From a practical perspective, if a winemaker wishes to conduct a low solids fermentation (e.g. 100 ntu) then these results suggest that the method of clarification will most likely not influence the total phenolic concentration of the wine.

The lack of difference in total phenolics between full solids on enzyme and full solids without clarifying agent suggests that there was little of the expected release of phenolics arising from any pectolytic breakdown of the grape particle (solids) cell walls.

Overall, the observation that total phenolic content is not linearly related to juice solid content suggests that a complex interplay between gains and losses by extraction and fining-like mechanisms by the grape solids both during the settling phase and during fermentation is likely.

Effect of juice clarification on white wine mouth-feel

'Oily' and 'metallic' characters were the only sensory attributes that significantly differentiated between low and high solids wines ($p<0.05$) (study 2) and these attributes were mostly associated with quercetin-glucuronide, ethanol and gentisic acid concentration, and with A420 which is a general indicator of phenolic compounds in white wine associated with browning (Figure 9).

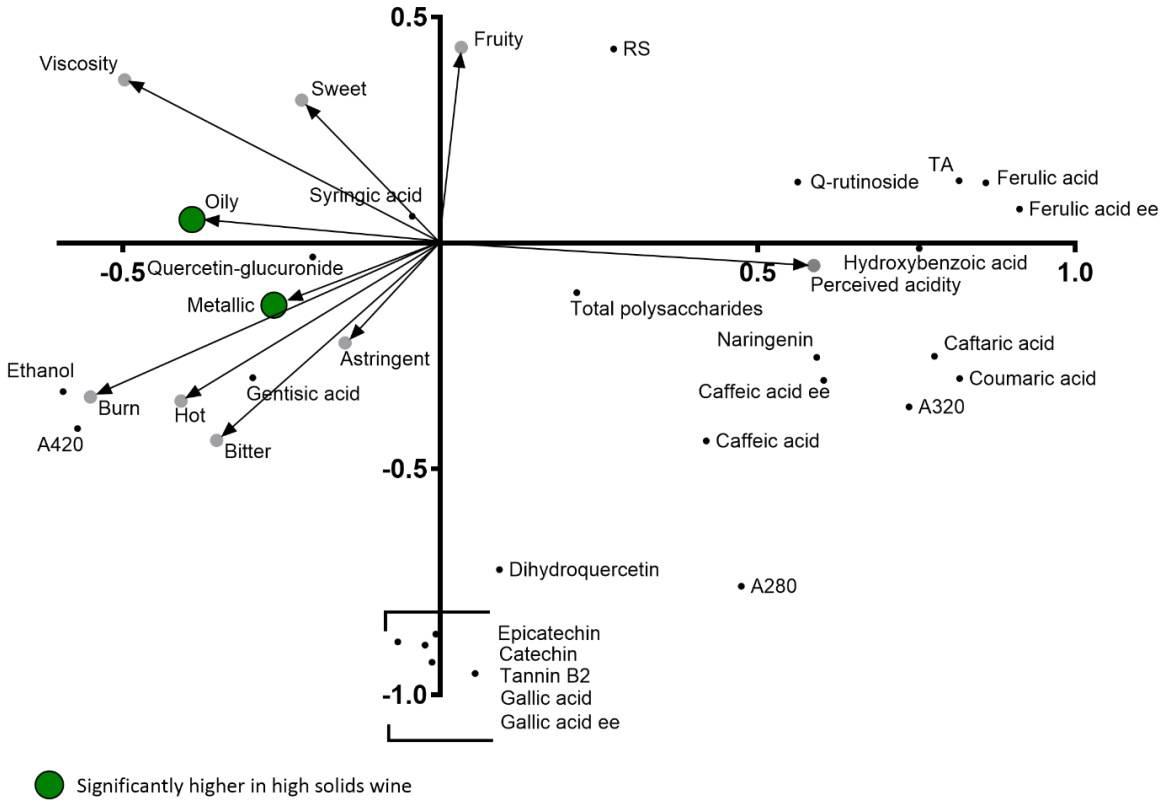

Figure 9. Total phenolic concentration of wines made from juices settled with different clarifying agents. Control indicates wines made from centrifuged juices (<40 ntu).

Using juices clarified in a medium-scale winery, higher juice solids resulted in wines that were perceived to be slightly more viscous and bitter in both varieties. Perceived viscosity was strongly associated with wine pH, alcohol, phenolic concentration and the concentrations of monosaccharides that mostly comprise rhamnogalacturonans. Increased perceived viscosity with increasing pH and phenolics (Gawel et al. 2013, 2014a), alcohol (Demiglio and Pickering 2008), and rhamnogalacturonans in model wine studies (Vidal et al. 2003) have been reported previously.

The most significant outcome related to volatile compounds and their impact on the flavour profile of these wines. Fermenting on solids resulted in both Chardonnay and Sauvignon Blanc wines having higher concentrations of esters, fatty acids, higher alcohols and thiols. In the case of Sauvignon Blanc, the four-fold increase in volatile thiol concentration in the wines produced by fermenting on solids resulted in the perception of significantly higher ‘passionfruit’ and ‘flinty’ characters.

Understanding protein haze in model wines

Individual proteins were isolated from juice for model wine studies of protein stability and aggregation. The influence of matrix parameters on protein stability was measured in multifactorial experiments conducted in collaboration with CSIRO. This involved exploring the influence of pH, ethanol, and salts on stability of four proteins. The results indicated that lower pH and higher salt concentration reduced protein stability and are likely to contribute to aggregation. This provides further evidence about the complexity of wine interactions and haze formation.

The strength of interactions between proteins and polysaccharides was assessed using isothermal titration calorimetry. When proteins were in their native state, there were limited interactions between these macromolecules. However, when proteins were unfolded, such as after heat

exposure, there were weak interactions between proteins and polysaccharides. Overall, this indicates that the protein-polysaccharide interaction is not likely to be a significant cause, or inhibitor, of protein haze formation.

Understanding protein haze in real wines

Grape protein classes, chitinases and thaumatin-like proteins, are the main wine components associated with haze in white wine; however greater protein concentration in wine does not necessarily indicate a greater haze potential (Van Sluyter et al. 2013). To better understand the variability surrounding haze formation, an analytical method was developed using high performance liquid chromatography (HPLC) to more rapidly identify and quantify specific proteins in white wine, reducing the analysis time from 60 minutes to 10 minutes per sample. To investigate the impact of haze formation on real wines, 60 protein-unstable wines were sourced from industry partners. The protein concentration and composition were measured using HPLC. Other components were also measured in each wine to assess the impact of the wine matrix on haze formation, including wine pH, sulfate, electrical conductivity, phenolics, polysaccharides, metal ions, and ethanol concentration. The only factors to have a statistically significant influence on protein haze in real wines were protein concentration and pH, however a highly accurate model for predicting haze based on these factors could not be developed due to the low correlation ($R^2 = 0.56$). It is likely that the other factors also play a role in haze formation that collectively has a great impact on haze; however, no other individual factor was shown to contribute significantly to haze formation.

Improving the efficiency of haze prediction

The number of different factors found to contribute to wine haze made the development of a predictive model based on wine matrix composition unfeasible. For this reason, a heat test was considered the best method for predicting haze and determining bentonite addition rates. However, the heat test can have issues with reproducibility and long turn-around times as well as suggestions that it can overpredict the amount of bentonite required. Further research was therefore warranted to improve reproducibility and potentially reduce turn-around time without compromising accuracy.

Preliminary trials assessed the impact of heating time and cooling time and temperature on haze formation. Heating wines at 80°C for 2 hours and cooling at 20°C for 3 hours produced haze ratings for wines that were similar to those reported by AWRI Commercial Services for the same wines (after heating 6 hours at 80°C and cooling for 20 mins at 20°C). The effect of bentonite fining and subsequent storage was also assessed on a range of unstable wines sourced from industry. A selection of wines were fined at bentonite dose rates predicted using the three methods below and stored for 12 months at 17°C and 28°C:

- 6 hours at 80°C, overnight cooling at 4°C, 1 hour at room temperature
- 2 hours at 80°C, 3 hours at 20°C in temperature-controlled bath
- 2 hours at 80°C, 2 hours at 0°C (ice bath), 1 hour at room temperature

Cooling temperature (20°C or 0°C) did influence the change in NTU, but did not influence the predicted bentonite dose rates. The results indicated that the amount of bentonite predicted from the 5-hour heat test (2 hours heat, 3 hours cool) was sufficient to stabilise wines for 12 months storage at 28°C (Figure 10). This suggests that the shorter heat test is a viable method for predicting haze.

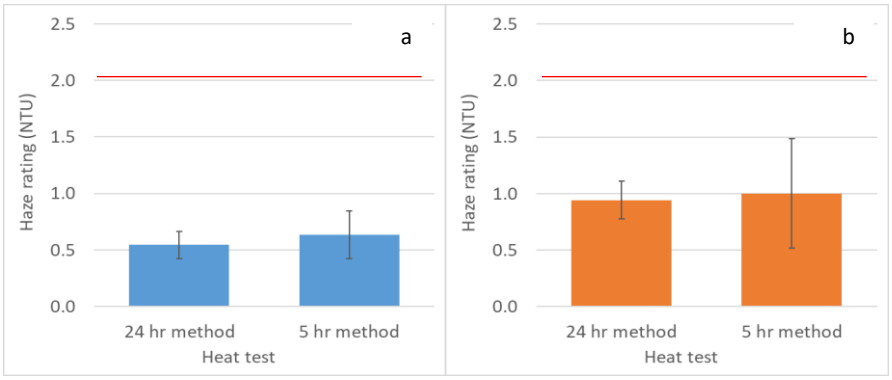

Figure 10. Turbidity of wines fined with a bentonite concentration predicted using the original heat test method (24-hour turnaround, 6 hours heating and 16 hours cooling) and the new heat test method (5-hour turnaround, 2 hours heating and 3 hours cooling) after 12 months storage at a) 17°C and b) 28°C. Results are shown as the average turbidity of seven wines in duplicate +/- one standard deviation. Wines less than 2.0 NTU are visually clear.

Preventing haze using alternatives to bentonite

Consumer and market expectations drive the push for clarity and absence of bottle deposits in white wines. Protein instability leading to haze in white wines is one of the most common non-microbial defects of commercial wine. Bentonite fining is currently used to remove haze-forming proteins from wine, but this has some major drawbacks including wine losses and waste disposal issues. The aim of this research was to develop rapid and selective methods for removing pathogenesis-related proteins from unfined white wines as alternatives to bentonite fining. These treatment options include new adsorbents for protein removal that are more selective, reusable and less wasteful, as well as protease enzymes with an ability to degrade proteins in wine.

New protein adsorbents trialled included surface-engineered silica, macrosponges and magnetic nanoparticles. Further investigations indicated that some types of surface-engineered silica are selective for proteins in wines and research should continue to evaluate these materials (

Figure 11). Macrosponges were provided by collaborators and preliminary trials suggested that while some proteins can be removed by this material, a haze is formed from treated wine during a heat test.

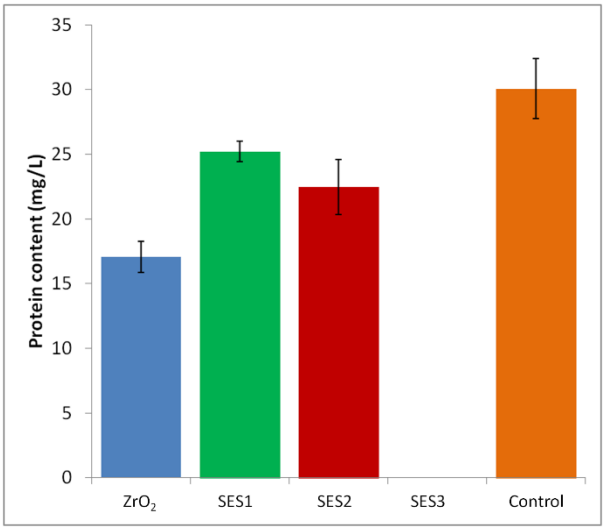

Figure 11. Protein concentration in Riesling wine after treatment with different surface-engineered silica (SES) at 10 g/L as compared with zirconium dioxide and the untreated control wine. SES3 removed all protein from the wine and therefore warrants further investigation.

A novel technology was also developed by the AWRI based on the use of acrylic acid (AcrA) plasma polymer coated magnetic nanoparticles (CMNPs) (Mierczynska-Vasilev et al. 2017). In the first step of the process, magnetic nanoparticles were coated by plasma deposition of gaseous acrylic acid to generate COOH-rich surfaces. The second step of the process involved conditioning of the acrylic acid-coated magnetic nanoparticles with white wine. The final step was a simple separation of coated magnetic nanoparticles from wine with the use of an external magnet.

The pathogenesis-related proteins in nine different white wines were selectively captured and removed by acrylic acid plasma-coated magnetic nanoparticles. Treated white wines were analysed for protein and phenolic content to assess the performance of the functionalised magnetic nanoparticles. The efficiency of the AcrA-coated magnetic nanoparticles to remove wine proteins was tested using different volume fractions of CMNPs. The effect of the coating conditions on the performance of the CMNPs in the removal of pathogenesis-related proteins from white wines was investigated in detail. It was found that using a plasma deposition power of 10 W, a plasma deposition time of 10 minutes and conditioning of CMNPs with wine for 10 minutes were the optimal conditions for the separation process. The new separation method is simple and very effective in removing both pathogenesis-related protein classes, TLPs and chitinases from white wines even when the wine contains an extremely high amount of proteins. Importantly, the phenolic content in the wines was not altered by the protein-removal treatment.

This new technology has potential to become an alternative to the conventional bentonite treatment. Furthermore, such rapid separation technology could benefit other areas such as water treatment, biotechnology and therapeutics.

As an alternative to removing proteins from finished wines by adsorption, proteases can potentially cleave haze-forming proteins. The AWRI has previously reported on success in using aspergillopepsin (AGP) enzymes to degrade wine proteins in combination with flash pasteurisation (Marangon et al. 2012). AGP has now been approved as a wine processing aid by Food Standards Australia New Zealand (FSANZ). Trials were also undertaken on other proteases to assess efficacy in grape juice without flash pasteurisation. Proteases isolated from *Botrytis cinerea* (BcAP8) and sunflowers (HaAEP1) were added to grape juice and the protein concentrations measured after storage at elevated temperatures and/or fermentation. BcAP8 decreased wine protein concentration by a small amount; however treated wines produced a haze in the heat test. HaAEP1 decreased the protein concentration of juice by around 50% after heating to 33°C for 7 days, warranting further research by directed evolution to produce an enzyme that can cleave haze-forming proteins in grape must without heating.

Trials of flash pasteurisation alone (without AGP enzyme) were also investigated as an alternative stabilisation strategy. Heating grape juice for one minute at 75°C was sufficient to produce stable Semillon and Sauvignon Blanc wines. The higher protein concentration of the Muscat Gordo must included in this study required three minutes of heating to produce stable wine (Figure 12). Therefore, pasteurisation for one to three minutes may be sufficient to stabilise wines and may be a viable alternative stabilisation strategy for the wine industry.

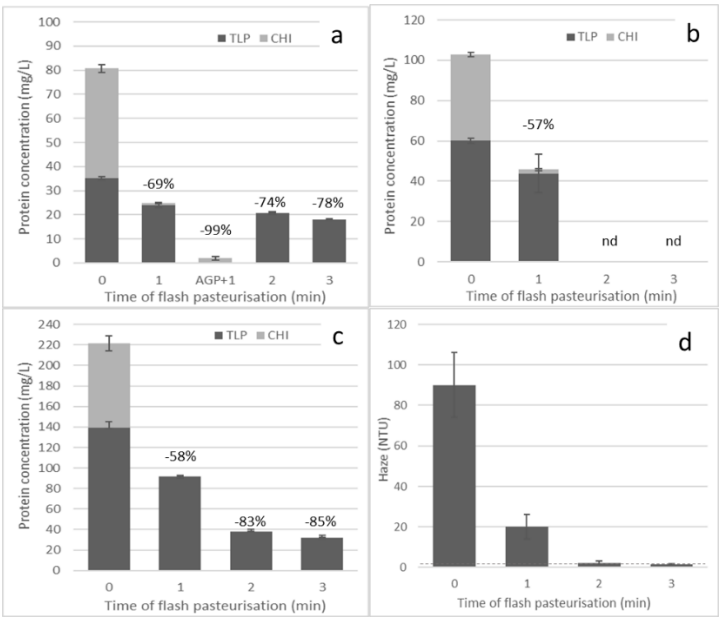

Figure 12. Total concentration of haze-forming proteins, including chitinases (CHI) and thaumatin-like proteins (TLP), in a) Semillon wines; b) Sauvignon Blanc wines and c) Muscat Gordo wines produced from must treated with flash pasteurisation (FP) at 75°C for 1-3 min. Numbers indicate the percent reduction in total concentration of haze-forming proteins compared to the control for significantly different results ($p < 0.5$); d) haze formation in the MG wines after FP for 1-3 min. The dashed line indicates the maximum haze for stable wines, nd = not determined for these samples and AGP+1 refers to aspergillopepsin I and II with 1 min FP. Results are given as the mean of triplicate ferments and error bars indicate +/- one standard deviation.

The use of flash pasteurisation in industry is not always possible and therefore alternative protein unfolding strategies were also investigated. A vortex fluidic device (VFD) uses shear force instead of elevated temperature to unfold proteins and has proven to be effective for unfolding and refolding pharmaceutical enzymes. However, applying this technique to grape juice was ineffective in unfolding haze-forming proteins. The limited availability of scale-up of the technology also suggested that flash pasteurisation would be a more effective technique to explore in future trials.

Sensory and macromolecule effects due to red wine filtration

Clarification and stabilisation, yeast and bacteria removal, final filtration for microbial stabilisation are all colloidal phenomena. Small-scale filtration investigations demonstrated the loss of colour and polysaccharides; however, the effect of commercial-scale filtration on red wines is unknown. For this reason, samples of four commercial wines (Cabernet Sauvignon and Shiraz from 2013 and 2014 vintages) were collected from two commercial bottling facilities before and after crossflow filtration and lenticular filtration, after 0.65 µm membrane filtration, and after 0.45 µm membrane filtration. Macromolecules and colour parameters were measured within a few weeks of bottling, after 9 months' ageing and after 18 months' ageing. Chemical analysis indicated no change in the tannin, colour, polysaccharide concentration or composition with any grade of filtration. The size of the particles in each wine at each filtration grade was assessed using NTA and particle size did decrease with crossflow filtration (Figure 13). This was likely due to the removal of residual microbes.

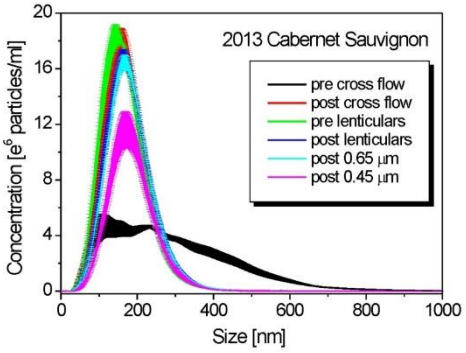

Figure 13. Measurement of particle size and concentration of 2013 Cabernet Sauvignon at various stages of filtration: cross-flow, followed by lenticular filtration and then 0.65 µm and 0.45µm membrane filtration.

After 18 months of ageing, the particle size of the 2013 wines increased in all wine samples at all filtration grades. This trend was not observed in the 2014 wines. Sensory analysis was undertaken on the pre-crossflow, post-crossflow and post-0.45 µm wines. There was no significant filtration-related difference in mouth-feel between samples (Figure 14). This suggested that winery-scale filtration does not significantly change wine macromolecules or mouth-feel but does remove the microbes associated with spoilage.

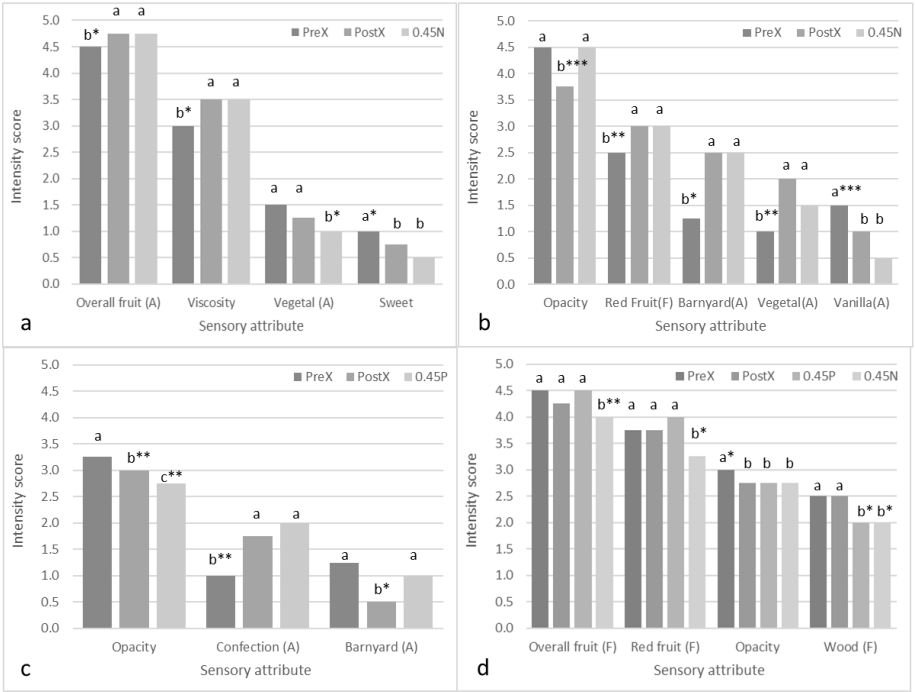

Figure 14. Intensity ratings of sensory attributes that showed significant differences between filtration grades for each wine: pre-cross flow (PreX), post-cross flow (PostX), 0.45 µm membrane of PES (0.45P) or nylon (0.45N). a) CAS13; b) SHZ13; c) SHZ14; d) CAS14. Different letters indicate significant differences between samples for each sensory attribute and asterisks indicate the significance level: * $p < 0.05$, ** $p < 0.01$, ***$p < 0.001$.

Outcome and conclusion

This project delivered improved knowledge and tools to allow winemakers to more objectively manage texture, stability, clarity and filterability during winemaking. It achieved this through the improved understanding of precursor grape and wine compositional drivers and a better understanding of the impact of winemaking processes on the macromolecules and colloids that are linked to these wine parameters.

Specifically, the project achieved the following objectives:

- compositional drivers for texture, hotness and bitterness were identified in red and white wines and include CO_2, tannin, polysaccharides and a new indole conjugate
- the role of macromolecules such as tannins, polysaccharides, proteins and their aggregate colloids in the expression of texture, stability, clarity and filterability have been investigated and shown to vary widely depending on treatment
- the impact of wine matrix components on macromolecule function and expression have been demonstrated
- some of the sources of these molecules or their precursors in grapes and yeast and the impact of winemaking processes such as clarification, flotation, vinification and filtration on their retention and/or transformation have been identified
- the impact of filtration on macromolecules has been demonstrated to be negligible
- alternative strategies for achieving protein stability have been developed and their efficacy demonstrated
- practical methods for wineries to determine likely extractability of macromolecules during winemaking were developed
- strategies for the stabilisation of colour independent of vintage effects were developed.

The knowledge generated by the project provides a framework for the development of winemaking strategies and practical recommendations for managing colour (and colour stability), astringency, viscosity, hotness, bitterness, filtration processes and protein hazes.

Recommendations

Molecular drivers of taste and texture
Bearing in mind that ethanol and pH most significantly impacted astringency and hotness respectively, low to medium molecular weight polysaccharides did cause modifications to a range of sensory characteristics including viscosity, hotness, astringency and bitterness. As such, it is recommended that more research into grape-derived polysaccharide fractions in wines is conducted to determine how significantly wine styles can be modified by manipulating them.

When deciding on ethanol concentrations in final wines, producers should be aware that across the wine-like range (10-15%), ethanol can influence astringency perception with lower levels of ethanol leading to higher astringency for a given amount of tannin. Decisions about the amount of tannin required for the desired wine style should be considered in the context of the target alcohol concentration.

A novel indole derivative has been identified as contributing to bitterness in white wine fractions. Further work should concentrate on determining the concentration range of this compound and compounds of the same class that might be found in white wine, establishing whether they have the potential to work additively like other known bitterants (Keast et al. 2003) to produce bitter tasting wines. It would also be beneficial to explore how the concentration of these compounds and the associated bitterness in wine are influenced by chemical and biochemical reactions.

The reasons for increased perceived sweetness and reduction in bitterness in the presence of increasing dissolved CO_2 levels are unclear and warrant further investigation. It is recommended that producers consider the CO_2 levels of their still wine products in the context of these potential sensory effects when deciding on wine style.

'Smart' surfaces for more efficient production
The evaluation of modified surfaces to reduce fouling should be pursued with collaborators to develop next generation low fouling membranes, tank materials, wine production surfaces and/or new sensing platforms that will reduce cost and improve productivity in wine and related industries.

Understanding wine haze
Wine haze is a complex process involving multiple factors that influence results. Higher haze is expected from wines with lower pH and greater concentrations of proteins, phenolics, sulfate, salts and metal ions. Due to the number of influencing factors, haze is best mitigated through protein removal and best predicted with haze-forming tests such as the newly proposed heat test rather than through wine component analysis.

Predicting haze formation
The new heat test method involves filtering wines at 0.45 µm, heating to 80°C for 2 hours, cooling at 20°C for 3 hours and measuring the turbidity before and after heating. It is recommended that wineries undertake their own cross-validation of this method with their current heat test method to provide additional confidence in the shorter method. This cross validation is currently underway at the AWRI and both the 2-hour heat method and the 6-hour heat method will be available for samples sent to AWRI Commercial Services in coming months.

Preventing wine haze
Accurate predictions of bentonite dose, the selection of the type of bentonite, partial addition during fermentation and post-addition treatment such as centrifugation can help minimise any adverse effects of bentonite additions. For many producers, bentonite addition currently remains the most effective method for preventing wine haze; however new bentonite alternatives are still being developed.

The use of aspergillopepsin (AGP) enzymes in conjunction with flash pasteurisation has been demonstrated as a potential alternative for bentonite. These enzymes have now been approved for use in winemaking and several companies have expressed an interest in developing them for commercial use. Flash pasteurisation without enzyme addition is also a useful technique for reducing protein concentration and bentonite use and can be considered a viable alternative where flash pasteurisation facilities are available.

Solids management effects on white wine style and composition
Producers interested in diversifying some of the textural and aromatic properties of their white wines might consider settling processes that increase the level of solids in their juices, without needing to be overly concerned about increased phenolic extraction.

Matrix effects on red wine astringency
Lower alcohol wines may have a more 'grippy' or puckering astringency than wines with the same tannin concentration but higher alcohol content. This should be considered when developing wines with lower alcohol concentrations and processes to modulate textural properties in lower alcohol wines should be considered.

Impact of winemaking methods on wine macromolecules and texture
The use of a newly developed 'wine-like' extraction protocol for measurement of grape tannins is recommended to producers interested in understanding the likely extractability of fruit prior to fermentation and using the data to support winemaking decisions. Monitoring of individual blocks over multiple seasons or after viticultural interventions will lead to an increased knowledge of the factors that influence extractability.

The use of yeast strain, enzymes and maceration techniques individually or in combination can have a marked effect on red wine tannin and polysaccharide. The magnitude of the effects varied depending on the maturity of the grapes and the effect of the particular yeast, enzyme or maceration protocol on the mechanisms driving extraction and retention processes. Nonetheless, experimentation by producers with these elements is likely to allow them to develop wines with a wide diversity of sensory and compositional profiles. Producers interested in effectively lowering alcohol whilst maximising wine macromolecule extraction and wine texture can consider must dilution treatments which may lead to more favourable outcomes than earlier harvesting.

Polysaccharides and red wine texture
Red wines produced using macerating enzymes had elevated levels of galacturonic acid and RGII, and may be associated with undesirable levels of astringency. However, early work into the sensory impacts of RGII on modulating tannin astringency showed that this was a key molecule which reduced tannin-derived astringency. Future research should seek to establish whether addition of purified RGII to wine and/or reducing galacturonic acid in wine can positively modify mouth-feel. Efficacy of RGII addition to wine should be tested in comparison to AGPs and mannoproteins as existing commercially available wine additives. Treating grape marc with enzyme can allow a high quantity of RGII to be extracted and grape marc could therefore be explored further as a significant source of RGII.

Red wine extractability and protein
The work on cell wall fractionation showed that pectic polysaccharides are a significant sink for tannin removal during winemaking, and that use of selected yeast/macerating enzymes can depolymerise this material to the extent that tannin and colour may be increased in wines. However, cell wall fractionation studies also showed that mesocarp- and juice-derived proteins are potential precipitation (fining) agents early during fermentation. This opens up a number of possible future directions for research:

- The concentration of red wine protein pre- and post-fermentation in *V. vinifera* is poorly understood. The extent to which extracted tannin and colour are precipitated and removed in an active ferment is also unknown. A survey of red wine-grape protein concentrations with associated tannin removal impacts is required and further studies of winemaking practices which affect wine protein (bentonite fining, pasteurisation) and tannin-protein interaction is recommended.
- Viticultural factors (climate, water availability, disease pressure, elicitors, cultivar) may significantly impact upon red grape proteins in the same way that they do white grape cultivars. This may be an important contributor to tannin/colour removal during red winemaking, yet these impacts are unknown and warrant investigation.
- The use of natural grape proteins as a fining agent has recently been identified as an alternative to animal-based proteins, or protein sources from non-grape plants. Protein recovered from bentonite application in white wine might be another valuable source of fining agents.

Regional or vintage-related effects and targeted winemaking

The research into extractability has provided an indication of the range of levels of extractable tannin and colour which can be found for the two principal Australian red varieties Cabernet Sauvignon and Shiraz. This has highlighted that for certain warmer regions the development of tannin and colour in grapes is limited by the environment. In other regions, grape tannin may be at the upper limit of desirable concentrations, leading to excessive astringency/bitterness. Future research should seek to identify context-specific winemaking techniques to optimise wine phenolics based on the extractable grape tannin and colour measured. For example, in regions or vintages where colour and tannin phenolic potential may be limited, particular enzymes or thermal must processing methods such as flash-détente could be applied to maximise extraction while minimising tank time.

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Project 3.3.2 – Influencing wine style through management of oxygen during winemaking

Abstract

Effective management of oxygen during winemaking can help create diverse wine styles. Oxygen exposure can be readily modulated throughout the winemaking process and a range of approaches are available to manage it. However, many of these are not based on scientific knowledge of their effects on fermentation and wine style, or are not underpinned by a clear and holistic understanding of the benefits and financial impacts across the entire wine production chain.

The aim of this project was to establish the impact of early use of oxygen at crushing or during fermentation on wine style, and on the efficiency of malolactic fermentation, using both model systems and pilot-scale fermentations. In addressing these questions this research also improved understanding of how oxygen management during processing and fermentation impacts on fermentation efficiency and fast track ageing of wine. Adoption of the outcomes from this research represent a significant opportunity for the Australian wine sector to manage oxygen exposure effectively, enhance stylistic diversity, improve fermentation efficiency and reduce costs derived from excessively reductive handling of wines.

Five pilot-scale vintage trials and numerous controlled laboratory experiments were carried out during this investment period. In parallel, several industry partners trialled the use of air additions at small, medium and large-scale wineries across the country.

The benefits of adding sizeable amounts of oxygen to red ferments include a reduction in the need for adding nitrogen supplements (a significant cost saving in itself) and prevention of low levels of sulfidic off-odours, thus bringing bright fruit characters to the forefront of the wine bouquet. In addition, softening of tannins during fermentation may reduce maturation time before bottling and make the wine available for market several months earlier.

In white winemaking, the research showed that oxygen additions can increase fermentation efficiency without having negative effects on sensory outcomes. This kinetic rather than stylistic effect could have a major impact on the efficiency of fermentation by allowing a wine to finish fermentation several days earlier than normal while maintaining style through unaltered fermentation temperatures. This is a particularly valuable outcome considering the growing need to manage fermentations in compressed vintages.

Executive summary

Effective management of oxygen during winemaking can help create diverse styles that are attractive to a range of different consumers. Many approaches to oxygen management are currently practised and oxygen management has predominantly been focused on: post-fermentation treatments; management during bottling; and the effects of closure selection on post-bottling development. However, the effects of oxygen management during the process of winemaking (from crushing through fermentation) are not well understood. The limited information at hand is mostly about the management of fermentation efficiency and reliability. Oxygen exposure is a valuable and readily available management option throughout the winemaking process and many practical approaches are available to manage it. However, many of these are not based on scientific knowledge of their effects on wine style, or are not underpinned by a clear and holistic understanding of the benefits and financial impacts across the entire wine production chain. For example, managing oxygen exposure at crushing and juice stage may be very capital-intensive and expensive (e.g. inert crushers), whereas management during fermentation can be achieved with modest capital modifications.

The aim of this project was to establish the impact of early use of oxygen at crushing or during fermentation on wine style, and on the efficiency of malolactic fermentation using both model systems and pilot-scale fermentations. These are critical to delivering the best quality product possible. Questions at the outset of this project included: how much oxygen (O_2) gets into juice through production? What does juice exposure to oxygen do to final wine style/composition? What does oxygen exposure during fermentation do to wine style? Can oxygen measurement be improved during winemaking or can markers for exposure be found?

This project focused on influencing fermentation efficiency and/or wine style through management of oxygen during winemaking. Five pilot-scale vintage trials and numerous controlled laboratory experiments were carried out during this investment period. In parallel, several industry partners have trialled the use of air additions at small, medium and large-scale wineries across the country.

The benefits of adding appreciable amounts of oxygen to red ferments have been demonstrated to remove the need for adding nitrogen supplements (a significant cost saving in itself) and prevent low levels of sulfidic off-odours, bringing 'bright fruit' characters to the forefront of the wine bouquet. In addition, softening of tannins may reduce maturation time before bottling and make the wine available for market several months earlier.

In wines made in 2012, greater oxygen exposure during fermentation produced wines with more 'aged' characteristics with respect to greater hue, fewer anthocyanins, lower tannin concentrations and smaller tannins with more modified structure. These changes were similar to those induced by 12 months of bottle-ageing in wines deprived of oxygen during fermentation. Treatments with 40% O2 and air scored lowest for 'bitter' and for 'astringency', while the protective N2 treatment scored highest for 'astringency'. This suggests that increased oxygen exposure during winemaking may reduce the need for extended wine ageing, saving winemakers costs associated with tannin fining and extended storage, and possibly increasing consumer preferences. Recent research on white wine phenolics adds to the body of evidence that oxygen is likely to impact wine texture: these results established that two of the major phenolics in wine (grape reaction product [GRP] and caftaric acid) that are influenced by oxygen exposure also modulate the perception of astringency and oiliness.

Additional beneficial effects of oxygen additions to ferments included decreased metal concentrations in wine post-ferment which may benefit the wine's shelf life and evolution, and significantly faster rates of malolactic fermentation which might provide a practical tool to assist in the reliable completion of malolactic fermentation.

In white winemaking, the research showed that oxygen additions can increase fermentation efficiency without having negative effects on sensory outcomes. Modulating the extent of oxygen exposure at the very earliest stages of juice preparation has been an important tool in understanding the effect of oxygen in white winemaking. Although the project did not set out to assess the merits of inert pressing, experiments highlighted some subtle effects that can be achieved from pressing under low oxygen conditions, if not totally inert environments. This is an area that should receive some further investigation, particularly looking at must from a range of grape varieties.

In a vintage 2014 experiment different oxygen levels that could be achieved simply through pressing and handling operations were investigated in Chardonnay, without further oxygen additions being conducted. The choice of pressing mode and the extent to which juice or wine was protected from oxygen during handling were both shown to affect a wine's final chemical composition and sensory characteristics, in this particular case potentially affecting 'floral' and 'citrus' characters. For juices prepared through normal (i.e. aerobic) pressing, no significant differences were introduced through the choice of handling method. This seems to suggest that, at least for Chardonnay, there is little need to invest too much time and money protecting juice and fermenting wine from oxygen, if it has been produced through aerobic pressing. However, other white varieties may behave differently so caution should be used before dispensing with inert gas blanketing altogether! On the other hand, if

a juice is produced by inert pressing it was shown that sufficient phenolics remain to be affected by further oxygen exposure during normal handling. Inertly pressed juices therefore need continued protection through reductive handling, if oxidation is to be avoided.

Having observed and quantified the chemical and sensory differences that occur through passive oxygen exposure in this study, trials during the 2015 vintage focused on making deliberate but controlled oxygen additions during fermentation which have potential for greater impact on wine style. These experiments demonstrated that addition of oxygen during white wine fermentation has positive benefits, with the main impact on the kinetics of fermentation rather than style of wine. This could lead to significant improvements in the efficiency of fermentation by allowing a wine to finish fermentation several days earlier than normal, while maintaining style through unaltered fermentation temperatures. This is a particularly valuable outcome considering the growing need to generate fermentation efficiencies during compressed vintages.

The sensory effects of adding oxygen in the 2015 experiments were minimal. The preferred timing of oxygen addition appeared to be in the first half of fermentation when sugars had dropped by 20% of the starting concentration. It was still beneficial, however, to make a late addition, even once the sugar concentration had dropped by 80 %. Although this did not give a considerable boost to the fermentation, it ensured that the ferment achieved dryness safely. The sensory analysis confirmed that there were no negatives issues associated with using a reasonable amount of oxygen.

The positive impacts of adding oxygen during red wine fermentation was initially explored in 2012 and are detailed above. During the 2016 vintage trials, the type of fermenter used and the way the aeration was carried out was modified to demonstrate how this could be achieved in wineries not equipped with rotary fermenters and with minimal capital outlay. The timings used in the 2015 trial were replicated with additional treatments of a daily dose and a post-press addition. In order to achieve the positive benefits of enhancing the ‘bright red fruit’ attributes through suppression of low-level reductive aromas, it was shown to be important to use an early aeration during the first few days of active fermentation. In addition, to achieve a decrease in astringency by softening the tannin, a repeated exposure may be necessary.

In summary, by adopting the outcomes from this research significant opportunities can be realised to manage oxygen exposure effectively, enhance stylistic diversity, improve fermentation efficiency and reduce the costs derived from excessively reductive handling of wines.

Background

Effective management of oxygen during winemaking can help create diverse styles that are attractive to a range of different consumers. Many approaches to oxygen management are currently practised but knowledge of oxygen management has predominantly been focused on post-fermentation treatments; management during bottling; and the effects of closure selection on post-bottling development. The effects of oxygen management during the process of winemaking (from crushing through fermentation) are not well understood. The limited information that exists is mostly on the management of fermentation efficiency and reliability.

However, the role of oxygen during winemaking is likely to have a profound effect on the final wine, and thus a significant opportunity exists for winemakers to use oxygen management before or during fermentation to impact on critical aspects of winemaking such as aroma, texture and post-bottling stability; in particular, to remediate or prevent the formation of reductive aromas during fermentation and possibly minimise the risk of reductive aroma formation post-bottling. The aim of this project was to establish the impact of early use of oxygen at crushing or during fermentation on wine style, and on the efficiency of malolactic fermentation using both model systems and pilot-scale fermentations. Furthermore, strategies were assessed for prevention of oxygen-related quality loss after fermentation. These areas are critical to delivering the best quality product possible.

Questions at the outset of this project included: how much oxygen (O_2) gets into juice through production? What does juice exposure to oxygen do to final wine style/composition? What does oxygen exposure during fermentation do to wine style? Can oxygen be better measured during the process or markers for exposure be identified? This project focused on influencing wine style through management of oxygen during winemaking.

The practice of winemaking in Australia has a tendency to be reductive in nature. Grapes are protected during crushing and pressing, and juices during transfer, with the liberal use of dry ice and/or early and frequent use of SO_2. Combined with the increasing availability of inert presses and inert crushers, the move to screw-caps and total package oxygen management post-production may increase the risk of 'in bottle' reductive characters, depending on the wine composition. While serving to protect against the negative effects of oxidation, blanket application of these approaches may also unnecessarily limit the tools available to winemakers to manipulate wine style.

Consultation with industry indicates sporadic and dispersed use of oxygen during primary fermentation, especially with red musts. Such treatment is carried out in a couple of the bigger wineries who use fixed air sparging systems in rotary or static fermenters, and in smaller boutique wineries where *ad hoc* solutions are employed. In the case of the rotary fermenters, air is used to minimise sulfidic (or 'reductive') aroma formation but in smaller wineries oxygen (sometimes 100%) may be used for colour stabilisation. There are limitations in the understanding of how these practices affect wine, or their efficiency in O_2 transfer, and very little scientific research has been reported on the effects on wine composition or sensory properties.

Oxygen exposure occurs to varying degrees during production of grape juice. Practices such as mechanical harvesting, crushing and pressing all contribute to oxygen contact with grape components, but it is unclear how much exposure occurs and what is the effect on the wine. However, studies of inert juice pressing (using nitrogen gas blanketing during the operation of a tank/membrane press) and hyperoxidation (an extreme example of juice oxidation which has the main goal of phenolic stabilisation by exposure to very large amounts of oxygen after pressing) representing both extremes have been carried out (Boselli et al. 2010, Cejudo-Bastante et al. 2011).These studies represent extremes and the work undertaken at AWRI aimed to study careful dose-controlled additions of oxygen and monitor effects on fermentation efficiency and wine composition.

In terms of fermentation efficiency, the role of oxygen in the stimulation of fermentation rates and assisting in the completion of difficult fermentations has been well described previously. Based on this work, the established time for the addition of oxygen to fermentation is at the end of exponential growth, 36 to 48 hours into fermentation. While fermentation efficiency has been a key driver of much work on oxygen use during fermentation, the sensory effects on the finished wine of oxygen exposure during fermentation have not been thoroughly investigated.

In terms of wine composition, the limited literature available on compositional effects from oxygen exposure shows that single dose oxygen exposure (added with the intention to manage efficiency) during fermentation can alter the ester profile, increase the production of higher alcohols and alter the composition of the volatile fatty acid pool compared to strictly anaerobic fermentations. Limited compositional effects have been reported, oxygen dose or duration has not been explored, and no sensory evaluations have accompanied existing literature.

Highlights

Measuring oxygen in the winery

Five different sensors were evaluated for their ability to measure oxygen in must and wine. Results showed that chemo-luminescence probes were suited to in-line dissolved oxygen (DO) measurements during must transfer or pump-over. 'Mini-DOT' sensors were suitable for monitoring DO inside a press or tank.

Practical tools to aerate ferments

Aeration of must is widely used to enhance fermentation performance, especially in red wine fermentations. A Venturi injector was trialled in a medium-sized winery and was shown to be very effective at high pump-over flow rates, giving up to 40% air saturation directly after the device. On a smaller scale, air-draw tubes gave constant and low dissolved oxygen (DO) pick-up, achieving 2-9% air saturation. Both approaches present viable alternatives to the classic method of aeration through 'cracking the fitting' which may cause pump cavitation and potentially burn-out the pump rotor.

Impacts of oxygen in white wine production can be positive

In continuing work on the effects of oxygen during winemaking, oxygen concentration in Chardonnay must at the time of inoculation was found to have no impact on fermentation duration or the concentration of yeast-derived aroma compounds in wine. However, aeration of fermentation later than typical practice still had a stimulatory effect on fermentation performance without negative consequences for wine sensory attributes. This suggests that, if required (e.g. for stimulation of a sluggish/stuck ferment), the use of oxygen outside the previously defined narrow window (24 – 72 hours post-inoculation) can be considered beneficial for ferment performance with limited risk to sensory outcomes.

Impacts of oxygen in red wine production can provide beneficial tannin softening and curtail reductive aromas

By adding oxygen during red fermentations on skins it is possible to modify the tannin composition of wine in ways that equate to several years of post-bottle ageing. Aeration during normal pump-overs is easily achieved if sub-cap recirculation is carried out as well. Alternatively, aeration devices using a sinter fed by compressed air sources may be used. Other equipment is also available for aerating red ferments without recourse to compressed gas, although care must be taken in the choice of pump.

Project objectives

Key objectives are to:

- determine how much oxygen gets into juice through production and what juice exposure to oxygen does to final wine style/composition;
- define key grape and yeast derived volatile and non-volatile compounds, which are affected by oxygen management during winemaking;
- determine the impact on wine style and sensory properties of key oxygen modulated compounds;
- develop improved measurement tools for monitoring oxygen exposure during wine production;
- provide practical advice about ways to introduce oxygen and the impacts of timing and dose of addition; and
- develop methods to improve the efficiency of malolactic fermentation through the use of oxygen during alcoholic fermentation.

Methods

Laboratory-scale fermentation suite

A laboratory-scale fermentation suite was assembled to investigate oxygen uptake rate, with the ability to supply input gas with variable O_2 content and measure outgas flow rates. The set-up used 15 custom-made glass fermenters fitted with dissolved oxygen (DO) probes, gas spargers, outgas flow rate monitoring and a sampling port. A standard fermentation volume of 250 mL agitated at 250 rpm and incubated at 17°C was used throughout.

The investigation into the effect of dissolved oxygen concentration at time of inoculation used Chardonnay juice which prior to inoculation was sparged with air to achieve three different levels of DO: 100 mg/L, 500 mg/L and 2,500 mg/L.

The effect of timing and dose of oxygen during fermentation was investigated using similar juice with gas of differing O_2 content sparged into the active ferment. Prior to inoculation all ferments were adjusted to a dissolved oxygen concentration of 2,500 mg/L. Two basic regimes of aeration during fermentation were applied. In the first, ferments were treated with nitrogen containing different concentrations of oxygen (0.1%, 1%, 10.5%, 21% v/v) or different flow rates of gas 48 hours post inoculation. The second regime fixed the concentration at 0.1% oxygen in nitrogen, delivered at a flow rate of 5 mL/min but varied the duration from 2 – 72 hours. When the effect of oxygen treatment timing was evaluated, a fixed dose of 10.5% oxygen in nitrogen was applied for 2 hours at 5 mL/min.

Pilot-scale investigation of passive oxygen exposure during juice preparation and primary fermentation in white winemaking (vintage 2014)

This trial was carried out using hand-picked Chardonnay grapes which were whole-bunch pressed using a Bucher-Vaslin Inertys press before transportation to the Hickinbotham-Roseworthy Wine Science laboratory at the University of Adelaide. Juice was cold settled then racked into 500 L temperature-controlled tanks before inoculation. Ferments for each treatment in the trial were conducted in triplicate.

Pressing: The first two juice lots were made in ‘inert mode’ in which the membrane press was configured to draw in nitrogen gas rather than air as the membrane deflated; the press was also sparged with CO_2 gas, prior to and during loading. The other two juice lots were made in ‘aerobic (or normal) mode’ allowing ambient air to be drawn in as the press membrane deflated. Dissolved oxygen (DO) measurements were made using a PME ‘miniDOT’ datalogger temporarily fixed near to the press’s juice channels. This type of device is typically used by hydrographers gathering data from the ocean, rivers or even waste-water lagoons.

Handling: After pressing, one of each type of juice (inertly pressed or aerobically pressed) was handled reductively and the other two juices were handled oxidatively. Overall, this resulted in four different treatments, from the four possible combinations of pressing and handling: inert-oxidative, inert-reductive, aerobic-oxidative and aerobic-reductive.

Reductive handling was achieved by blanketing the source and receival tanks during transfer or racking operations with either dry ice or an inert gas mixture. This handling regime was continued from the initial filling of the press holding tank until after post-fermentation racking when SO_2 was added. The ullage was minimal but the tank headspace was regularly sparged. Conversely, the oxidative handling regime did not involve any use of inert gas until after the post-ferment racking. After post-ferment racking the four treatments were handled identically, with all subsequent operations carried out in a standard reductive manner under inert gas cover.

Investigation to assess different O_2 concentrations during pressing

A commercial-scale trial was carried out to assess the chemical and sensory characterisation of different O_2 concentrations only during the pressing stage, with subsequent fermentation under laboratory conditions. Handpicked Pinot Gris grapes were whole-bunch pressed using a Bucher XPlus 30 Inertys with five different O_2 exposure regimes: N_2 (99.9%), 5, 10, 15% O_2 and air (20.9 % O_2). The juice was fermented at laboratory scale (5 L) and subsequently bottled for sensory and chemical analysis.

Pilot-scale investigation of validation of aeration during fermentation of white wines (vintage 2015)

Commercially prepared Chardonnay juice was distributed into 12 x 500 L fermenters and inoculated with active dried yeast. Three aeration treatments were carried out in triplicate with an additional 'no-treatment' variant also performed in triplicate. Two timing variants were incorporated into the experiment: 'early' treatment started once 20% total soluble sugars (TSS) had been consumed by the yeast and 'late' when 80% of initial sugars had been consumed. Two oxygenation durations were carried out at the 'early' time point lasting two hours ('early-short', ES) and 20 hours ('early-long', EL). The 'late' treatment lasted 20 hours. These timings and durations were determined from the laboratory experiments described above. Wines were subsequently handled identically through to bottling.

Pilot-scale investigation of the effect of timing and dose of oxygen addition in red wine (vintage 2016)

The effect of timing of oxygen addition in red wine, determined by laboratory experiment, was verified at pilot-scale using donated commercial Shiraz grapes (Langhorne Creek), fermented in pilot-scale (500 L) fermentation vessels using standard pump-over techniques with a fixed irrigator placed just below the opening of the tank. Sub-cap recirculation was carried out during which time air was introduced in the flow of must. One treatment received a one-off addition during pump-over to saturate the fermenting must with air when sugars had dropped by 20% ('Early') while another treatment consisted of repeated aerations to achieve air saturation repeatedly over five consecutive days ('Daily'). Another single treatment occurred later when the initial concertation had dropped by 80% (Late) and a final treatment on previously unaerated wines was carried out after pressing (Post-Press). All wines were then handled in a standard manner until bottling. Sensory analysis was carried one year after vintage.

Pilot-scale investigation of the effects of aeration of red ferments in closed rotary fermenters (vintage 2012)

Donated hand-harvested Shiraz grapes (Barossa Valley) were crushed into rotary fermenters (730 kg each) fitted with three stainless steel sintered sparging heads (2 µm frit size) and fed with three separate gas treatments (10 mL/min at 200 kPa): 40% O_2/N_2 ($O_2$40), air (containing approximately 21% O_2) or pure N_2, applied for 60 min every 12 h starting 24 h after inoculation. The treatments

were compared with a 'no treatment' control. After fermentation, the wines were drained, the marc pressed and the wines settled for 24 h, prior to undergoing malolactic fermentation (MLF) in 200 L drums. Tartaric acid (1.5 g/L) wasadded prior to inoculation with the malolactic bacteria (VP41, 10 mg/L, 20 °C). Finished wines were bottled in 375 mL antique green bottles and sealed under screw cap with Saran Tin™ or Saranex™ liners. Samples were analysed for tannin and colour at time 0 (finished wine, samples taken after MLF), at 8 months (2 months post-bottling), and 18 months (12 months post-bottling).

Pilot-scale investigation of the effect on malolactic fermentation of oxygen addition in red wine (vintage 2017)

The effect of oxygen addition during red wine fermentation on simultaneous and sequential malolactic fermentation (MLF) was verified at pilot-scale in a 2 x 2 factorial experimental design. Using commercial Shiraz grapes (McLaren Vale), pilot-scale (500 L) fermentations were carried out in triplicate using standard pump-over techniques with a fixed irrigator placed just below the opening of the tank. Fixed cross-shaped sparging devices were placed at the bottom of six tanks and fed with compressed air (5 L/min). Half of the tanks were inoculated with commercial *Oenococcus oeni* bacteria two days after the addition of rehydrated yeast while the remainder were left uninoculated until they were sugar dry. Half of the simultaneously malolactic inoculated ferments and half of the uninoculated ferments received five additions of air nominally every 12 hours when the total soluble solids were between 11 Bé and 4 Bé. Dissolve oxygen was monitored in tank using oxoluminescent probes. All tanks were pressed on day 10 and after settling were transferred to 50 L stainless steel kegs. SO_2 was added to the co-inoculated wines when the residual L-malic acid was consumed; commercial *Oenococcus oeni* bacteria was added to the remaining wine previously uninoculated for MLF. Wines were bottled three months after the end of fermentation.

Results and discussion

Measuring oxygen exposure in the winery during white winemaking (vintage 2014)

The first stage of this project involved investigating the effects of oxygen during standard types of processes undertaken during production. The primary aim of this work was to determine how much oxygen gets into juice through production and specifically to investigate if passive oxygenation – that amount of O_2 which is introduced during winemaking by virtue of the choices a winemaker makes – can have a measurable difference in terms of both wine chemistry and sensory impact. As soon as a grape is crushed the enzyme polyphenol oxidase (PPO) is activated and O_2 will react with phenolic material (Macheix 1991). The extent of that phenolic oxidation may influence the aroma precursors and aroma compounds (Patel et al. 2010) as well as how the yeast metabolise their nutrient source (Salmon 2006).

The way in which the must/juice is prepared (mechanical harvesting, crushing, whole-bunch press) determines the window of first exposure of O_2. This is obviously very difficult to control in scientific experiments so a proxy technology was employed. If whole bunch pressing is used then the oxygenation that occurs is only from the air that is pulled into the press as the press membrane deflates for crumbling; the extent of this is obviously influenced by the press program as well: more frequent deflate cycles increase the overall O_2 exposure. It is for this reason that the development of the inert press (Ardilouze 2006) has given more control to the winemaker over O_2 inputs to grape must. It is because of this ability to control the O_2 atmosphere in which a grape is initially crushed that this trial has used whole bunch pressing in an oxygen-controlled environment to regulate O_2 exposure.

Some authors (Boselli et al. 2010, Motta et al. 2014) have characterised the composition of juice made using inert-gas cover during pressing while others (Antonelli et al. 2010, Mattivi et al. 2012, Motta et al. 2014) have analysed wine made using reductive handling techniques. However, the relative effect of using reductive handling compared to early protection during pressing has not been

assessed within the same experiment. The first winery-scale experiments in the 2014 vintage involved using two pressing techniques on white fruit (inert and aerobic pressing) followed by two common handling methods for transfer to tank (reductive and oxidative handling), resulting in four very different final wines which reflect the extremes of oxygen use under 'usual' production conditions.

A 2 x 2 factorial design allowed the relative merits of each technique to be assessed. To assess the oxygen inputs during juice preparation several DO measuring devices were employed at different stages of the winemaking process. The PME 'miniDOT' datalogger was ideal to measure the oxygen environment inside a modern membrane press. The dissolved oxygen (DO) profiles inside the press during the two modes of operation are shown in Figure 1. In the normal/aerobic mode each time the membrane is deflated prior to crumbling a spike in the DO is observed. As this occurs multiple times during a press cycle juice from partially pressed grapes can be exposed to a considerable amount of oxygen. Conversely once the press chamber is flushed of air, the environment remains anoxic until the end of the cycle and the press doors are opened.

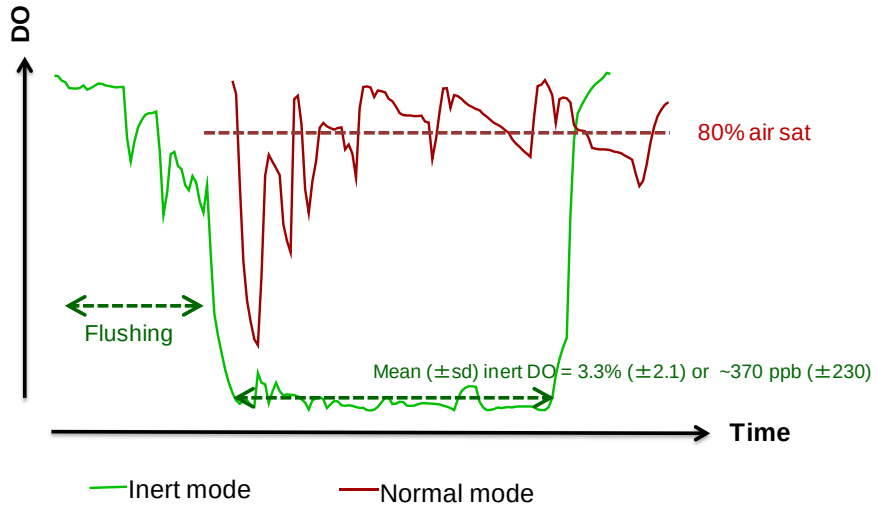


Figure 1. Dissolved oxygen during an inert and an aerobic (or normal) press run

To assess the amount of exposure experienced by a juice or wine, the DO was measured before and after cellar operations. Each time juice or wine was moved from one tank to another, usually for racking to remove supernatant liquid from juice or yeast solids, a probe integrated into the transfer line after the pump allowed the DO to be measured or a handheld DO meter was used to measure headspace O_2 (HSO) concentration. Juice and wine racking was carried out with a variable speed displacement pump. A 'tee-piece' with an appropriate fitting on the side arm allowed an optical process-grade DO to be placed on the delivery side of the pump, positioned tangentially to the flow. During each transfer or racking, the DO was recorded manually. *Ad hoc* measurements of tank headspace were made using a hand-held DO meter.

During the racking operations, juice or wine was moved from the fermentation tank into a temporary buffer tank which had either been inerted with sublimed solid CO_2 during 'reductive' handling or left unprotected for the 'oxidative' handling. The effectiveness of these operations is demonstrated by HSO concentration. During inerted racking operations, mixed gas flowed onto the surface of the juice or wine using a floating gas diffuser to prevent surface gas exchange. As juice or wine moved out of the source tank, the DO was measured in the transfer line and averaged out over the few minutes the operation took; it was measured on its return to the same source tank. The success of this reductive handling is indicated by +9% change in DO for the Inert-Reductive juice and a 16% decrease for Aerobic-Reductive juice; the margins of error in measuring the DO within a

commercial pilot-scale winery are likely to account for these negative changes. In contrast, the DO increased 37% and 59% when oxidative handling was carried out. During post-fermentation wine racking (B), however, there is discrepancy in the DO for the reductively handled juices compared to the previous juice-racking operation since the DO increased by 71% and 132%, although HSO conditions in the temporary buffer tank were similar. This may be because wine is more sensitive to O_2 than recently pressed juice.

Assessment of the effect of O_2 on final wine style with identification of key grape and yeast-derived sensory impact compounds

Effect of passive oxygen exposure during juice preparation and primary fermentation in white winemaking on chemical composition and sensory outcomes (vintage 2014)

The first pilot-scale winery trial in 2014 investigated the effect of passive oxygen additions during white winemaking; that is the oxygen that gets into wine during pressing and handling but is not actively bubbled into the juice or ferment. By separating the very early oxygen exposure that occurs at pressing from the later exposure which happens through different ways of handling juice or wine after pressing until the end of fermentation, it was possible to find out at which stage oxygen has the greatest effect. In the trial two pressing modes (inert and aerobic) and two forms of post-pressing handling (reductive or oxidative) were used to create four distinct Chardonnay wines, allowing the effects of oxygen timing to be closely examined. Figure 2 outlines the trial design.

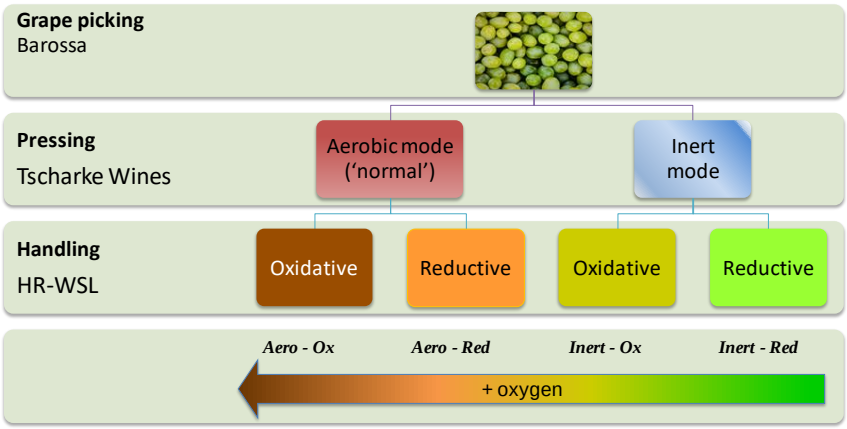


Figure 2. Flow chart of experimental pilot-scale set-up (vintage 2014)

Analysis of aromatic compounds and phenolic composition showed that oxygen exposure during the phase when grapes are first burst open by pressing (as a controlled proxy for mechanical harvesting or crushing) is significantly greater than the effect of oxygen exposure during post-pressing handling. The large amounts of oxygen to which white grapes are exposed during pressing (in this case whole-bunch) resulted in a juice with lower phenolic load, increased higher alcohols, and modified fermentation esters, amino acids and volatile organic acids.

Compositional differences, resulting from either pressing mode (particularly for total phenolics) were far greater than the differences brought about by using reductive handling techniques (with extensive dry ice cover) compared to passive oxidative techniques. This was particularly the case for aerobically pressed juice where the chemical differences between handling techniques were not statistically valid. There were, however, subtle differences between reductive and oxidative handling techniques.

The first indication that there were real differences in this experiment came from Somers' white wine phenolic measures, which showed that the total phenolics and total hydroxycinnamic acids

were highest in the inert press treatments and lowest for the aerobic press treatment (Figure 3). Wines with higher levels of phenolics and hydroxycinnamic acids may have potential for improved texture, as a recent study demonstrated that wines with added phenolics received higher sensory scores for texture (Gawel et al. 2013). Even more interesting is that the type of handling had an influence on the phenolic content of the inertly pressed juices but not the aerobically pressed juices.

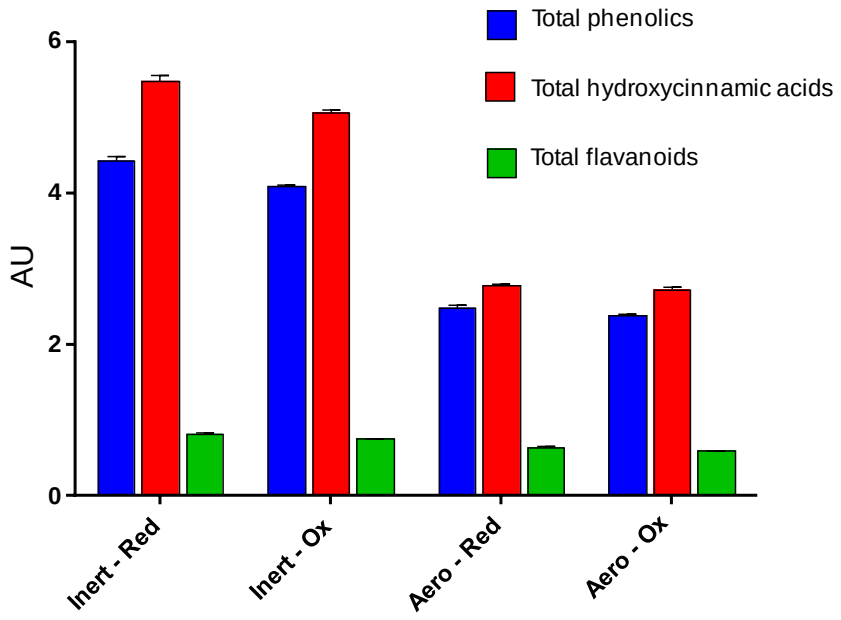


Figure 3 Somers' white wine phenolic indices for the four wines. Treatment codes: Inert-Red and Inert-Ox are inertly pressed and reductively or oxidatively handled. Aero-Red or Aero-Ox are aerobically/normally pressed and reductively or oxidatively handled

Accelerated browning test

Another difference between the wines was found after the assessment of their tendency to undergo oxidative browning. This was done using a simple accelerated browning test (Singleton and Kramling 1976) which compares the absorbance at 420 nm (A_{420}) between wines which are either saturated with air or flushed with nitrogen gas and then stored at 55°C for eight days and assessed using simple colour measures. The results of this test on the four wines are shown in Figure 4, which plots the percent increase in A_{420} caused by the excess of oxygen. It can be seen that wines made from inertly pressed juices have a greater potential to brown than normally pressed juices. This highlights the balance that must be considered in protecting a juice to retain fresh aromas versus the increased potential for browning.

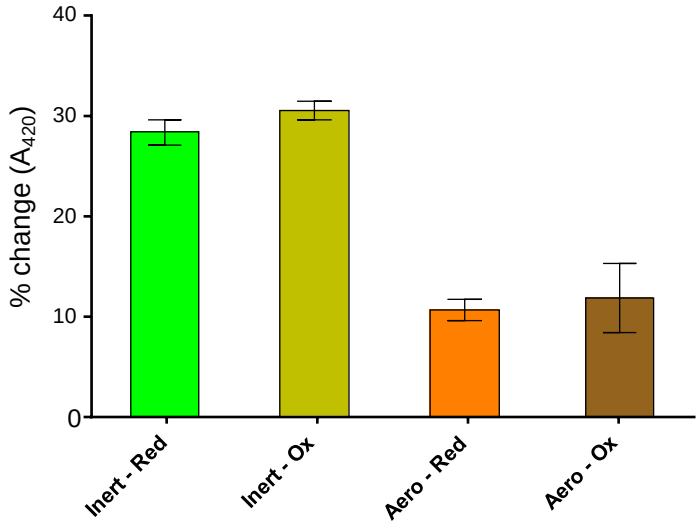


Figure 4. Accelerated browning test (55°C for 8 days) measured with A420, higher values indicate higher potential for browning

Analysis of chemical data for the wines indicated variations in certain aroma compounds (methanethiol, methional, furfural, benzaldehyde, ethyl propanoate and ethyl octanoate) were significantly impacted only through the very early oxygen exposure during pressing, while other compounds (glycine, glutamic acid, tyrosine, 2-methylpropyl acetate, and hexyl acetate) were only affected by oxygen introduced through oxidative handling. A number of other amino acids and volatile esters were influenced by both pressing mode and handling.

Sensory outcomes

The wines were bottled under controlled DO pick-up conditions six months after fermentation and descriptive sensory analysis was conducted by a trained panel of tasters at the AWRI six weeks after bottling. Attributes where the panel found the most significant differences among the wines are shown in Figure 5. The results show that the inert-reductive treatment was significantly higher in 'floral' and 'confection' aroma, and lowest in 'yellow colour' compared to the other three treatments which did not differ significantly from each other in the two aroma attributes. However, for 'acid' taste, the aerobic-oxidative treatment was rated lowest, while for 'yellow colour' the inert-oxidative and the aerobic-oxidative treatments were rated significantly higher than aerobic-reductive, and significantly lower than inert-reductive.

The sensory data were also analysed statistically to separate out the effects of press mode and handling mode. This showed that pressing had a larger effect on the sensory perception of the attribute 'confection' while the attributes 'acid' and 'citrus' were more affected by the handling treatments. While the sensory differences observed at this time point are relatively small, when considered in conjunction with differences found in the chemistry of the wines, they suggest that more significant differences may appear as the wines develop.

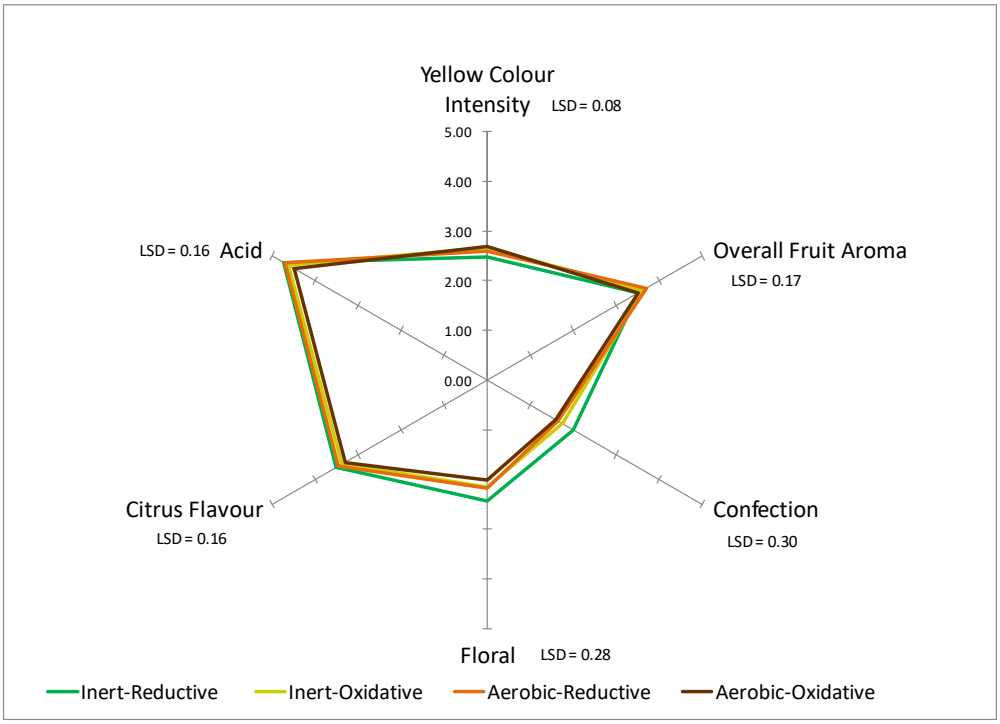


Figure 5. Plots of significant sensory attributes (P < 0.1). (Least Significant Difference values (LSD) are the smallest difference between treatments for them to be noticeable with a probability of 95%.)

Linking aroma compounds to sensory attributes

Aroma compounds which contribute positive characters, such as the fruity and floral esters, the varietal thiols and volatile acids, were analysed after fermentation and stabilisation. A series of aldehydes and other aromatic compounds that contribute to the 'oxidised' aroma of wine were also analysed along with amino acids which are potentially their precursors, giving a total of 72 analytical parameters and eight sensory attributes to analyse. A data reduction technique known as partial least squares analysis (PLS) was used to understand both sets of data, and the results are shown in Figure 6. Looking at how the individuals from each treatment group together and their relative positions on the plot, it is possible to describe the horizontal axis, Factor-1, as the 'pressing axis' and the vertical axis as the 'handling axis'. A lot more of the initial variance is represented by horizontal axis, showing that pressing has a bigger impact on both chemistry and sensory characteristics than handling.

Wines from inertly pressed grapes showed higher concentrations in some ethyl esters and medium-chain volatile acids as well as total phenolics. These wines should display some fruiter notes and may develop enhanced texture. The wines from aerobically pressed grapes had higher concentrations in the varietal thiols, different medium-chain volatile acids and ethyl esters. The volatile sulfur compounds hydrogen sulfide (H_2S) and methanethiol (MeSH) were also higher in wine from aerobically pressed grapes giving them a potentially more reductive character. This is directly opposite to the situation seen in red wines (Day et al. 2013) and has been confirmed in laboratory experiments.

Fewer chemical attributes were affected by the handling mode of wines. For wines handled oxidatively there was greater influence from 2- and 3-methylbutanoic acid ('sweaty/cheesy') and hexyl acetate ('sweet/perfume') while for reductively handled wines there was more benzaldehyde ('marzipan') and benzylmethanethiol ('struck flint'). Sensory results, however, do not reflect a higher 'flint' character in the reductively handled wines, most likely because the levels of benzylmethanethiol are very close to the aroma threshold.

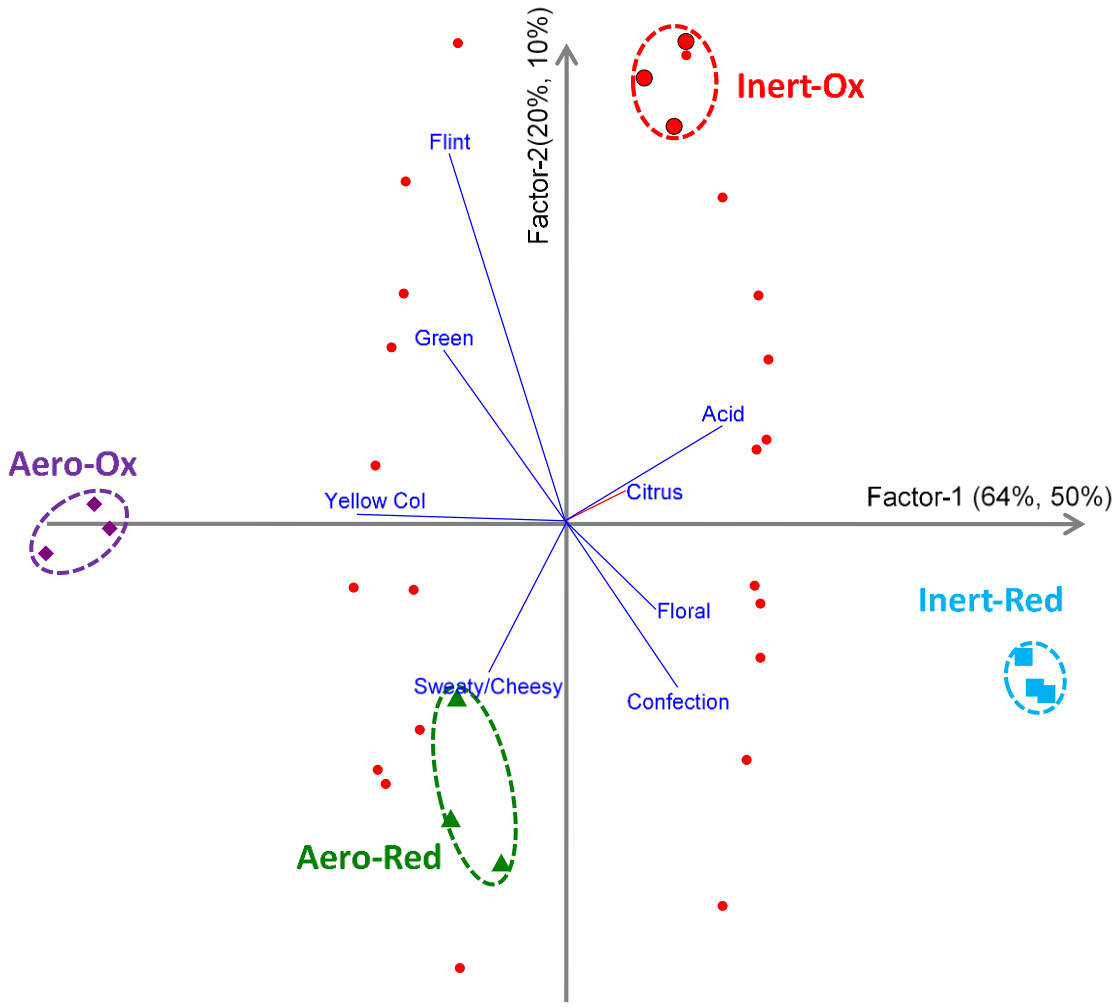


Figure 6. Partial Least Squares analysis (PLS) biplot of significant volatile compounds (red dots) and sensory attributes (blue text)

Effect of different O_2 concentrations on grape crushing/pressing

As demonstrated above, the impact of very early O_2 exposure had the most impact during winemaking. There were clear chemical markers which differentiated wines made from normally pressed juice and wines made in an entirely inert atmosphere. In the case presented above, this occurred during pressing of whole bunches. The extreme represented by totally inert-pressed whole bunches led to a higher browning potential because of the higher residual phenolic matter and tendency to 'pink'. It may be possible to modulate these effects by having a less-inert pressing environment that minimises oxidation of varietal aromas (Makhotkina et al. 2014). This hypothesis was tested by whole-bunch pressing Pinot Gris grapes with different gas compositions. Three gas blends of O_2 in N_2 (5%, 10% and 15%) along with 100% N_2 and air (21% O_2) were introduced into the previously evacuated/deflated gas reservoir of a Bucher-Vaslin XPlus 30 Inertys. A small portion of the juice produced from 1,500 kg grapes was transferred to a 30 L keg and transported back to the AWRI laboratories where the juice was settled, fermented, racked and bottled under strict inert conditions. An operational incident at the winery resulted in the 0% O_2 treatment having juice-clarifying enzymes added inadvertently and therefore any comparison with other O_2 treatments needs to be treated with caution.

The phenolic content of the wine resulting from pressing with different O_2 concentrations is shown in Figure 7. There is an exponential decrease in the total phenolic content with increasing O_2 concentration ($y = 2.07e\text{-}0.075x$; $r^2 = 0.989$). This would indicate that, in the presence of excess PPO,

the rate limiting step is based on the O_2 concentration at the time of initial grape rupture and that modulation of phenolic content can be carefully controlled with O_2 environment. Another observation from this experiment is that the concentrations of the haze-forming proteins, chitinase and thaumatin-like protein, decrease with increasing O_2 concentration exposure when the grape PPO-mediated oxidation first occurs Figure 8. The increased quinone activity with higher O_2 concentrations leads to the increased aggregation of polyphenolics which can bind grape proteins (Poncet-Legrand et al. 2007) and eventually precipitate out of solution during fermentation. The effect to the winemaker of pressing in a diminished O_2 environment will be an increase in the need for protein fining agents such as bentonite (Sauvage et al. 2010).

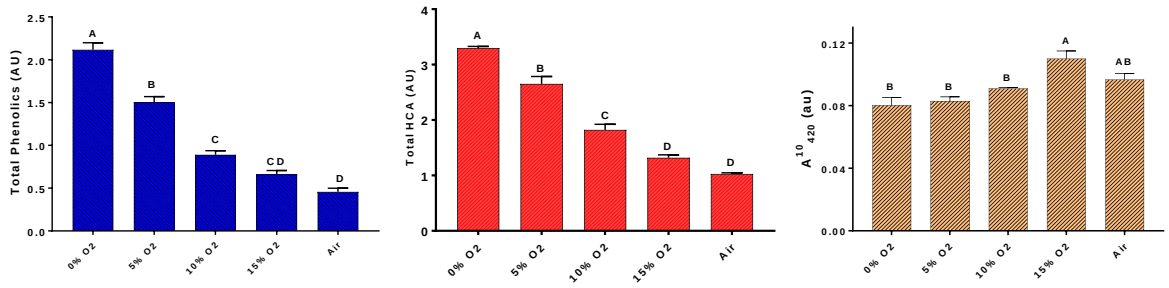


Figure 7. Comparison of phenolic indices with O_2 concentration at pressing

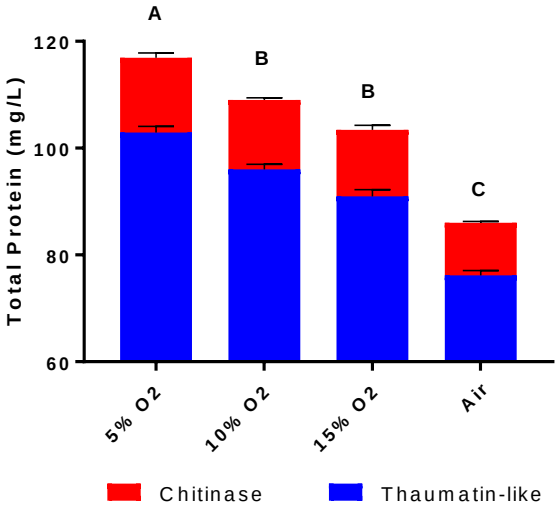


Figure 8. Effect of O_2 concentration at pressing on concentration of haze-forming proteins

The wines were bottled in the laboratory soon after post-fermentation clarification. Fermentation-derived acids, alcohols and esters were analysed, within a few weeks of bottling, to observe any aroma differences arising from early O_2 exposure. Because of the inadvertent addition of pectolytic enzymes to the 0% O_2 treatment with fully inert pressing, it is possible that aroma precursors would have been released from the grape skins and pulp before fermentation, making any comparison of the aroma profile of these wines not possible and therefore these data were excluded from analysis. Data from the remaining treatments were mean-centred and scaled by standard deviation before a PCA was performed. Of the original variance in the data, 62% is represented in the first two principal components (Figure 9). In this plot the replicate fermentations from the 5% O_2 treatment are clearly separate from the other groups of samples, with the air treatment also being slightly separate from the 10% and 15% O_2. The parameters that associate with the 5% O_2 samples are: butanol, acetic, propanoic, butanoic and decanoic acids, ethyl acetate, 2-phenylethyl acetate, 2-methylbutyl acetate. Those that are associated with air are: 2- and 3-methyl butanol, 2- and 3-methyl butanoic acid, 2-methyl propanol and ethyl 2-methyl propanoate. The samples arising from pressing with 10% and 15% O_2 associate with ethyl octanoate and ethyl decanoate.

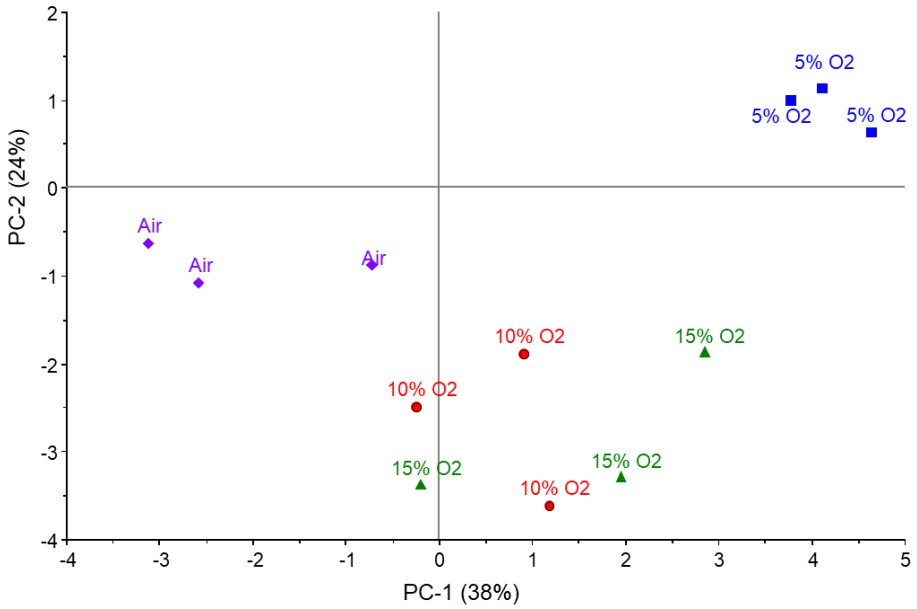


Figure 9. PCA scores plot of fermentation volatile compounds grouped by O_2 concentration at pressing

Sensory analysis was carried out using a full descriptive panel on all the treatments carried out. Of the 3 appearance terms, 13 aroma terms and 16 palate descriptors, only the attributes 'yellow colour intensity', 'pink tinge', 'sweaty aroma', 'apple/pear flavour' and 'hotness' were significant at $P < 0.05$. Calculation of the 95% least significant difference (LSD) indicated that these statistically significant differences were driven by the 0% treatment samples in which a low yellow colour intensity and observable pink tinge was seen; all other aroma or flavour descriptors were maximum for this treatment too. As explained above, these differences should be discarded because of the accidental use of pectolytic enzyme in this treatment.

Laboratory investigation of the effect of DO at inoculation
Must oxygen concentration at the time of inoculation had no impact on fermentation duration or the concentration of yeast-derived aroma compounds in wine. However, aeration of ferments later than might normally be recommended still had a stimulatory effect on fermentation performance without negative consequences for wine sensory attributes. As such, if required (e.g. for stimulation of sluggish/stuck ferments), the use of oxygen outside the previously defined narrow window (24-72 hours post-inoculation) could be considered beneficial for ferment performance with limited risk to the sensory outcome.

Impact of rate, length and timing of oxygen addition during fermentation
Oxygen is a key nutrient in the context of fermentation despite wine fermentation being conducted largely anaerobically. Supplementation of ferments with oxygen has been shown to be beneficial to fermentation progress, especially if added at key growth stages. The effects of oxygen addition during white wine fermentation outside of these narrowly defined time points were examined, looking particularly at efficiency and chemistry impacts. Small (250 mL) and winery-scale (500 L) fermentations were used to evaluate the impacts of oxygen addition on fermentation performance and sensory characteristics of Chardonnay.

Laboratory-scale exploration
The effect of oxygen addition, both its quantity and timing, was explored in a series of laboratory fermentation trials. The primary finding was that fermentation performance and wine chemistry were predominantly influenced by the **total amount of oxygen** consumed by the fermentation, **not the duration** over which it was delivered. Ferments that received a large amount of oxygen in a short period and those that received a small amount over a longer period, with equivalent overall consumption, exhibited similar performance and chemical profiles.

Specifically, it was observed that:
- volatile acids such as acetic, octanoic, and decanoic acids, normally associated with negative sensory attributes in wine, decreased with increasing oxygen dose
- branch chain acids and their associated esters, such as 2-methyl butanol and ethyl-2-methyl butanoate increased proportionally with oxygen treatment
- in particular, the concentrations of branch chain esters were modulated around their aroma thresholds and therefore may change sufficiently to influence sensory qualities
- significant stripping of oxygen by CO_2 occurred, with oxygen uptake rates inversely proportional to CO_2 production rates, at least at low oxygen input concentrations.

The extent of must aeration at the time of inoculation, which can be influenced by tank filling operations, had minimal impact on fermentation performance and production of yeast-derived volatile compounds. This suggests that variations in must oxygen concentration at the time of inoculation are unlikely to influence wine sensory attributes.

In addition to investigations on the effects of total oxygen consumption, the effect of oxygen addition timing was also explored. From a fermentation performance perspective, oxygen additions when ferments had reached 80% to 60% of initial sugar had the biggest impact, which is consistent with the work of others. Fermentation duration was still reduced by treatment at 40% initial sugar, but was substantially longer than observed for the earlier treatments. No difference in fermentation duration was found between ferments treated at 20% initial sugar or no oxygen treatment. The effects on wine chemistry largely mirrored that of fermentation performance, with exposure of fermentations to oxygen at 80% of initial sugar having lower concentrations of medium chain fatty acids and later additions showing increasing concentrations of these acids.

In summary, laboratory experiments demonstrated that oxygen addition between 80 to 60% of initial sugar for a period of 2 to 48 hours, depending on the concentration and flow rate of gas used,

had maximal impact on fermentation outcomes and that these parameters can be modulated to shape the extent of the effect.

Pilot-scale validation (vintage 2015)
The vintage 2015 trial examined the effect of 'actively' adding oxygen during active fermentation. Short (2 hours) and long exposure (20 hours) treatments were applied when sugars had dropped to 80% of their initial concentration, designated as Early-short (ES) and Early-long (EL); a third treatment, long exposure, was applied when sugars had dropped to 20% of initial level, designated as Late-long (LL).

The progress over time of the fermentations was followed by measuring the sum of the concentrations of glucose and fructose and is shown in

Figure **10**. The 'early' treatments (ES and EL) started on the afternoons of day 4 and the 'late' (LL) on day 11, although samples were taken for sugar concentration determination several hours prior to the aeration treatments. It was not until day 6 that significant differences occurred, with only EL being statistically lower. By day 8, ES and EL were significantly different from each other until day 13 when there was no significant difference between the treatments. Although no statistical differences were calculated in the sugar concentrations between LL and the control, the LL ferments finished by day 15 at which time there was still residual sugar in the control.

The Somers' phenolic indices were measured directly after post-ferment cold setting and are shown in Figure 11 (Tukey *post hoc* pairwise comparisons). The increased exposure to oxygen during the EL treatment significantly decreased the total phenolic content when compared to wine which did not receive any aeration. This treatment also significantly increased the yellow colour. This rise in yellow colour may be due to an increased generation of the xanthylium ion from enhanced quinone production when oxygen and PPO are at their peak activity (Guo et al. 2017).

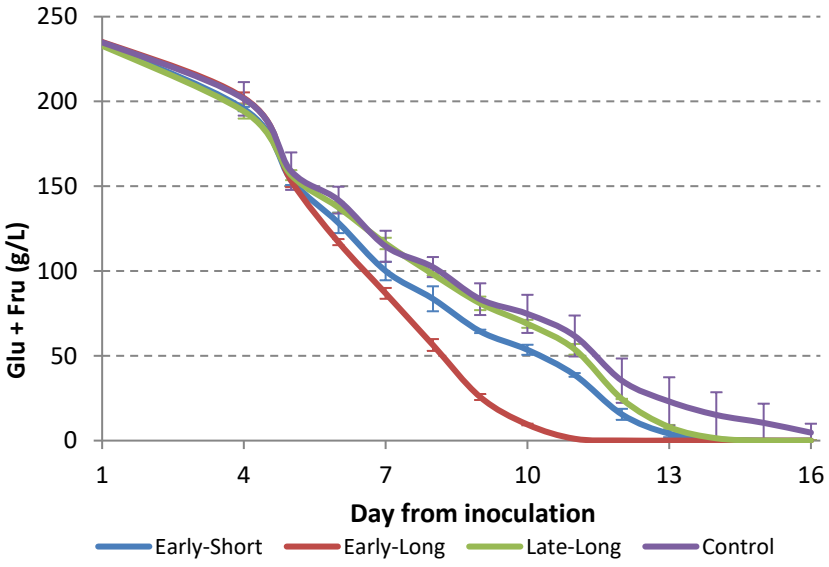
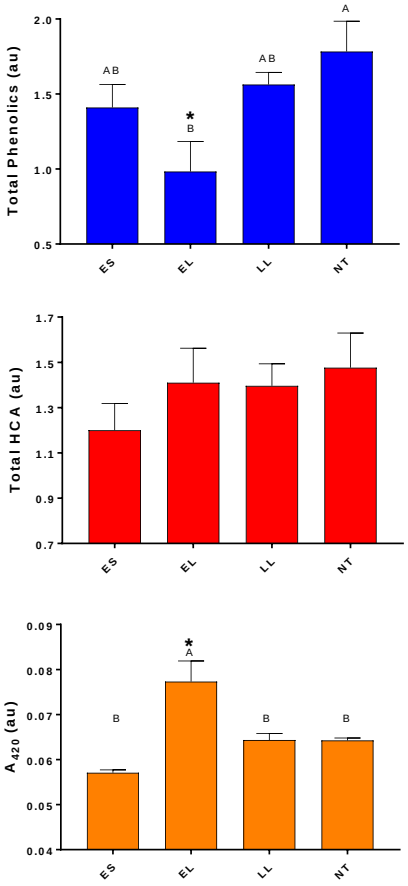


Figure 10. Sugar consumption (glucose + fructose) as a function of time in Chardonnay juice subjected to aeration during fermentation for different timing and duration



* indicates significant difference compared to 'No treatment' using Dunnett t (P = 0.05) *post-hoc* test

Figure 11. Spectral indices indicating phenolics contents as a function of timing and duration of aeration of Chardonnay fermentations

The chemical aroma profiles of the finished wines were determined six months after bottling, at the same time as sensory analysis. Mean concentrations of the fermentation-derived alcohols, organic acids and esters were subjected to ANOVA along with significant treatment differences using the Tukey *post hoc* test at 95% confidence level. All compounds analysed showed significant differences between the treatments ($P < 0.005$) with over 50% being present above the odour threshold values given by Siebert et al. (2005).

Sensory analysis was carried out after the wine had been stored in bottle for exactly six months to replicate a reasonable parallel with industry. Following the last training session, the descriptive terms were finalised as containing one 'appearance' term, twelve 'aroma' terms (eleven defined and 'other') and fifteen 'palate' terms. The ANOVA indicated the attributes 'green aroma', 'green flavour' and 'sweet' differed significantly between treatments ($P < 0.05$), as did 'stone fruit aroma' and 'chemical' at a lower significance level ($P < 0.1$). There were no significant differences among ferment replications for any attribute, signifying consistency in the winemaking replication.
The mean data for each treatment are shown Figure 12. The LL treatments gave rise to wines with significantly higher ratings for 'green' aroma and flavour, and slightly lower ratings for sweetness, compared to the control, with the EL also being rated somewhat higher than the control in 'green' aroma. The EL and ES treatments showed a trend for a lower 'stone fruit' aroma, indicating the application of oxygen early in the fermentation gave a slight less fruity wine.

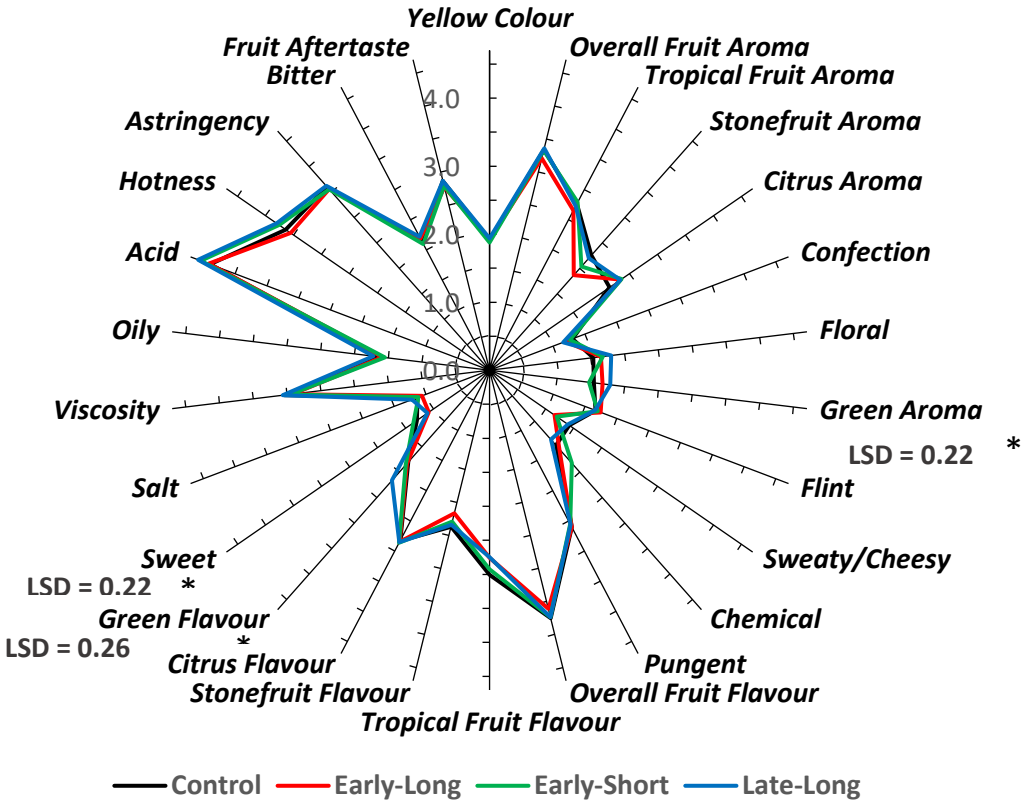


Figure 12. Radar plot of the mean scores for the Chardonnay wines made with varied timing and duration of air sparging during active primary fermentation

Effect of timing and dose of oxygen addition in red wine

Pilot-scale validation using pump-overs and a sub-cap aeration (vintage 2016)
Having established that the timing of oxygen additions during primary fermentation was crucial for maximum advantage in efficiency, aroma and palate structure in white wines, focus was shifted to red fermentations to see if similar effects occurred. In the initial foray into red winemaking with O_2 at the AWRI in 2012, it was established that stylistically diverse red wines could be created by use of 'macro-oxygenation' (introducing large volumes of oxygen) during fermentation in rotary tanks using air (21% oxygen) or 40% oxygen. These wines showed bright red fruit characters, softer astringency, no reductive aromas and much lower residual metal concentrations than the wines made without oxygen. In vintage 2016 macro-oxygenation was further explored using air in red fermentations (during pump-overs), examining the timing of air addition as well as the duration or treatment.

Optimum timing of oxygen addition (from a performance gain perspective) was compared to later addition and repeated aerations in pilot-scale (500 L) Shiraz fermentations. One treatment received a one-off addition during pump-over to saturate the fermenting must with air when sugars had dropped by 20% ('Early') while another treatment consisted of repeated aerations to achieve air saturation over five consecutive days ('Daily'). Another single treatment occurred later when the initial concertation had dropped by 80% ('Late') and a final treatment on previously unaerated wines was carried out after pressing ('Post-Press').

All on-skins aeration treatments showed lower H_2S concentrations post-MLF and at sensory; the 'Daily' treatment showed significantly lower methanethiol (MeSH) than 'No Treatment' with methanethioacetate (MeSAc) being totally absent for this treatment. The differences in chemical aroma composition were a lot subtler. Only half of the 15 significant aroma compounds were above odour thresholds (Siebert 2005) but were characterised by descriptors of 'berry', 'fruit' or 'sweet' in

aeration treatment. The concentration of the only varietal thiol to show significant differences (3-MH) was suppressed during aeration treatments on skins although the trends in the precursor hexenal were not as clear cut. Textural descriptors from sensory analysis also showed there were differences in astringency, viscosity, hotness and acidity due to the treatments which were not correlated with the aroma characteristics. Only the 'Daily' treatment had significant effects on the tannin composition: total phenolics were lower, tannin polymer length was shorter (mDP of 8 vs 10), skin-like tannins were reduced with a corresponding increase in wine/seed-like tannins. The convoluted or knotted character (inverse of % mass conversion) of tannins was measured to be statistically lower in the 'Early' treatment compared to no oxygen exposure. Wine colour density and total anthocyanins were depressed and hue was increased with less obvious corresponding trends in improvements to stabilised colour (Figure 13). Sensory analysis one year after vintage showed 'Early' and 'Daily' treatments were found to be higher in 'fruity' and 'floral' characters in comparison to the 'No Treatment', 'Late' and 'Post-Press' treatments which were found to possess higher levels of reductive 'vegetal', 'earthy' and 'black olive' characters and lower intensity of fruit characters.

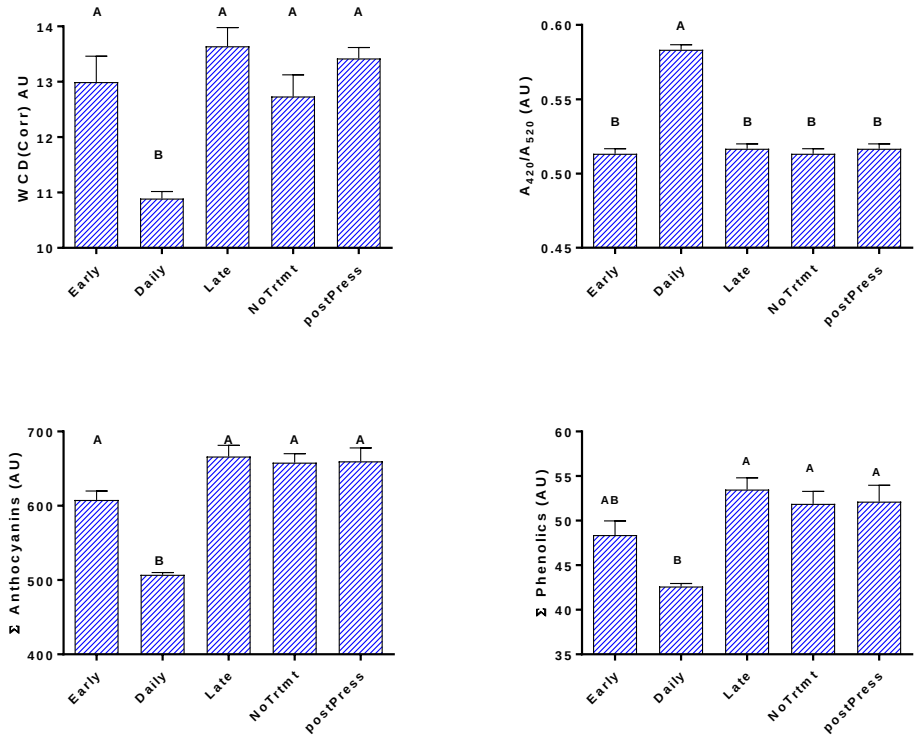


Figure 13. Somers' phenolic indices, prior to inoculation for MLF

Further understanding on the addition of oxygen during red fermentation (vintage 2012).
The effects of oxygen treatment during red wine fermentations on phenolics, metals and malolactic fermentation were determined during this project, stemming from a vintage trial in 2012. In this 2012 vintage experiment, Shiraz wines were made in triplicate using rotary fermenters and treated with different levels of O_2 exposure during fermentation, namely air (containing approximately 20% O_2) and 40% O_2/60% N_2. The impact of the physical displacement of volatile compounds and mixing effects by gas was assessed using gas injections of pure N_2, and the controls were fermented without any gas addition. Wine colour and tannin characteristics were measured after fermentation (Time 0), and after 2 and 12 months of bottle ageing under two different screw cap liners, Saran-Tin and Saranex.

Different levels of O_2 exposure can dramatically alter the colour and texture of red wines. Gradual exposure to O_2 over many months, for example during barrel ageing, can impart a softer mouth-feel to the wine and a more reddish, rather than purple, hue (Cheynier et al. 2006). With O_2 exposure, the purple, monomeric anthocyanins become more stable and resistant to SO_2 bleaching by directly or indirectly forming polymers with condensed tannins as well as acetaldehyde-mediated derivatives such as pyranoanthocyanins (Bakker and Timberlake 1997).This induces a change to red-orange hues and can lower wine astringency, as anthocyanin-bound tannins are less astringent than non-pigmented tannins (Weber et al. 2012). O_2 exposure also alters the structure of wine tannins by decreasing the proportion of acid-labile interflavan bonds, which is related to a decrease in percent conversion yield in depolymerisation reactions, as calculated from the molar mass of cleaved tannin subunits relative to the mass of tannin used in the reaction (Sauvage et al. 2010). Lower percent tannin yield also decreases the extent of protein binding (McRae et al. 2010) and may lead to a less intense wine astringency. Ageing of wines under bottle closures with high oxygen transfer rates (OTRs), such as cork or Saranex lined screw caps, can modify wine colour and tannin structures to a greater extent than closures with lower OTRs, such as SaranTin screw caps. This effect is particularly enhanced for wines at lower pH (McRae et al. 2010). The importance of O_2 exposure on colour stability and mouthfeel without the use of extended barrel ageing has led to the development of microoxygenation (MOX) techniques in red wines although there is still significant debate about the best time to apply MOX and the ideal dosage rate (Schmidtke et al. 2011).

As an alternative to MOX, changing the level of O_2 exposure during fermentation may alter the colour and mouth-feel of the resulting wine by inducing chemical and enzymatic oxidation of polyphenols, including catechin and caffeic acid, and potentially modifying the extraction of tannin from grape cells. In the production of red wine, O_2 exposure may occur whenever the ferment is plunged or pumped over but the level of O_2 exposure can vary significantly, depending on the number, duration and modality of pump-overs. As an alternative to pump-overs or cap plunging, appropriate levels of grape skin contact during fermentation can be achieved during winemaking with the use of rotary fermenters. These fermenters are horizontal tanks that rotate axially and, while effective in improving skin contact, the vessels are enclosed and can produce reductive conditions. Grape tannins extracted from skins and seeds during winemaking may be directly impacted by chemical oxidation, as well as enzymatic oxidation via such enzymes as laccase and polyphenol oxidases (PPO). PPO can polymerise flavan-3-ols and smaller phenolics such as caffeic acid which may react with tannin to form complex structures. The production of acetaldehyde as a fermentation product as well as from oxidation of ethanol may also increase with greater O_2 exposure during fermentation, changing the structures of extracted tannins. Oxidation reactions at the grape cell surface (i.e. cell wall-bound PPO) may also potentially enhance the retention of tannins and anthocyanins by grape solids during winemaking (Bindon et al. 2010).This may reduce the amount of tannin extracted into the wine and thus the intensity of wine astringency.

Colour and tannin effects

The O_2-treated (40% O_2 and Air) wines were significantly lower in total anthocyanin than the control or N_2-treated wines, and were slightly higher in non-bleachable pigments. This suggested that the pigments formed in the presence of higher O_2 concentrations were more stable. The wine colour density (WCD) remained relatively consistent regardless of fermentation treatment. Tannin composition was significantly different between the wines produced by the O_2-treated ferments (40% O_2 and Air) and those produced from the control or N_2-treated ferments. The tannins from the 40% O_2 and Air wines were less susceptible to depolymerisation reactions (more 'cross-linked'), were more coloured and smaller (McRae et al. 2015). In terms of sensory effects, the 40% O_2 and Air treatments scored lowest for 'bitter' and for 'astringency', while the N_2 treatment was scored highest for 'astringency' emphasising the anecdote that oxygen diminishes the astringency of red wines.

Impact on metal concentrations

After bottling, the O_2-treated wines contained significantly lower concentrations of iron, copper and zinc than the control or N_2-treated wines (McRae et al. 2014). Recent studies have shown the particular importance of iron and copper, as well as others, in the formation of both positive and negative flavour and texture compounds, and on the shelf life of wine (Viviers et al. 2013). The impacts of different metal ions on wine flavour and aroma remains an ongoing area of research at the AWRI and this research indicates that early oxygen exposure in red wines is likely to influence metal-catalysed changes in composition.

Impact on 'sulfidic' or 'reduced' aromas.

The evolution of H_2S was monitored during fermentation; after only two days of treatment, the amount of H_2S gas generated by the fermentations treated with O_2 (40% O_2 and Air) was significantly decreased and after a further two days all production had stopped with no subsequent evolution, even when O_2 treatment stopped. Analysis of volatile sulfur compounds (VSCs) after pressing showed that O_2-treated wines had much lower EtSH and MeSAc and a complete absence of EtSAc. The data demonstrated that the O_2 treatment during fermentation created an environment that favoured VSC removal or modification, either through increased yeast activity or potentially through the incorporation of VSCs into other wine compounds. Comparison of the O_2-treated wines with the no treatment/control showed that there were no statistically significant differences in VSCs, thus indicating that 'splashing' or other aerative cellar operations do not physically displace H_2S gas. The VSCs of the wines sealed under two OTR closures were monitored over 12 months; although there were variations during extended bottle maturation the O_2-treated wines showed consistently lower VSC concentrations (Bekker et al. 2016). Oxygenated handling during fermentation produced wines with desirable 'red' and 'dark fruit' aromas, and correspondingly, the lack of O_2 during fermentation resulted in sensory characters associated with unpleasant 'reductive' aromas, such as 'sewage', 'rotten egg' and 'rubber' aromas

Impact of wine ageing and closure type on wine composition

After 12 months of bottle ageing of the control and N_2 wines, the colour measures and two tannin compositional characteristics associated with ageing (specifically size and degree of 'cross-linkage') were similar to those of the Air and 40% O_2 wines as measured after 2 months of bottle ageing. This highlights the effect of O_2 during fermentation in producing aged wine-like characteristics. Thus the level of O_2 exposure during fermentation may also improve the mouth-feel of young red wines through the influence of modified tannins.

The effect of O_2 exposure during fermentation was compared with the impact of relatively limited O_2 exposure during bottle ageing with two different screw caps: Saranex, which allows a reasonable O_2 ingress, and Saran Tin, which restricts O_2 ingress. The impact of closure type on colour measures and tannin composition after 12 months in bottle was greater in the wines fermented in the absence of O_2 than the O_2-treated wines. The reason that the tannins from the control and N_2-treated ferments were susceptible to this change more so than the O_2-treated wines may relate to differences in the formation of tannin under each treatment type. Greater O_2 exposure during fermentation may increase the oxidised proportion of tannins, resulting in the formation of stabilised tannins with modified interflavan bonds and increased intramolecular interactions to such an extent as to restrict further oxidation due to O_2 ingress through bottle closures. Thus, the oxidation induced by slight O_2 ingress through the Saranex closures was more pronounced in the control and N_2-treated wines than in the air/$O_2$40 wines. Increased O_2 exposure during wine fermentation had a much greater impact on tannin structure in the resulting wine than closure type and this highlights the significance of O_2 exposure during fermentation to tannin formation, development and stabilisation.

Effect of oxygen addition on malolactic fermentation efficiency in red wine
One of the serendipitous observations from the 2012 pilot-scale vintage trial was that aeration into red Shiraz fermentations in vinimatic tanks had a marked effect on the speed of subsequent malolactic fermentation (inoculated after post-press settling). MLF was completed after 8 days for the air and $O_2$40-treated wines and after 17 days for the control and N_2-treated wines. This effect has been examined several times in the laboratory with no confirmations of this acceleration effect having been made.

An investigation into the potential negative effects of fermentation oxygenation on effectiveness of co-inoculation of malolactic acid bacteria or sequential inoculation was performed in Shiraz wines in 2017. Aeration was carried out with distributed sinter points across the bottom of the tank. DO values approaching 50 % air saturation were achieved. Malic acid consumption showed no overall differences between co-inoculated ferments whether aerated or not, nor for sequentially inoculated which had previously received aeration or not. Sensory and chemical analysis will occur at the end of the calendar year. However, the VSCs were analysed at the end of MLF once all treatments had received an addition of SO_2. ANOVA indicated that there were no statistical interactions between the use of oxygen and timing of inoculation for the detected VSCs: MeSH, DMS, CS_2 or MeSAc. A low-level interaction ($P = 0.075$) was observed for H_2S. There were significant differences for the timing of inoculation for MeSH, DMS and MeSAc. Further analyses will be carried out after 4-5 months in bottle.

Practical measurement tools and oxygenation equipment for use in the winery
One aspect of this project was to develop practical experience using tools available for monitoring oxygen exposure during different stages of wine production. In brief, several optical-based dissolved oxygen (DO) measurement tools were used in both the pilot-scale experiments and a large commercial winery. It was found that process-grade probes in specialist housings were best suited for DO measurement during pump-overs or transfers due to their fast equilibration and response time and that they had appropriate configuration for use in a commercial winery. Hand-held meters were equally adaptable to measuring in-tank DO during racking operations. Techniques for introducing oxygen into an active ferment were also assessed. A Venturi injector was trialled in industry and proved a simple and effective device.
There are various analytical technologies that allow the determination of dissolved oxygen (DO) and or headspace oxygen (HSO) in grape and wine products. The industry-standard for many years has been the Clark electrode which contains an oxygen-permeable membrane linked with an electrochemical detector. These are known popularly as the ‘Orbisphere‘ and are mostly adapted for use in a production laboratory or bottling line. They require careful maintenance and fouling of the membrane is a common problem in the routine use of such devices. A more robust technology makes use of the quenching effect of oxygen on the chemiluminescent properties of certain ruthenium diimide complexes or similar compounds and work by measuring the phase-angle shift of an exciting light source. This technology was initially used for good effect in the Presens or Nomasense devices using in determining DO inside wine bottles (O'Brien et al. 2009). The technology has now been transferred to more traditional style immersion probes as well as process-suitable probes. Because of increased robustness and ease of use, only devices using chemiluminescent DO determination were assessed. This technology is often referred to as LDO.

A number of devices were trialled throughout the project, including:
- Presens ‘Fibox Trace [sensor spot/dipping probe]
- Pyroscience Firesting [sensor spot/dipping probe]
- Hach HQ 30d [handheld DO meter]
- Mettler-Toledo 6870i + M400 Controller [process probe]
- PME miniDOT [deploy and forget data logger]

Some observations of experiences with these tools are outlined below.

The AWRI has been using Presens devices for in-bottle measurements for a number of years and has used this as a useability benchmark, based on experience from several vintages in industry settings as well as the Hickinbotham-Roseworthy Wine Science Laboratory's pilot winery. In addition to in-bottle measurements which employ a chemiluminescent sensor spot glued to the bottle wall, it is possible to measure DO in the winery by attaching a sensor spot inside a sight-glass to measure liquid flowing through a pump. It is also possible to use a 'dipping probe' for this device in which the chemiluminescent material is bonded inside a narrow stainless steel probe and linked to the measuring device with a fibre optic cable. Other manufacturers produce this type of equipment and the AWRI selected the Firesting from PyroScience (Germany) as a comparison and also for work in the laboratory where multiple channels are required. A comparison (del Alamo-Sanza et al. 2014) of the above-mentioned equipment has recently been published and the Firesting trace was assessed to provide the most accurate data with the least noise error. The Firesting and the Presens are more suited to the laboratory environment, so more appropriate adaptations of the chemiluminescent technology were also assessed during the 2014 vintage. Handheld DO meters have been available for a number of years and the Hach HQ30d was taken as an example of this type of meter found in many wineries. It is battery operated, comes with a 5-metre lead and a weighted probe cowl allowing easy deployment into deep tanks – a rugged stainless steel version is also available. This particular model has a several reading modes: continuous, interval and press-to-read; the data generated can be saved and downloaded or reviewed on the handheld meter. The active part of the unit is the LDO sensor cap containing the chemiluminescent material and a separate memory chip 'iButton' which holds the calibration data. The cap has a fixed lifespan of 365 days and replacement is very inexpensive. Regular calibration checks are recommended using a two-point calibration (see below).

Most of the above devices are portable and do not integrate into a winery environment or on a fixed platform. The Firesting and the Presens have separate thermocouple wire and a fibre-optic cable which is inconvenient (the Hach has the thermocouple integrated into the probe housing). The process-style probes, represented in this study by the Mettler-Toledo InPro 6000 LDO probes, (Hach equivalent is the 410 Orbisphere with a M1100 or K1100 probe) present as much more robust. The probe housings are stainless steel and the controllers carry an IP6x rating which can also be mounted into power control unit on a pump/filtration/skid.

Measurement of DO is important in the waste-water sector and several devices which differ from those typically used in the wine industry may have potential for a technology transfer. Many of those devices are integrated data loggers that are deployed in the field for considerable periods of time. They are relatively inexpensive and small in format. The miniDOT from Precision Measurements Engineering (USA) was selected. This has the ability to store up to 60 days data, depending on the data measurement frequency which can be up to one reading per minute. The unit is autonomous and can be constructed from food grade plastics. The advantage of this device is that it can be placed inside equipment without the need to run cables back to a control unit. One example that will benefit oenology research is the ability to monitor DO inside a membrane press. The device can also be deployed in fermenters or other vessels that do not have access ports for classical DO probes. Frequently the measurement of DO is compromised by the sampling methods or even the potential of introducing oxygen into a vessel in which a probe is being deployed or removed. Therefore, another added advantage of this type of device lies in the 'deploy and forget' nature of a watertight data logger.

More information on this work can be found in Day and Wilkes (2014) and a fact sheet entitled 'Ways to introduce oxygen into an active red ferment' available from the AWRI website at: https://www.awri.com.au/wp-content/uploads/2015/02/introducing_oxygen.pdf

Industry adoption

Case study from The Oxford Landing Winery

Research of this type has most value when trialled by, and ideally adopted by, industry. Following a workshop held at the 16th Australian Wine Industry Technical Conference in 2013, the AWRI was approached by The Yalumba Wine Company about trialling oxygen additions to production-scale ferments at The Oxford Landing Winery (OLW) during the 2014 vintage. The following summary highlights some of the practical experiences of working with industry partners to support adoption of this research.

2014 vintage trials

The trials were conducted in four 100-tonne sweeping arm Potter (SWAP) fermenters using Cabernet Sauvignon fruit from the Oxford Landing vineyards. The project team decided to use a more conservative dose of 1.6 g/L oxygen compared to the doses added during the pilot-scale trials (2.8 g/L and 5.5 g/L), based on the site equipment and an achievable cost/benefit return.
Three different types of oxygen introduction device were tested:

- 3-inch venturi injector (Mazzei) placed at bottom or top of pump-over line
- Pulsair tanks in normal operation
- Air sparger at bottom of tank.

Results from the trial were very encouraging, with all of the wines ending up in their intended blends, a decision which was a small leap of faith for the winemakers! No sulfidic aromas were detected, although this is not normally a significant problem for Cabernet Sauvignon wines. With the venturi configuration, the DO measured before and during the aerated pump-over (probe placed just before irrigator) rose to 19.9% air saturation which from the given total soluble sugars (TSS) at the time of aeration gave a DO of 1.43 mg/L. When the venturi injector was at the top of the SWAP fermenter, the DO rose to 42.2% air sat or 2.92 mg/L. From a practical point of view, the venturi injectors – placed directly after the pumps at the bottom of the tanks – did not work well in the set-up at OLW because the in-place pump-over pumps are a high flow, low pressure design. This meant that the flow rate was dramatically reduced and changed the dynamic of the pump-over system, creating another variable in the trial. It also would not be feasible to retrofit the venturi injector into the existing pump system. With the right pump design a venturi system might be suitable for a new installation. Because of the passive nature of the venturi device and the fact that the inlet pressure was not measured, which meant the actual volume of gas delivered could not be calculated, the project team reluctantly decided not to continue with this device.

2015 vintage trials

Following the positive outcomes from the 2014 vintage, the OLW project team decided to continue and expand their trials in 2015 to include rotary fermenters as well as SWAPs. The necessary compressed air lines were attached to the existing Pulsair fittings and connected to the SCADA system for solenoid control and logging. Compressed air was only supplied to the fermenter while it was being pumped over to ensure mixing. The air flow rate was set at 200 L/min and 130 kg O_2 were delivered over five days to 100 t of Cabernet and Shiraz grapes from the Riverland. Treated wines were compared with similar untreated batches.

The rotary fermenters had a capacity of 30 t and were fitted with a manifold containing several stainless steel sinters. The air supply was connected to a gas turret fitted with a safety switch to prevent rotation with the gas line attached, and they were also connected to the SCADA system. The air flow rate in this case was 100 L/min delivering 33 kg of O_2 over the five days of fermentation. Because of the smaller head height in the rotary fermenters compared to the SWAPs, the bubble residence time was much lower. However, with the mixing that occurred during rotation, it was hoped that the headspace oxygen would be incorporated back into the fermenting must. Three

batches of Cabernet Sauvignon and one each of Merlot and Shiraz (all from the Riverland) were used in the trial. Control wines were made with the same fruit without addition of air. After the first day or so, the sinters in the rotary fermenters became blocked and were replaced with 1-2 mm holes drilled along the length of the gas supply line in the fermenter. Fortunately, these did not block and were well suited to purpose. No other major engineering problems occurred. One key observation during the trial was that none of the ferments treated with oxygen required the addition of DAP to stop 'stinky' sulfur odours, compared to three out of the five control experiments.

The trial wines were tasted at OLW and Yalumba three months after bottling. For the Shiraz wines made in the rotary fermenters, six of the nine tasters preferred the oxygen-treated wines over the control and one had no preference. No negative impacts on colour were noted and the tannins were considered to be 'smoother'. A slight 'sour' and 'aldehydic' note was picked up by the two tasters that preferred the control Shiraz. All tasters preferred the oxygen-treated Cabernet wines made in the rotary fermenters. For the wine fermented in SWAPs there were unfortunately no control wines available at the time of tasting; however, the oxygen-treated samples looked very good, in particular the Shiraz wines, which had smoother tannins than other wines of similar age.

Analysis of the wines was undertaken by the AWRI at the time of tasting. Volatile sulfur compounds were analysed by GC-SCD (gas chromatography with sulfur-chemiluminescence detection) which detected the presence of hydrogen sulfide (H_2S), methanethiol (MeSH), dimethylsulfide (DMS) and carbon disulfide (CS_2). As with the AWRI trials, the concentration of MeSH was lower in the air-treated wines; however, there was little difference for the other compounds. The trends in tannin composition were also similar to the AWRI pilot-scale trial. Free anthocyanins and total tannin concentrations were lower in oxygen-treated wines as they had been combined into more complex and evolved tannins in which the colour is stabilised. Such tannins tend to be less astringent and are associated with wine ageing.

Further contact with industry

During preparation and execution of the 2017 vintage, several industry players contacted the AWRI for advice in using oxygen during red ferments. These wineries ranged from premium boutique wineries in the Western Australia and Limestone Coast, as well as two large-scale wineries in Victoria and Barossa.

Some quotes from email with one happy winemaker:

Sent: Friday, 7 April 2017 4:02 PM

Hi Martin
Working well, We are running about 20 litres minute of filtered compressed air on a 10 Tonne ferment. Normal pumpover would have been 30 minutes, air for 15minutes is working well during the pumpover. I'm basically looking for the milkshake vision.
cheers

Sent: Tuesday, 11 April 2017 8:51 AM

Hi Martin
Basically I've been rotating it on all shiraz and merlot ferments, about a dozen so far
Each one at 9-10 Baume is getting 15-30 minute intervals, then again at 6-7 Baume.
Seen no foaming issues, its appears frothy but that subsides very quickly.
The effects have been excellent, when compared to my traditional aerative pumpovers into bins, then back over the top.
The team prefer it as well! Only because its less work!

Sent: Wednesday, 9 August 2017 11:35 AM

Hi Martin
All went really well.
We removed the necessity to do any traditional rack and returns on the static fermenters. The oxygen sparger lead the charge and was utilised on all Cabernet, Merlot and shiraz static ferments at least twice through fermentation.
Our rates were 15-30 minutes of air, set at approximately 20 litres/minute, so half the actual pumpover time allocated depending on batch sizes; which were between 6 and 14 tonnes.
The ferments appeared aromatically brighter, with no reductive issues in the ferments and fermentation timing/temperature etc saw no difference to normal practices.
I think we will push parcels harder next year, we really were quite cautious I think.
cheers

Outcome and conclusion

Greater oxygen exposure during fermentation trials in vintage 2012 produced wines with more 'aged' characteristics with respect to greater hue, fewer anthocyanins, lower tannin concentrations, and smaller tannins with more modified structure. These changes were similar to those induced by 12 months of bottle ageing in wines deprived of oxygen during fermentation. The 40% O_2 and Air treatments scored lowest for 'bitter' and for 'astringency', while the N_2 treatment scored highest for 'astringency'. This suggests that increased oxygen exposure during winemaking may reduce the need for extended wine ageing, saving winemakers the cost of tannin fining and extended storage, and possibly increasing consumer preferences. Recent research on white wine phenolics adds to the body of evidence that oxygen is likely to affect texture; the research established that two of the major phenolics in wine (GRP and caftaric acid) that are influenced by oxygen exposure also modulate astringency and increased oiliness (Gawel et al. 2014).

In addressing these questions this research also improved the understanding of how oxygen management and use during processing and fermentation affects areas other than fermentation efficiency and continued to explore how oxygenation during fermentation can be used to remediate or prevent reductive aromas and enhance attributes generally considered to have a positive impact on wine style. In addition, decreased metal concentration in a wine post-ferment may benefit a wine's shelf life and evolution. Finally, the significantly faster rates of malolactic fermentation might provide a practical tool to assist in the reliable completion of malolactic fermentation. As such, investigations into the MLF implications of oxygen exposure are continuing.

The different oxygen levels that occurred in the vintage 2014 trial arose simply through pressing and handling operations – no active oxygen additions were conducted. The choice of pressing mode and the extent to which juice or wine was protected from oxygen during handling were both shown to affect a wine's final chemical composition and sensory characteristics; in this particular case affecting 'floral' and 'citrus' characters. For juices prepared through normal (i.e. aerobic) pressing, no significant differences were introduced through the choice of handling method. This seems to suggest that, at least for Chardonnay, there is little need to invest too much time and money protecting juice and fermenting wine from oxygen, if it has been produced through aerobic pressing. Other white varieties may behave differently so caution should be used before dispensing with inert gas blanketing altogether! On the other hand, if a juice is produced by inert pressing then sufficient phenolics remain which can be affected by further oxygen exposure during normal handling. Inertly pressed juices therefore need continued protection through reductive handling, if oxidation is to be avoided.

Having observed and quantified the chemical and sensory differences that occur through passive oxygen exposure in the 2014 study, trials during the 2015 vintage focused on making deliberate but controlled oxygen additions during fermentation which were expected to have a greater impact on wine style. The addition of oxygen during white wine fermentation had a positive effect on the kinetics of fermentation, rather than style of wine. This observation could have major impact on the efficiency of fermentation by allowing a wine to finish several days earlier than normal, while maintaining style through unaltered fermentation temperatures. This is a particularly valuable outcome considering the growing need to manage fermentations in compressed vintages. The sensory effects of adding oxygen were minimal. The effect of the timing of oxygen additions was also assessed and the preferred timing of oxygen addition appeared to be in the first half of fermentation when sugars had dropped by 20% of the starting concentration. It was still beneficial, however, to make a late addition, even once the sugar concentration had dropped by 80%, as this ensured that the ferment achieved dryness safely. Sensory analysis confirmed that there were no negatives issues associated with using a reasonable amount of oxygen.

During 2016 vintage trials, type of fermenter used and the way the aeration was carried out were modified to demonstrate how oxygen addition could be achieved in wineries not equipped with rotary fermenters and with minimal capital outlay. The timings used in the 2015 trial were replicated in 2016 with additional treatments of a daily dose and a post-press addition. In order to achieve the positive benefits of enhancing the 'bright red fruit' attributes and suppressing low-level reductive aromas, it was important to use an early aeration during the first few days of active fermentation.

Recommendations

Five pilot-scale vintage trials and numerous controlled laboratory experiments were carried out during this funding period. In parallel, several industry partners trialled the use of air additions at small, medium and large-scale wineries across the country.

In white winemaking oxygen additions can lead to increases in fermentation efficiency without having negative effects on sensory outcomes. Modulating the extent of oxygen exposure at the very earliest stages of juice preparation was an important tool in understanding the effect of oxygen in white winemaking. Although the project did not set out to assess the merits of inert pressing, results have highlighted some subtle effects that can be achieved from pressing in low, if not totally inert environments. This is an area that should receive some further investigation, particularly looking at sensory variations and fermentation efficiency gains in a range of grape varieties.

There is still a need to establish the appropriate dose of oxygen for any given must, red or white, and to monitor this in large-scale fermentation tanks. The observed DO during active ferment will most probably be a strong indication of the amount of oxygen consumed by the ferment and accurate oxygen measurement is the 'missing link' in defining a universal approach to controlling dosage. Some engineering solutions may be required to ensure correct DO measurement on large-scale fermenters, which is where the greatest benefits could be achieved. Work on redox potential probes to complement DO measures may also contribute to improved dosage control. The ultimate aim would be to create a 'calculator' to decide the amount of oxygen to add from easily measured juice parameters.

Many winemakers have already attended workshops on the use of oxygen in the winery, and further extension activities would be highly desirable to support adoption and regional trials.

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Project 3.5.3 - Formation and fate of positive and negative sulfur compounds

Abstract

Low molecular weight sulfur compounds (LMWSCs) can contribute both positive and negative sensory attributes to wines. Positive aromas include 'passionfruit', 'tropical' and 'blackcurrant' and negative characters include 'rotten egg', 'rubber', 'sewage' and 'canned corn'. This project focused on increasing understanding of the formation and fate of sulfur compounds responsible for these sensory attributes and applying this knowledge to develop practical strategies to manipulate and modulate LMWSCs during winemaking. A further aspect was to ensure that positive and negative compounds do not undergo any further unwanted changes in tank or bottle, once a wine has a desirable LMWSC profile.

As part of this project, new advanced analytical methods were developed to enable characterisation of the very challenging and reactive LMWSCs at part per billion (ppb) concentrations and below. Another very significant breakthrough was the identification of precursors to several key LMWSCs. The work also showed that the addition of copper significantly influenced the evolution of LMWSCs. Importantly, the effects of copper differed depending on dissolved oxygen concentration post-bottling and also as a function of the residual copper concentration. In addition, copper effects were further influenced by pH and SO_2 and it was demonstrated that post-bottling formation of H_2S, MeSH, DMS, and CS_2 is significantly impacted by both copper additions and wine pH. Beyond copper, the project showed that the formation of LMWSCs from their precursors in wine is influenced by the presence of other metal ions that naturally occur in wine, especially when present in high concentrations. Data on factors modulating the chemical formation of LMWSCs in wine was augmented by the discovery of significant differences among wine yeast in their ability to release both positive and negative LMWSCs. Taken together, this information will help winemakers to make informed choices regarding yeast strain and winemaking conditions to suit their wine style, potentially reducing the formation of negative LMWSCs in a winery environment.

Executive summary

Low molecular weight sulfur compounds (LMWSCs) can contribute both positive and negative sensory attributes to wines. Positive characteristics can include 'passionfruit', 'tropical' and 'blackcurrant' but the negative characteristics include 'rotten egg', 'rubber', 'sewage' and 'canned corn'. Their control in a winery environment is an important avenue to increasing the value of wine by either increasing positive sensory attributes or decreasing those deemed to be negative. Their occurrence can be influenced by factors including yeast selection and fermentation conditions; the nature and quantity of precursor compounds; the availability or absence of oxygen at different points of the winemaking process; and availability and speciation of transition metal ions such as copper. Broadly, this project focused on increased understanding of these factors; this knowledge was then used to develop practical strategies to manipulate and modulate LMWSCs during winemaking and to ensure that once a wine has produced the expressed LMWSCs, positive or negative, they do not undergo any further unwanted changes in tank or bottle.

More specifically, the project aimed to develop an in-depth understanding of the role of compounds suspected to be the main precursors of the sensorially important LMWSCs, with a focus on negative sensory characters deriving from hydrogen sulfide (H_2S), methanethiol (MeSH), ethanethiol (EtSH), dimethylsulfide (DMS), phenylmethanethiol (BnSH) and the generally positive varietal thiol sensory characteristics of 3-sulfanylhexan-1-ol (3-MH) and 3-sulfanylhexyl acetate (3-MHA). The project sought to understand the metabolic and chemical pathways that lead to formation of these thiol compounds and the chemical and environmental switches that lead to otherwise innocuous sulfur-based compounds being converted to those that have a significant sensory impact.

As part of the enabling technology required for this project, new advanced analytical methods were developed to enable characterisation of these very challenging and reactive LMWSCs. A new HPLC-

MS method for the analysis of thiols and disulfides in wine was developed and validated which quantifies a wider range of LMWSCs than previous methods. In addition, to support investigations of sulfur-containing amino acids as LMWSC precursors, an amino acid assay method was developed in collaboration with the AWRI Metabolomics group for cysteine (Cys), methionine (Met) and glutathione (GSH).

A very significant breakthrough from this project was the identification of precursors to several key LMWSCs. At the outset of this project, it was suspected that the presence of certain compounds such as Cys, GSH, Met, disulfides, and thioacetates such as methyl thioacetate (MeSAc) and ethyl thioacetate (EtSAc) were contributing factors in determining H_2S, MeSH, EtSH, and DMS concentrations in wines post-bottling. However, these hypotheses had yet to be tested in real wines. This study demonstrated that the direct desulfurisation of Cys, Met, or GSH did not pose an obvious risk of H_2S or MeSH formation in wine post-bottling. However, the presence of MeSAc, or disulfides such as DMDS, significantly increased the risk of MeSH formation, with up to a 20% MeSH yield and a 70% MeSH yield obtained from MeSAc and DMDS, respectively, as measured in wines over a 12-month storage period. Additionally, model studies do not support the theory that BnSH forms from benzaldehyde reacting with H_2S, leaving unanswered the question about how BnSH and the associated 'struck flint' character are formed. Based on these findings it is recommended that thioacetates and disulfides are monitored to assess potential risk of post-bottling LMWSC release, in addition to the 'total packaged oxygen' (TPO) concentration of wine.

It was shown that the addition of copper significantly influenced the evolution of LMWSCs, but the effects of copper differed depending on dissolved oxygen concentration post-bottling and with the residual copper concentration. As expected, at higher oxygen concentrations, some metals such as copper, significantly reduced the concentration of the thiols in the wine tested. During wine maturation, once the oxygen concentration decreased to non-detectable, the effect of copper was reversed, with the presence of copper now being associated with a significant increase in MeSH concentration, regardless of the presence or absence of other metals. Higher residual copper concentrations also resulted in significantly higher H_2S concentrations in wine, but interestingly, these differences did not become evident until 12 months post-bottling. This work highlights the copper concentration dependency of H_2S formation and the weakness of traditional benchtop trials used to determine copper additions, where only the immediate effects are assessed and not the long-term impacts in-bottle resulting from residual copper.

In addition, copper effects can be influenced by pH and SO_2 and this study has demonstrated that the post-bottling formation of H_2S, MeSH, DMS, and CS_2 is significantly impacted by both copper additions and wine pH. For some 'reductive' aroma compounds, the interaction between pH and copper treatment was an important factor in determining their final concentrations in Chardonnay and Shiraz wines post-bottling. The current results established that both wine pH and copper additions have significant impacts on H_2S, MeSH, and DMS concentrations in wines post-bottling. Specifically, less H_2S and MeSH were produced through copper-catalysed reactions in wines at a lower pH than in wines at a higher pH level. It should be noted also that different effects were observed in red wines and white wines, likely due to the differing nature of the wines' matrix components. Investigations also showed that the presence of SO_2 plays a fundamental role in the modulation of H_2S profiles through copper interactions in both red and white wine post-bottling.

One possibility for managing copper effects is to bind copper to chelating compounds that completely remove it from the wine matrix or chemically isolate it so that it can no longer participate in the formation of reductive characters. The results from this project suggest that differing metal chelation environments can be established using additives and that these can have a significant effect on H_2S generation. It is possible to optimise copper's beneficial effects through the timing of its addition and in terms of limiting residual copper concentrations it is more desirable to add copper at 0° Brix (during the final phase of active fermentation) rather than at the end (post-

active ferment) which is a common industry practise. This timing ensures that yeast and solids adsorb copper and limit the residual copper concentration in a final wine.

The project has shown that the formation of LMWSCs from their precursors in wine is influenced by the presence of not only copper, but also by other metal ions that naturally occur in wine when present in sufficiently high concentrations. These metals include manganese, aluminium, iron and zinc, and it is recommended that producers monitor these and keep metal concentrations as low as possible in wine, to minimise the post-bottling evolution of wine LMWSCs.

As part of this project the AWRI collaborated with a project led by Dr Andrew Clark at Charles Sturt University (CSU). The results from the collaboration demonstrate that major non-volatile matrix components (red wine tannin, white wine protein, white wine polysaccharide, red wine polyphenol, white wine polyphenol) had limited impact on the form of copper present in wine, most likely because the major form of copper in most wines is as copper(I) sulfide (Cu_2S), rather than as complexes with matrix components as suggested by previous studies.

In further research to understand the chemical forms of copper in wine, differences in particle size and concentration of copper-tartrate complexes suggested that the various types of copper-tartrate complexes produced at varying pH levels may affect the binding sites of copper that are available to either catalyse the formation of LMWSCs such as H_2S, or quench the thiols produced to form copper sulfide complexes. This may explain some of the pH-related effects observed in these experiments.

This project has also discovered significant differences among wine yeast in their ability to release both positive and negative LMWSCs. Small-scale fermentation experiments with a large number of yeast strains have shown the great diversity in the production of LMWSC by yeast. This analytical information, combined with genomic data on these yeast strains, has allowed the identification of several yeast markers associated with the formation of important LMWSCs, such as the ‘tropical’ thiols 3-MH and 4-MMP and H_2S, from their precursors. This information will help winemakers to make informed yeast strain choices to suit their wine style, and potentially reduce the formation of negative LMWSCs in a winery environment.

Background

Volatile sulfur compounds (LMWSCs) can contribute both positive and negative attributes to wines, and their control in a winery environment is an important avenue to increasing the value of wine by either increasing positive sensory attributes or decreasing those deemed to be negative. Their occurrence can be influenced by factors including yeast selection and fermentation conditions, the nature and quantity of precursor compounds; the availability or absence of oxygen at different points of the winemaking process; and availability and speciation of transition metal ions such as copper.

The project sought to develop an in depth understanding of:

- the role that compounds suggested as the main precursors to of the sensorially important LMWSCs, with focus on hydrogen sulfide (H_2S), methanethiol (MeSH), ethanethiol (EtSH), dimethylsulfide (DMS), phenylmethanethiol (BnSH), 3-sulfanylhexan-1-ol (3-MH) and 3-sulfanylhexyl acetate (3-MHA);
- the metabolic and chemical pathways that lead to their formation
- the chemical and environmental switches which lead to otherwise innocuous sulfur based compounds being converted to those that have a significant sensorial impact.

This framework of understanding is required to develop practical strategies in winemaking to manipulate and modulate LMWSCs during the winemaking process. A further aspect was to ensure that positive and negative compounds do not undergo any further unwanted changes in tank or bottle, once a wine has a desirable LMWSC profile.

Highlights

Precursors to LMWSCs identified

Key precursors to several key LMWSCs were identified. It was demonstrated that the direct desulfurisation of Cys, Met, or GSH did not pose a risk of H_2S or MeSH formation in wines post-bottling. The presence of Cys and GSH was associated with smaller increases in H_2S concentrations compared to the increases in H_2S concentration seen with the presence of copper (acting on yet to be identified precursors) in Shiraz wines. For MeSH and EtSH, important precursor compounds were their corresponding disulfides and thioacetates, such as dimethyldisulfide (DMDS), methylthioacetate (MeSAc), and ethylthioacetate (EtSAc). These precursor compounds produced large concentrations of MeSH and EtSH, respectively, of between 30% to 70% yields of the thiols as measured in wines over a twelve-month storage period. External factors such as copper addition to wine, and the pH of the wine were important factors that determined 'reductive' aroma accumulation in wines post-bottling.

The effect of copper differs depending on dissolved oxygen concentration post-bottling and residual copper concentration

The addition of copper was found to significantly influence the evolution of LMWSCs. Oxygen concentration played a significant role in copper's effect on the formation of LMWSCs in wine. Initially, at high oxygen concentrations, the presence of some metals such as copper, significantly reduced the concentration of the thiols in the wine tested. During wine maturation, the oxygen concentration decreased to 0 ppb after four months of anaerobic storage and the effect of copper was reversed, with the presence of copper now being associated with a significant increase in MeSH concentration, regardless of the presence or absence of other metals.

Managing copper effects

It is possible to manage copper through the timing of its addition. To limit residual copper concentrations in wine it is more desirable to add copper at 0°Brix (during the final phase of active fermentation) rather than at the end (post-active ferment) which is a common industry practice. This ensures the yeast and solids adsorb copper and limit the residual copper in the final wine.

Impacts of metals on reduced aroma formation

The formation of LMWSCs from their precursors in wine was found to be influenced by the presence of not only copper, but also other metal ions that naturally occur in wine, when they are present in high concentrations. Winemakers looking to reduce the risk of aromas from LMWSCs can consider taking steps to minimise metal concentrations in wine.

Understanding differences among wine yeast strains in their ability to release volatile sulfur compounds (LMWSCs)

New understanding has been gained of the differences among wine yeast in their ability to release both positive and negative LMWSC. Small-scale fermentation experiments with a large number of yeast strains showed the great diversity in the production of LMWSCs by yeast. This analytical information, combined with genomic data on these yeast strains, has allowed the identification of several yeast markers that modulate the formation of important LMWSCs, such as the 'tropical' thiols 3-MH and 4-MMP and H_2S, from their precursors.

Objectives

The core objective of the project is to develop successful strategies to manipulate and modulate key LMWSCs (i.e. H_2S, MeSH, EtSH, DMS, BnSH, 3-MH and 3-MHA) in a commercially practical winemaking context. This was achieved through the investigation of the core precursors of the sensorially important LMWSCs; the metabolic and chemical pathways that lead to their formation and the chemical and environmental switches which lead to their sensorial expression or repression.

Specifically, this involved the development of an understanding of:

- the origin of LMWSCs (H_2S, MeSH, DMS, BnSH, 3-MH and 3-MHA)
- the role of transition metal ions, in particular copper, in the release of LMWSCs via their interactions with other wine components
- the impact of the quantity and timing of copper and sulfur dioxide (SO_2) additions when producing wine to ensure the optimum sensorial outcome and eliminate the development of negative LMWSCs both in tank and in bottle
- the influence of yeast strain on LMWSC formation (in collaboration with AWRI Projects 3.2.2 and 3.2.3), by observation of levels produced in model fermentations, and through qualitative assays of key enzymatic activities;
- which yeast genes encode enzymes that mediate formation of H_2S from Cys (Cys sulfhydrylase or desulfhydrase), 3-MH from Cys and GSH conjugates, and MeSH from Met (Met γ-lyase or methioninase); and how yeast genetic determinants and/or fermentation conditions regulate the accumulation of S-reserve compounds (Cys, GSH) and LMWSCs, which may be released via autolysis into the wine once fermentation is finished, and contribute to the subsequent chemical formation of LMWSCs.

Methods

Gas chromatography (GC) coupled to sulfur chemiluminescence detection was used to analyse low molecular weight sulfur compounds (LMWSC). Wines were analysed for their LMWSC profiles using an Agilent 355 SCD sulfur chemiluminescence detector coupled to an Agilent 6890A gas chromatograph as described by Siebert et al. (2010). The varietal thiol 3-MH was quantified as the pentafluorobenzyl derivative using a stable isotope dilution assay, by means of solid phase microextraction (SPME) coupled with GC-MS as described by Ugliano et al. (2011).

Wines and stock solutions were analysed for their metal concentrations by Flinders Analytical, Flinders University (Adelaide, Australia) using an Agilent 7500 ICP-MS (Agilent Technologies, Tokyo, Japan) as described in Thiel et al. (2004).

The amino acid concentrations of the wines were determined by the AWRI SA Metabolomics Facility using the method described by Ovalles et al. (2005) that was adapted for wine analysis.

The LMWSCs present in the liquid phase of the wines were analysed using the method described by Seiwert and Karst (2007) that was adapted for wine analysis.

The reaction between benzaldehyde and BnSH was monitored using high performance liquid chromatography (HPLC) performed on an Agilent 1100 liquid chromatograph (LC) with diode array detection (DAD) (Agilent, Australia) using a Phenomenex Synergi C18 Hydro column (4 µm particle size, 80Å, 150 mm, 2.1 mm ID) (Lane Cove, NSW, Australia). The mobile phase was 50% acetonitrile in MilliQ water and the flow rate was 0.25mL/min.

Results and discussion

The origin of LMWSCs (H_2S, MeSH, DMS, BnSH, 3-MH and 3-MHA)

As part of this project, a comprehensive review of the sources of volatile sulfur compounds in wine was compiled and published (Smith et al. 2015).

New methods developed to enable monitoring of sulfur compounds

A new HPLC-MS method for the analysis of thiols and disulfides in wine was developed and validated which quantifies a wider range of LMWSCs than previous methods. The new method also allows for a comparison between the LMWSCs present in the headspace of wine and the LMWSCs present in the liquid phase to gain better insight into the fate of LMWSCs in wine and to possibly predict the way a wine will age in bottle. To assess a potential role of sulfur-containing amino acids as LMWSC precursors, an amino acid assay was developed with the SA Metabolomics Facility and samples are now being assessed to quantify the sulfur-containing amino acids in wine, Cys and Met and the tri-peptide, GSH. The new methods were applied to determine the formation and fate of H_2S and other LMWSCs throughout the winemaking process and during wine bottle storage.

What are the main precursors of the sensorially important LMWSCs?

At the outset of this project, it was suspected that the presence of certain compounds such as Cys, GSH, Met, disulfides, and thioacetates such as MeSAc and EtSAc were contributing factors in determining H_2S, MeSH, EtSH, and DMS concentrations in wines post-bottling. However, these hypotheses had not been tested in real wines.

Experiments were performed to determine the ability of sulfur containing amino-acids such as Cys, Met, and the tripeptide GSH; and other LMWSCs to act as precursors to H_2S and MeSH in real wines (Figure 1) (Bekker et al. 2017). Cysteine and GSH did not act as sensorially relevant precursors to H_2S. Their presence was associated with small increases of H_2S concentrations, but this could also be an indirect effect where the reducing characteristics of Cys and GSH promoted an environment that favoured H_2S formation and not necessarily a direct desulfurisation of the thiol moiety of Cys or GSH. In these experiments, the presence of copper remained the main factor that contributed to significant increases in H_2S concentrations from yet to be identified precursor compounds. Cysteine and GSH were associated with lower H_2S formation from copper-catalysed reactions in Shiraz wines. Cysteine and GSH may inhibit H_2S formation from copper-catalysed reactions; or possibly form mixed disulfides or polysulfanes through the reaction of Cys/GSH with H_2S.

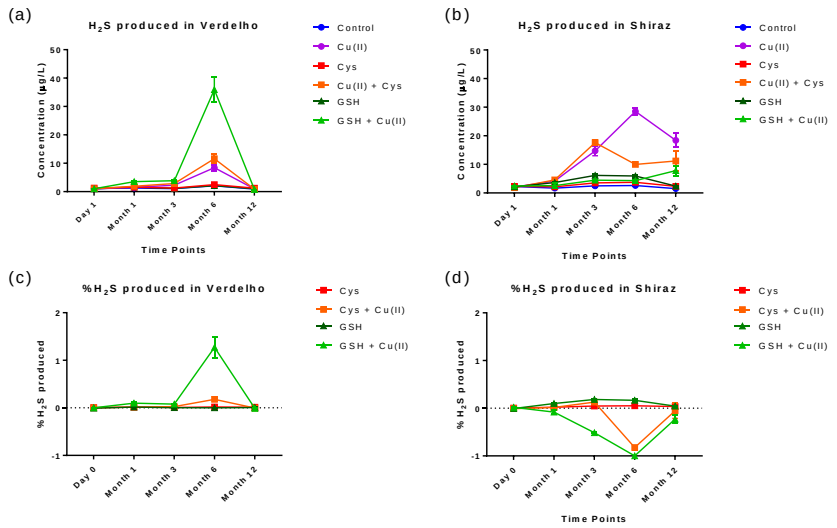


Figure 1. Hydrogen sulfide (H_2S) produced from cysteine (Cys) and glutathione (GSH), with and without the addition of copper, in Verdelho and Shiraz wines.

Notably, DMDS and MeSAc did contribute significantly to MeSH concentrations in the wines post-bottling on a sensorially relevant scale (Figure 2). In all cases, the presence of copper had significant effects on the ability of the precursors to produce H_2S and MeSH, as well as the rate of H_2S and MeSH formation. Wine pH also significantly affected MeSH formation, with higher release of MeSH at lower pH, most likely due to increased rates of acid catalysis (discussed subsequently).

The accumulation of LMWSCs in white wines and red wines were also different, with significantly less H_2S, MeSH, and EtSH accumulating in red wines that in white wines. This suggests that these thiols may have interacted with wine compounds that inhibited their accumulation in the red wines. However, the risk remains that under changing wine conditions any thiols incorporated into other molecular forms may again be released.

This study demonstrated that Cys, Met, or GSH did not pose a risk of H_2S or MeSH formation from direct desulfurisation in wines post-bottling. However, the presence of MeSAc, or a disulfide such as DMDS, posed a significant risk of MeSH formation, with up to a 20% MeSH yield and a 70% MeSH yield obtained from MeSAc and DMDS, respectively, as measured in wines over a twelve-month storage period.

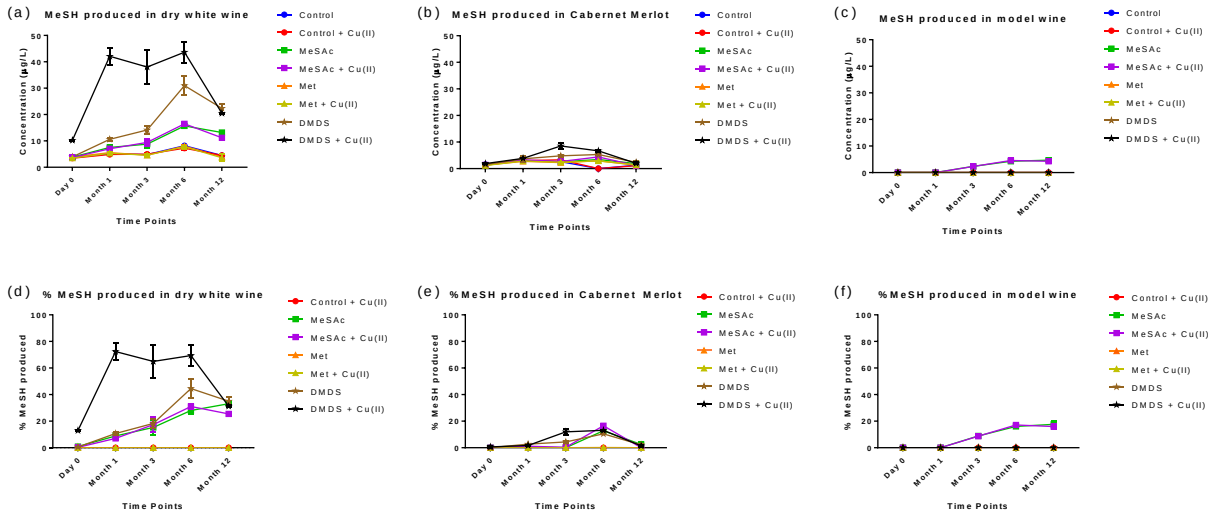


Figure 2. Methanethiol (MeSH) produced from methylthioacetate (MeSAc), methionine (Met), and dimethyldisulfide (DMDS), with and without the addition of copper, in dry white wine, Cabernet Merlot, and model wines.

The reactivity of benzaldehyde with H_2S was studied in model wine systems over a nine-month period. Benzaldehyde and H_2S were added to model wines, with benzaldehyde at a concentration of 9.42 µM and the H_2S concentration varied (9.42 µM, 94.2 µM, 0.942 µM) to give molar ratios of 1:1, 1:10, and 10:1 of benzaldehyde to H_2S. Model wines were either treated with 0.5 mg/L of copper and 4.0 mg/L of iron, or treated with no metals. Samples were duplicated with one set prepared with the total exclusion of oxygen and the second set prepared in an environment rich in oxygen. After nine months of storage no BnSH could be detected using HPLC analysis, or by LCMS analysis with a detection limit of 0.040 µM for BnSH. As such, these studies do not support the theory that BnSH forms from benzaldehyde reacting with H_2S.

Management of LMWSCs can be effectively performed using oxygen during fermentation

The final report chapter on Project 3.3.2 outlines in detail the effects on LMWSCs of using oxygen during fermentation (Bekker et al. 2016c). In summary, conditional on the dose and timing, oxygen can be very effective at limiting the accumulation of LMWSCs during fermentation and for significant periods of storage time after treatment.

The role of transition metal ions, in particular copper, in the release of LMWSCs via their interactions with other wine components

A series of experiments aimed to generate detailed information about how 'reductive' sulfur aromas form post-bottling, and the potential catalytic role of copper and other metals in such reactions. There are many possible precursors to LMWSCs in wine, making it important to not only understand the chemical nature of precursor sources but also the mechanisms or chemical switches that are involved in the release of LMWSCs from their various precursor compounds. Investigating the role of metal ions as catalysts, as well as the synergistic effects of the metals during their catalytic action in the formation of LMWSCs, is crucial to gain a better understanding of the chemical processes governing the formation of post-bottling 'reductive' aromas.

As part of the current management strategy to deal with 'reductive' aromas, copper is often added to wine prior to bottling to treat unpleasant 'sulfidic' aromas such as 'rotten egg'. It is thought that the copper ions react with H_2S to produce an insoluble solid (copper sulfide), which results in the removal of both copper and H_2S from the wine. However, previous work at the AWRI (Ugliano et al. 2011) established that copper additions at bottling can promote the accumulation of H_2S at later stages of bottle ageing.

It was found that the addition of metals to wine significantly influenced the evolution of LMWSCs. Oxygen concentration also played a significant role in the effect of the metals on LMWSC formation. Initially, at high oxygen concentrations, some metals such as copper, significantly reduced the concentration of thiols in the wine tested. During wine maturation, the oxygen concentration decreased to 0 ppb after four months of anaerobic storage and the effect of copper was reversed with the presence of copper now being associated with a significant increase in MeSH concentration, regardless of the presence or absence of other metals (Viviers et al. 2013).

A series of boxplots in Figure 3 describe the evolution of MeSH in Shiraz samples (n = 96) with or without added copper. The boxplots graphically display differences among MeSH concentration in the samples; the median (white line); the mean (star) with the red area depicting the 95% confidence interval for the mean; as well as outliers (black dots). At Day 1 (a) no MeSH was present in samples with or without added copper, but, after one month of storage, the scavenging ability of copper can be observed in the significantly decreased MeSH concentration in all samples with added copper (Figure 3b). However, as the oxygen concentration in the wine decreased, the MeSH concentration slowly increased to nearly the same levels in both samples with or without added copper (Month 4, Figure 3c). After 6 to 12 months of anaerobic storage, the MeSH concentration had significantly increased in all samples with added copper and reached concentrations above MeSH's odour threshold value of 1.8 µg/L (Siebert et al. 2010) (Figure 3d and 3e). This shows that the formation of MeSH is not only influenced by the presence of metals, but that the oxygen concentration in wine also significantly affects the evolution these compounds.

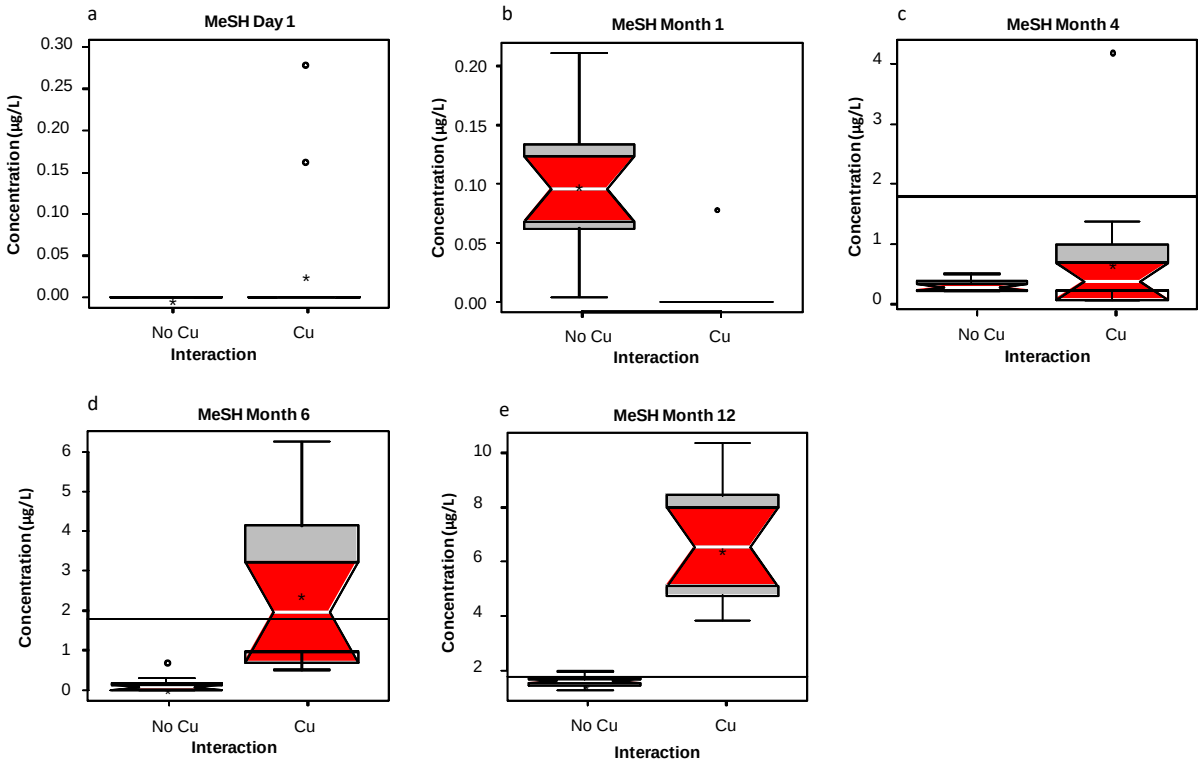


Figure 3. Notched boxplots of the MeSH concentration (µg/L) in Shiraz wine samples during storage show initially a significant decrease (b) and later significant increases (d) and (e) due to copper addition. The line parallel to the x-axis in (c), (d) and (e) indicates the odour threshold value for MeSH at 1.8 µg/L.

Residual copper concentrations are influenced by timing of copper additions and affect reduced aroma formation.

Another study investigated the dose effect of copper on Chardonnay wine LMWSCs. The combination of an early (post-ferment, on solids) treatment and a late (pre-bottling) treatment

resulted in higher residual copper concentration (0.55 mg/L residual copper) compared with just an early treatment (0.17 mg/L residual copper). These higher residual copper concentrations resulted in significantly higher H_2S concentrations in the wine, but interestingly, these differences did not become evident until the 12-month time point, with earlier time points showing essentially equivalent results for both treatments (Figure 4). This work highlights the copper concentration dependency of H_2S formation and the weakness of traditional benchtop trials used to determine copper additions, where only the immediate effects are assessed and not the long-term impacts in-bottle resulting from residual copper.

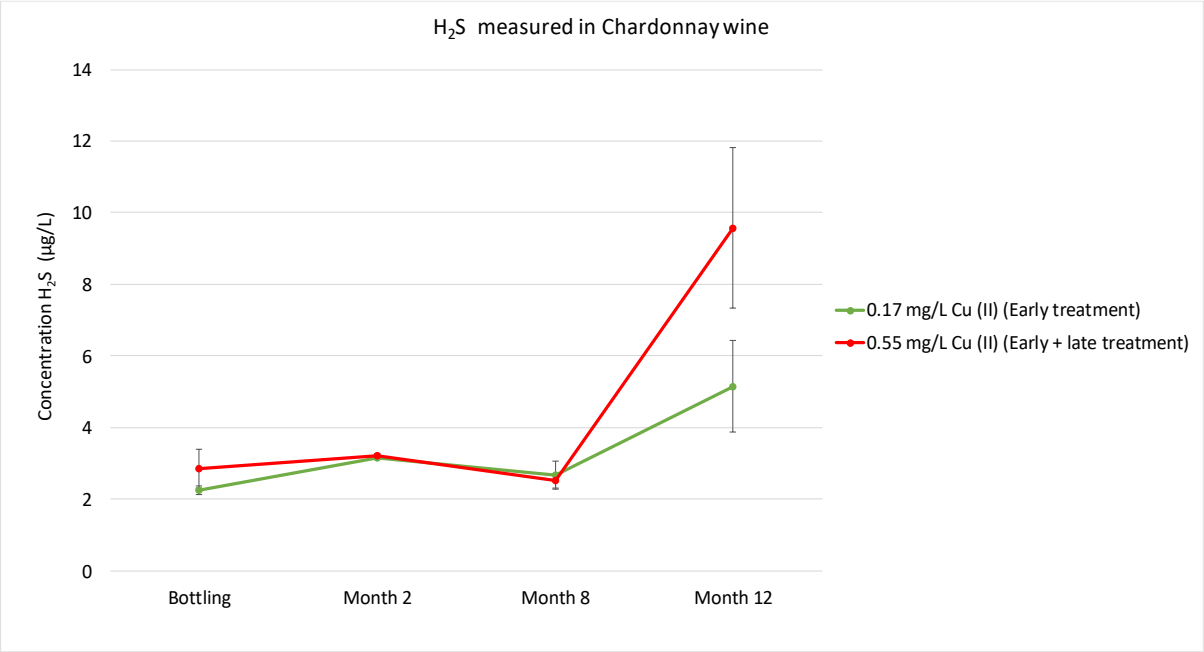


Figure 4. The effects of timing of copper addition on the evolution of hydrogen sulfide (H_2S) as measured over 12 months post-bottling.

To more deeply investigate the effects of a wider range of metals on the formation of the 'reduced' aroma compounds MeSH, H_2S and DMS during bottle ageing, a large experiment was designed in which five metals (copper, iron, manganese, zinc, aluminium) were added to Chardonnay and Shiraz wine samples in all possible combinations (resulting in 31 treatments and 1 control for each wine). The metals were present at either their native level in the base wine (the base wines selected exhibited relatively low metal levels), or a high level spiked to approximately ten times that native concentration. The concentrations of LMWSCs were analysed over a 12-month period. At bottling, the wines contained oxygen at around the recommended level of 1 mg/L (Chardonnay 1.11 ± 0.34 mg/L; Shiraz 1.43 ± 0.35 mg/L) and after four months of anaerobic storage the dissolved oxygen (DO) of both Chardonnay and Shiraz samples decreased to undetectable levels.

Significant changes in volatile sulfur compounds were observed over the 12-month period, with the Chardonnay samples showing increases in H_2S and DMS, and the Shiraz samples showing increases in H_2S and MeSH (Figure).

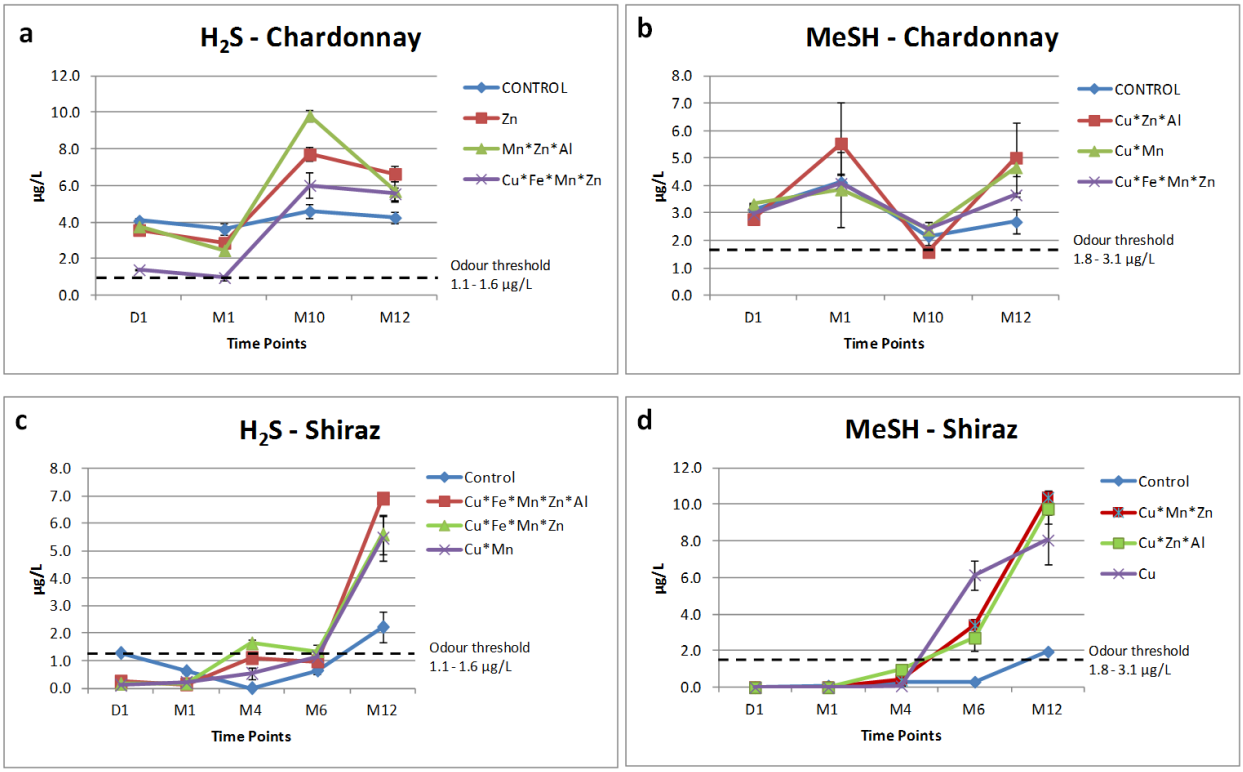


Figure 5. The impact of metals and metal combinations on the concentrations of H_2S and MeSH in Chardonnay and Shiraz wines over time

The most remarkable results of this current study, however, were the effects observed due to additions of metals (i.e. manganese, zinc, and aluminium) that have not previously been considered in the context of wine LMWSCs, as well as the interactions between the five metals. Not all metals and metal combinations had significant effects on the formation of H_2S throughout the experiment, and in some instances the metals were only associated with significant effects at one analysis time point. To distinguish between the different significant metal effects, multivariate statistical methods were used. For example, the Chardonnay samples that displayed the largest decrease in H_2S concentration at Day 1 and Month 1 were all samples that were treated with copper, and it seemed that copper was the only metal associated with significant decreasing effects. But by making use of multivariate statistical analysis it was possible to distinguish between the significant effect of copper on the evolution of H_2S at Day 1, as well as the significant effects of copper*iron, that was also associated with significant decreasing effects on H_2S concentrations at Day 1 in the Chardonnay samples. This reaction between thiols and metals is routinely used in copper fining trials to reduce the impact of unwanted thiols in wines (Ugliano et al. 2009). If both the Chardonnay and Shiraz samples are considered, only 7 of the 31 metal treatments significantly affected the evolution of H_2S in both wines, and they were copper, iron, zinc, aluminium, copper*iron, copper*manganese*aluminium, and copper*zinc*aluminium. Three of the metal treatments, zinc, manganese*zinc*aluminium, and copper*iron*manganese*zinc were associated with the largest increases in H_2S concentrations in the Chardonnay samples at Month 10. Using multivariate statistical analyses it could be concluded that the increases in H_2S concentrations are due to the significant effect of either zinc, aluminium, zinc*aluminium, or manganese*zinc*aluminium in these metal combinations.

There were more metals and metal combinations that were associated with significant effects in the Shiraz samples than in the Chardonnay samples, with four metal treatments associated with significant effects in the Chardonnay samples, compared to the nine metal treatments that were associated with significant effects on MeSH concentration in the Shiraz samples. This could be due to higher concentrations of polyphenols and anthocyanins present in the red wine samples which are

likely involved in the redox cycling of the metal ions. Three examples of metal treatments associated with some of the largest increases in MeSH concentration in the Shiraz samples are shown in Figure 5d. The increased MeSH concentration in samples with added copper*manganese*zinc, copper*zinc*aluminium, and copper were driven by the significant effect of copper, and not due to the other metals (Figure 5d).

Overall, fewer metals produced significant effects on DMS evolution, and the metal treatments were mostly associated with an overall decrease in DMS concentration. The only metal treatments associated with significant effects on DMS concentration in both Chardonnay and Shiraz samples were aluminium and zinc*aluminium. The decreasing effects of the metals could possibly be due to metals inhibiting the formation of DMS from its precursor molecules already present in the wine, or due to the catalytic degradation of DMS.

This study showed that metals can act singly or in combination to greatly influence evolution of undesirable 'reduced' aromas. Winemakers that wish to lower the risk of such characters should therefore take steps to minimise metal concentrations in wine. From these results, it is clear that the formation of LMWSCs from their precursors in wine is not only influenced by the presence of metals, but oxygen concentration in wine also significantly interacts with metals in the evolution of LMWSCs.

The impacts on LMWSC formation due to interactions between copper and pH

Many factors affect the formation of LMWSCs in wines post-bottling, including early oxygen treatment during fermentation (Bekker et al. 2016), the presence of the precursor compounds in wine, elevated residual copper concentrations post-bottling, and anaerobic storage conditions (Ugliano et al. 2011, Viviers et al. 2013). Wine pH also has the potential to influence the chemical reactions that are related to the formation or degradation and loss of flavour compounds. This can be through its effects on precursor compounds or through its effects on catalytic compounds that facilitate the release of LMWSCs from their precursor compounds or through loss mechanisms. Although the role of copper in the formation of H_2S and MeSH has recently been established (Ugliano et al. 2011, Viviers et al. 2013), the ability of other ubiquitous winemaking variables, including pH, to modulate the formation of LMWSCs had not yet been studied.

As part of this project, the effects of wine pH, and the effects of interaction between pH and copper, on the LMWSCs associated with 'reductive' aroma formation in wines post-bottling were assessed (Bekker et al. 2016d). A Chardonnay and a Shiraz wine were selected to determine whether wine pH had significant effects on the formation of 'reductive' aroma formation post-bottling. Each wine was divided into two portions. The pH of one portion of wine was not adjusted (Chardonnay pH 3.46, Shiraz pH 3.72) and the pH of the second portion of the wine was adjusted using tartaric acid to pH 3.0 for both the Chardonnay and the Shiraz wines. To investigate the interaction between residual copper and wine pH, all wines were also either treated with copper to give a final concentration of 0.5 mg/L residual copper or received no added copper (0 mg/L residual copper).

The LMWSC profiles of all wines were monitored of the course of six-months post-treatment using a gas-chromatograph coupled to a sulfur chemiluminescence detector as described by Siebert et al. 2010. All experiments were conducted in an oxygen-free environment and all wines were also stored in an oxygen-free environment (Bekker et al. 2016).

The effects of wine pH on H_2S and MeSH formation in Chardonnay and Shiraz wines were only observed in samples that were also treated with copper. In samples without added copper, pH had no effect on the amount of H_2S and MeSH produced in the Chardonnay or Shiraz wines post-bottling (Bekker et al. 2016a).

In samples with added copper, however, significantly less H_2S was produced in Chardonnay wines when the pH levels of the wines were adjusted to pH 3.00 relative to Chardonnay samples with added copper at the unadjusted pH level of pH 3.46 (Figure 6a). On average 51% less H_2S was

produced in Chardonnay wines with added copper at pH 3.00, which shows that wine pH significantly impacts H_2S formation caused by elevated residual copper concentrations. The effects of pH and copper interactions on H_2S formation in Shiraz wines were not as pronounced as in the Chardonnay wines, with significant decreasing effects of lower pH levels only measured directly after treatment and again after a month post-treatment.

Methanethiol was not as affected by lower pH conditions. Lower pH was only associated with significant effects on MeSH concentration at Day 0 in the Chardonnay samples. Similarly, decreasing the pH of copper-treated Shiraz samples produced significantly less MeSH after one month of storage, and this effect was also measured three months post-treatment (Figure 6d). It is not clear why the interaction between pH and copper significantly impacted MeSH formation only at certain stages of the experiment. It could be that the lower pH is affecting the reaction rate of the formation of MeSH. The differences may be a reflection of the time needed for the wines to reach the same end-point MeSH concentration.

Dimethyl sulfide was significantly affected by the pH of the wines, with lower concentrations of DMS measured in both Chardonnay and Shiraz samples throughout the course of the six-month experiment in wines with a lower pH (Figure 6e, 6f). Six months' post-treatment there was 27% less DMS measured in Shiraz wines with a pH of 3.00 compared to Shiraz wines with a pH of 3.72. The effect of pH on DMS formation in wines post-bottling is remarkable given that DMS is a stable molecule that remains unaffected by oxygen treatment during fermentation (Bekker et al. 2016) and is unaffected by metals such as copper, iron, or manganese (Viviers et al. 2013). The decreased DMS measured in this study is most likely due to the precursor compounds of DMS becoming less prone to release DMS at a lower pH level. It is known that one of the main precursors to DMS (Segurel et al. 2005), namely S-methyl methionine (SMM), is stable in acid conditions but it rapidly decomposes at pH greater than 7.00 (Cantoni 1960). The decrease in pH from 3.72 to 3.00 most likely prevented the formation of DMS from SMM by stabilising SMM.

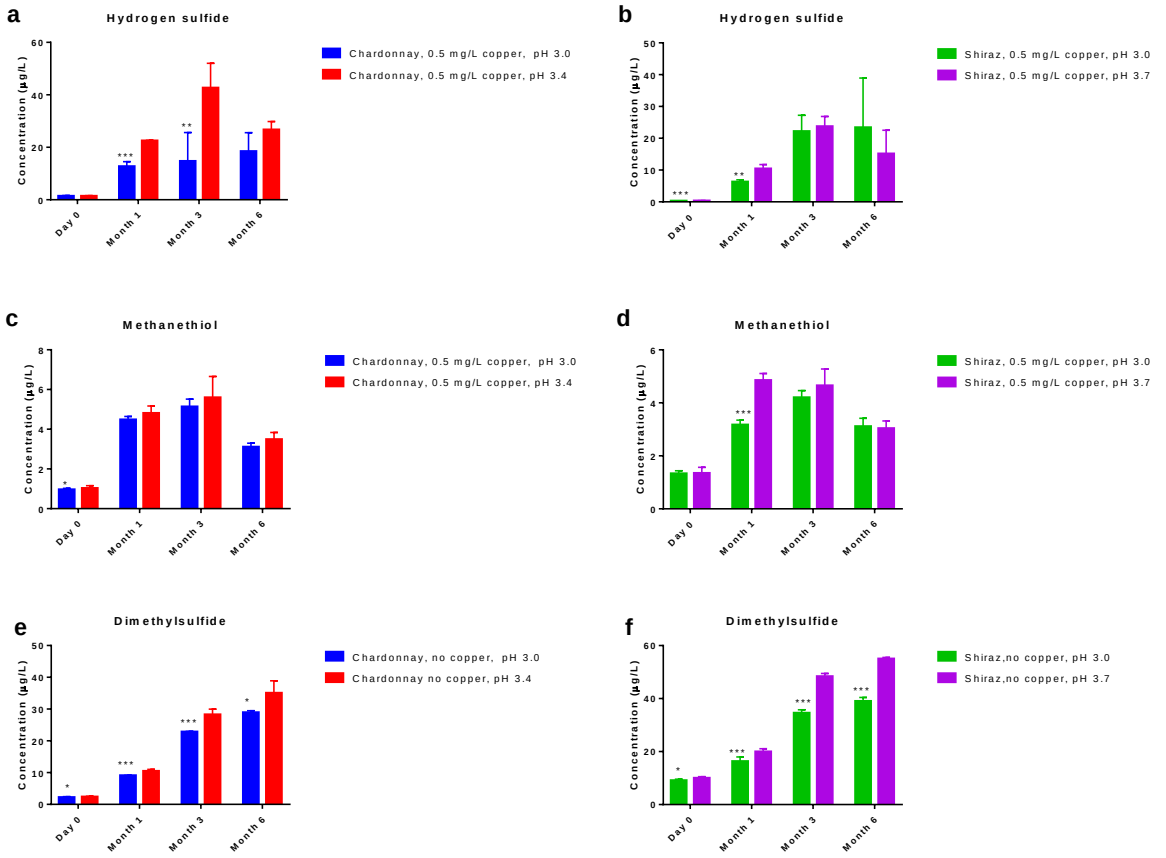


Figure 6. The effects of pH, and the interaction between pH and copper, on the formation of hydrogen sulfide [(a), (b)], methanethiol [(c), (d)], and dimethylsulfide [(e), (f)] in Chardonnay and Shiraz wines measured over the course of six months post-treatment. Significance of pH effect: P-value ≤ 0 (***), P-value ≤ 0.001 (**); P-value ≤ 0.01 (*).

This study demonstrated that the post-bottling formations of H_2S, MeSH, DMS, and CS_2 were significantly affected by both copper additions and wine pH. For some 'reductive' aroma compounds, the interaction between pH and copper treatment was an important factor in determining their final concentrations in the Chardonnay and Shiraz wines post-bottling. It is known that pH levels affect the rate of chemical reactions, and this will in turn impact the final concentration of LMWSCs present in wines. Winemakers have some flexibility in managing wine pH levels as well as the option to add copper to their wines through copper fining treatments. The current results have demonstrated that both wine pH and copper additions have significant impacts on H_2S, MeSH and DMS concentrations in wines post-bottling. In this study, less H_2S and MeSH were produced through copper-catalysed reactions in wines at a lower pH than in wines at a higher pH level (Bekker et al. 2016).

Use of chelating agents to isolate copper from the LMWSC formation process

While the risks of residual copper in packaged wine are becoming increasingly apparent, copper is still an important tool for winemakers dealing with volatile sulfur characters. For this reason it is important to identify ways to manage residual copper after it has been added to wine. One possibility is to bind copper to chelating compounds that completely remove it from the wine matrix or chemically isolate it so that it can no longer participate in the formation of reductive characters. To investigate this, a range of possible chelating agents were trialled in wine. A commercial polyphenol extract was found to suppress H_2S formation at the 12-month time point in comparison to controls (Figure 7). As seen in the trial on the timing of copper additions, this suppression did not

become evident until the 12-month time point, suggesting an interesting interaction of polyphenols, metals and other wine components occurring over time in bottle. Ethylenediaminetetraacetic acid (EDTA), a common chelation agent used in food production, also demonstrated significant effects both at equimolar concentrations (to copper and iron) and when present in excess. The results suggest that differing metal chelation environments can be present and that these can have a significant effect on the mechanism of H_2S generation.

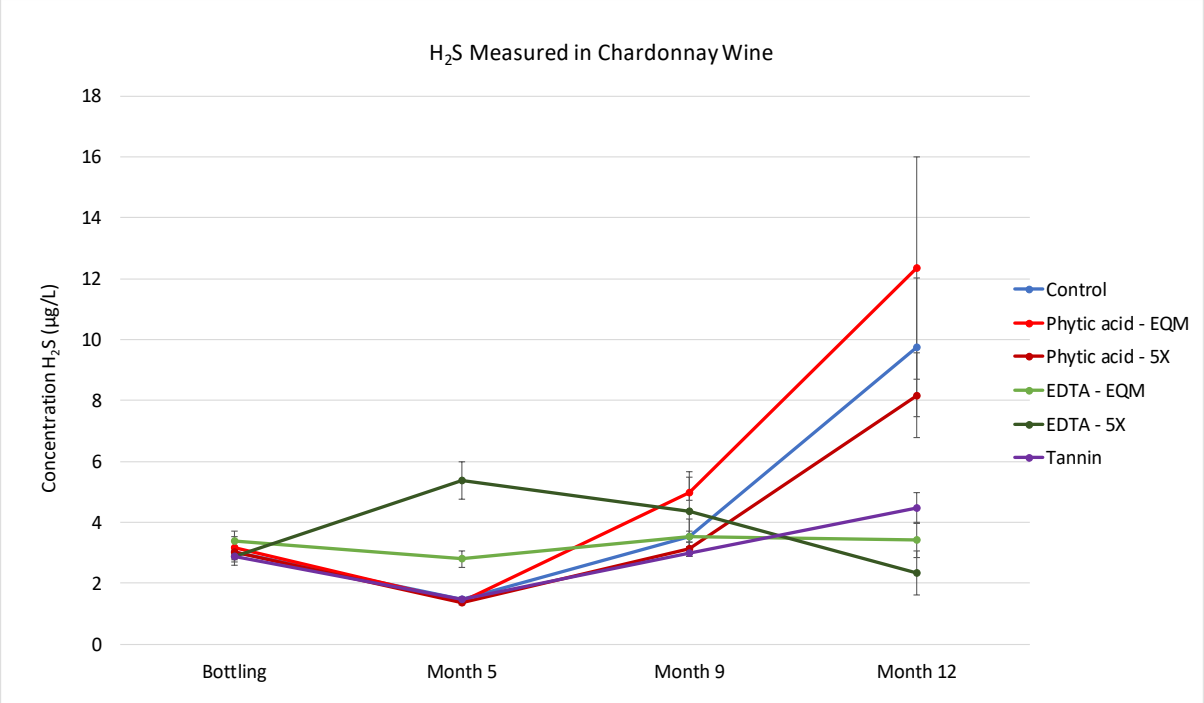


Figure 7. Effect of copper chelating agents on LMWSC accumulation over 12 months in a Chardonnay wine.

Managing copper addition(s)

Research was also performed on copper additions during ferments and the effects on the resulting wines (Reschke et al. 2015). The main purpose of this study was to determine what copper concentrations could be added at what stage of fermentation to minimise negative effects of sulfides on the resulting wines while limiting the residual copper in the final wine.

During laboratory-scale fermentation, copper was added at concentrations between 0 and 50 mg/L with the additions made at three time points: at the beginning of fermentation, at 0° Brix and after the fermentation was finished. The released hydrogen sulfide, residual copper concentrations, formation of volatile sulfur compounds, aroma profile and colour were determined where appropriate during, or at the end of fermentation. The results indicated that lower copper additions have similar impacts on wines as higher additions and that in terms of limiting residual copper concentrations it is more desirable to add copper at 0° Brix (during the final phase of active fermentation) rather than at the end (post-active ferment) which is a common industry practice. This ensures the yeast and solids are available to adsorb copper and limit the residual copper in the final wine. It should be noted that copper additions earlier in the fermentation process were also shown to pose greater disadvantages than the addition at 0° Brix. By adding copper at 0° Brix positive wine aromas can form in the log phase of culture growth and any reduced characters formed can be removed at this stage as well. Copper concentrations up to 5 mg/L can be added without significant

residual copper concentrations remaining in the wines and volatile sulfur containing compounds can be reduced by these levels of copper additions. Although the addition of copper has many advantages, the aromas of copper-treated wines can be affected negatively at the copper addition rates used in this study. All copper-treated wines reviewed in the informal sensory study showed a detectable aroma of 'nail polish remover'. Further study is needed to investigate the sources of the nail polish remover aroma.

Understanding the forms of copper in wine

It has been commonly thought that when sulfides in wine interact with copper, they simply precipitate out as copper sulfide and are removed from the wine through racking and filtration. Recent work by collaborators at Charles Sturt University (CSU) has shown, however, that this is not necessarily the case (Clark et al. 2015); in fact, copper sulfide remains in the wine. This finding, along with increased understanding of the risks of residual copper species in wine, has made it important to gain a more thorough understanding of the chemical nature and role of copper sulfides formed in wine.

Many wine compounds have the capacity to bind copper, including organic acids (such as tartaric acid), tannins, polyphenols, and LMWSCs. The binding of copper to these various compounds may affect the particle size of the copper-complexes, which in turn may affect the binding sites of copper that are available to catalyse the formation of LMWSCs. To investigate this, the impacts of different ratios of H_2S and copper in model wine were studied using nanoparticle tracking analysis (NTA). NTA affords the opportunity to monitor very small particles and their behaviours in liquids and was used to study the effects of variable pH concentrations in a model system containing copper, tartaric acid, and H_2S. (Bekker et al. 2016).

A decrease in the mean particle diameter was seen as the ratio of H_2S to copper increased. Furthermore, the copper-tartrate complex concentration increased with H_2S addition. At equimolar concentrations of H_2S and copper the particle size was smaller than in the other treatments, suggesting strong and uniform binding of H_2S and copper that prevented copper from further interacting with other compounds to produce larger particles. As the ratio of copper was increased in relation to H_2S, the particle size increased, suggesting that the unbound copper was available to interact with other compounds (in this case tartaric acid) to form larger particles. Further studies are needed to determine whether the smaller particle size is associated with lower catalytic ability, perhaps due to the binding sites of copper being occupied and prevented from further interaction with other wine compounds.

The impact of pH on complex formation was also investigated. Lower pH decreased copper-tartrate complex size and lower particle concentrations were measured when compared to copper-tartrate complexes produced at higher pH. The difference in particle size and concentration of copper-tartrate complexes suggests that the types of copper-tartrate complexes that are produced at varying pH levels may affect the binding sites of copper that are available to either catalyse the formation of LMWSCs such as H_2S, or quench the thiols produced to form copper sulfide complexes. As discussed previously, research in this project has also shown that wine pH, copper additions and the interaction between pH and copper significantly influence LMWSC formation in Chardonnay and Shiraz wines post-bottling.

A collaborative project led by CSU with the AWRI also investigated the speciation of the copper, by investigating a range of 52 commercially available wines. Using anodic stripping voltammetry Dr Andrew Clark and co-workers at CSU showed that only below very low levels (25 µg/L) of 'electrochemically labile' copper (able to react with H_2S) could free H_2S be liberated from the wine. While confirming the ability of copper to bind H_2S, the study also showed that in the vast majority of the wines tested, the copper was present in non-labile forms and was not available to inhibit the formation of free H_2S by reacting with it. This correlates with earlier findings that residual copper in

wine rarely serves to inhibit the formation of volatile sulfur compounds and in many cases appears to facilitate it.

To understand what the possible forms (chemical speciation) of the non-labile copper were, the collaboration between CSU and the AWRI focused on whether copper was bound to some of the major matrix compounds found in wine. The components of wine that can interact with Cu(II), Fe(II) and Fe(III) are numerous and vary widely in general type. They can consist of the wine macromolecules, including proteins, polysaccharides, tannins and their aggregate combinations, as well as monomeric species, including organic acids, phenolic compounds, and sulfur-containing compounds. Although many studies have been conducted on the complexation of metal ions to these components in non-wine-like conditions (higher pH, ethanol-free or without organic acids), the specific conditions of wine are likely to be critical in indicating the dominant binders of metals. This study was conducted to identify which wine components influence the measurement of copper and iron in their fractionated/electrochemical forms and to enable an improved understanding for the use of such measurements in following metal activity in wine.

The wine components provided by the AWRI that were assessed by CSU included those extracted from wine (red wine tannin, white wine protein, white wine polysaccharide, red wine polyphenol, white wine polyphenol that had been isolated and characterised at the AWRI), and commercially available monomeric compounds, including phenolic compounds and sulfur-containing compounds such as H_2S. The wine components to be assessed were added at typical wine-like concentrations: control (no further addition), white wine protein (50 mg/L), white wine polysaccharide (200 mg/L), white wine polyphenol (200 mg/L), red wine polyphenol (2000 mg/L), red wine tannin (1000 mg/L) and H_2S (two-fold mole excess over metals ions, 2:1). Further experimental details can be found in Kontoudakis et al. (2016).

It was evident that only the addition of H_2S to the model wine system afforded a significant change in the classification of copper by the electrochemical technique (by forming non-labile CuS). The formation of Cu(I) sulfide (Kreitman et al. 2016) in the model wine system is responsible for the non-labile copper, some of which would be removed as particulate Cu(I) sulfide by the filtration step prior to electrochemical analysis of the sample (Clark et al. 2015). Part of the non-labile copper is also suspended Cu(I) sulfide particles that are not detected by the electrochemical sensor (Clark et al. 2016). For all other model macromolecules tested with copper, the predominant form of copper was labile copper, identical to the classification of copper in the model wine without any binding component added, that is, with copper present as Cu(II) tartrate and likely to be available for electrochemical reactions that may lead to changes in LMWSCs.

The results demonstrated that it is likely that the majority of copper in wine is in the form of Cu(I) sulfide (Cu_2S), and the presence of the macromolecules and other matrix compounds tested are unlikely to change the electrochemical state of the copper, regardless of the wine being white or red.

The impact of the quantity and timing of copper and SO_2 additions when producing wine to ensure the optimum sensorial outcome and eliminate the development of negative LMWSCs both in tank and in bottle

This study explored the interaction of copper and SO_2 in the evolution of reduced aromas as the use of SO_2 is an essential part of winemaking. The dosage and timing of SO_2 addition are similarly key activities under winemaking control and may impact H_2S formation. Sulfur dioxide naturally occurs in wines at very low concentrations (10–30 mg/L, Jackson 2008); however, the main contributor to the final concentration in commercial wines is added SO_2 at concentrations in the range of 50–200 mg/L. Sulfur dioxide exists in free and bound forms, with the majority of free SO_2 present as bisulfite ions (HSO_3^-) at normal wine pH (all of the different species of sulfur dioxide in equilibrium found in wine, which includes the molecular sulfur dioxide, bisulfite, and sulfite, will be referred to generically as 'SO_2' throughout the text). Sulfur dioxide plays a critical role in the prevention of microbial spoilage, and is important as an antioxidant, through reaction with hydrogen peroxide (H_2O_2) generated from oxygen via a series of redox steps, as well as with quinones to regenerate polyphenols (Danilewicz, 2011). It is also known that SO_2 influences the loss of thiol compounds post-bottling (Waterhouse and Laurie 2006). It has also been proposed that SO_2 may act as a source of LMWSCs. Rankine suggested that the reduction of SO_2 may occur through the interaction of metal ions such as manganese and zinc with tartaric and malic acids during fermentation (Rankine 2004). No evidence for the post-bottling formation of H_2S via the metal-catalysed reduction of SO_4^{2-} or SO_3^{2-}during low oxygen conditions has yet been shown, even though this pathway has been proposed by Ribéreau-Gayon et al. (2006) and Lopez et al. (2007). Another possible pathway through which SO_2 can be involved in the modulation of 'reductive' LMWSCs post-bottling is through reactions with wine compounds, such as quinones. Nikolantonaki et al. (2012) described the reaction and kinetics between certain o-quinones and nucleophilic thiols and has shown that the addition of SO_2 directly influenced the rates and the yields of all of the o-quinone sulfur adducts.

The separate effects that copper and SO_2 additions may have on H_2S formation in wines post-bottling have been discussed above, however, copper and SO_2 are usually both present in wine and may have synergistic effects with wine matrix compounds. Given that the final concentrations of copper and SO_2 in finished wines, as well as timing of the addition of copper and SO_2, are under a winemaker's control it is important to understand the synergistic interactions between them and the effects that these compounds may have on H_2S formation. The synergistic effects of the combined treatment of copper and SO_2 on H_2S formation in a white wine (Verdelho) and a red wine (Shiraz) were investigated. Some of the factors modulating the formation of H_2S involving copper and SO_2 interactions during wine storage under low oxygen conditions were also studied.

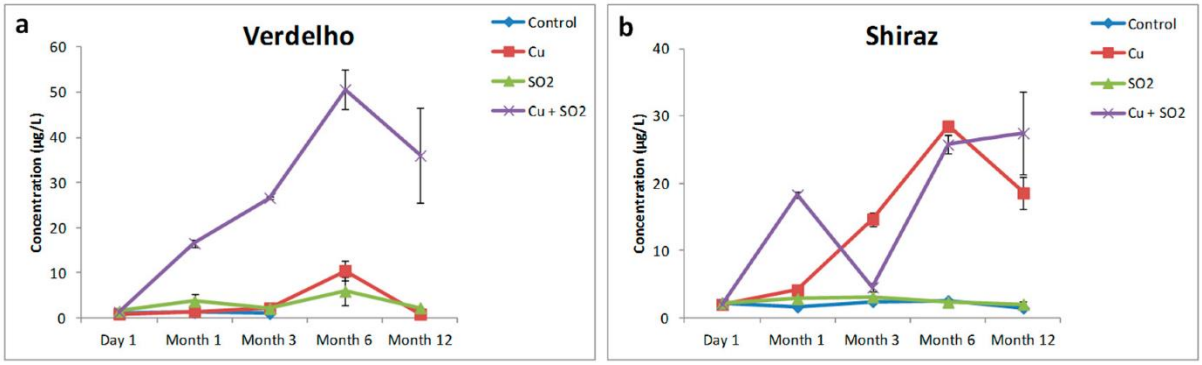
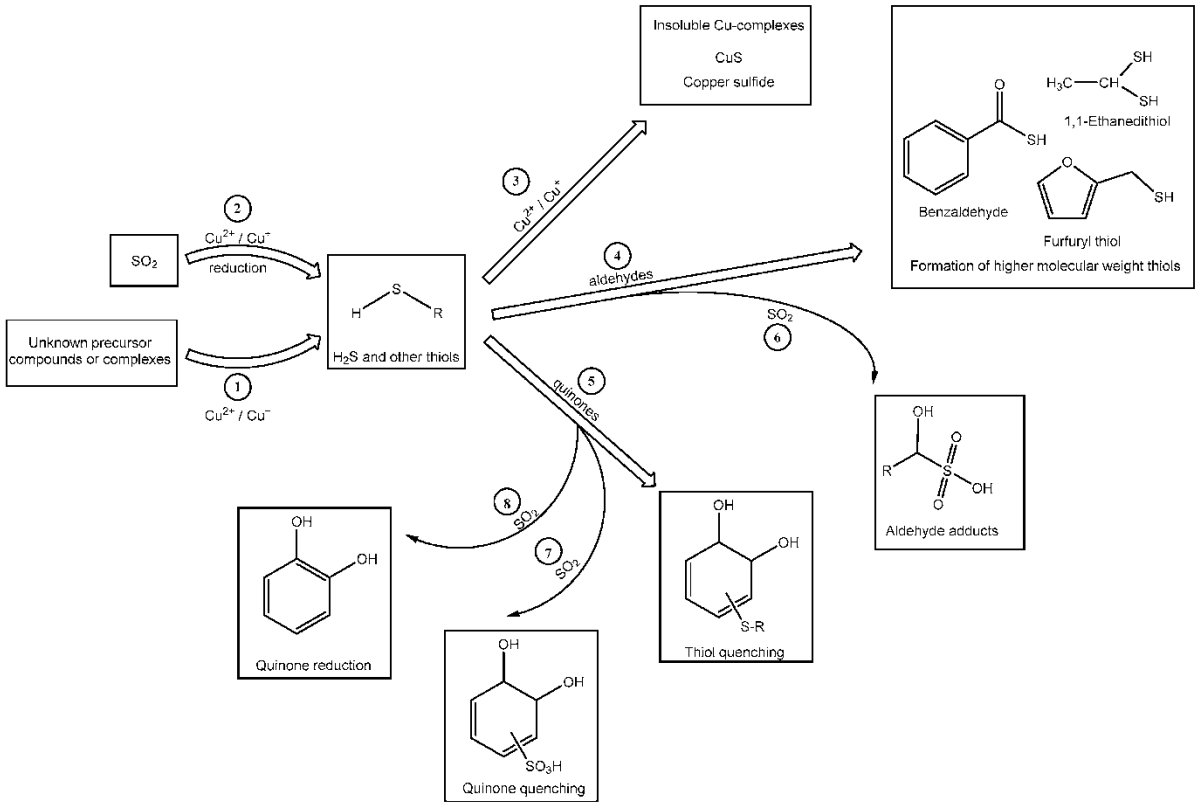


Figure 8. The effects of Cu^{2+}, SO_2, and Cu^{2+} + SO_2 on the formation of H_2S in (a) Verdelho and (b) Shiraz wine samples that were stored under nitrogen (N_2) and measured over the course of 12 months.

The combined copper and SO_2 treatment in the Verdelho wine samples resulted in significant and large increases in H_2S concentrations (Bekker et al. 2016b). For the Verdelho samples more H_2S was measured in samples treated with copper + SO_2 than by treating samples with copper alone (Figure 8a). Using multi-way ANOVA it was possible to separate the effects of copper treatment from the combined copper and SO_2 effects, with the increase in H_2S concentration significantly associated with copper + SO_2 treatment in the Verdelho samples ($p < 0.001$). The concentration of H_2S produced in Verdelho samples treated with copper + SO_2 was 36.01 (±18.1) µg/L compared to the 0.879 (±0.028) µg/L of H_2S measured in the control samples after 12 months of storage under low oxygen conditions, which is a significant increase in H_2S concentration.

Significantly increased H_2S concentrations were also measured in the Shiraz samples treated with copper + SO_2 (Figure 8b). By making use of multi-way ANOVA it was clear that these increases were only associated with a significant effect of copper ($p < 0.001$) and not associated with the combined copper and SO_2 treatments (Figure 8b). If the evolution of H_2S in Shiraz samples is considered over the course of the 12 months of storage in a low oxygen environment, the trends of H_2S formation for Cu^{2+}-treated samples and samples treated with both copper and SO_2 are remarkably different (Figure 8b). Shiraz samples treated with only copper displayed a steady increase in H_2S concentrations from Month 1 to Month 6, followed by a decrease to Month 12, whereas samples treated with copper and SO_2 displayed an initial increase from Day 0 to Month 1, followed by a significant decrease in H_2S concentrations at Month 3, which was then followed by increased H_2S concentrations from Month 6 through to Month 12. The exact mechanism for these different H_2S evolution trends are not clear; however, this demonstrates the complex underlying interactions of different wine compounds with one another and how the addition of SO_2 to a wine already containing copper could have significant implications for H_2S evolution. Previous studies have also shown the non-linear increase in thiols such as H_2S and MeSH evolution over time, which suggests that compounds such as H_2S and MeSH may be dynamically bound and released by other wine compounds after bottling and during storage.

Although the exact reaction pathway for the formation of H_2S associated with the combination of copper and SO_2 is not yet known, there are a few possible mechanisms (Scheme 1). When a higher ratio of free SO_2 to H_2S is available, it can form adducts with wine quinones or reduce them back to their corresponding phenols, and in doing so prevent the quenching of the newly formed thiols. Competing reactions between H_2S and SO_2 could also result in a smaller percentage of H_2S lost through, for example, reactions with acetaldehyde. This could indirectly lead to a relative increase in thiol concentration in the treatments with a high concentration of carbonyl compounds.



Scheme 1. Possible mechanisms for the formation of H_2S and other low molecular weight thiol compounds and the interaction between copper, SO_2, and copper + SO_2 with wine compounds such as aldehydes and o-quinones, during H_2S formation (from Bekker et al. 2016).

In summary, these investigations have shown that the presence of wine additives, such as copper and SO_2, play a fundamental role in the modulation of H_2S profiles in both red and white wine post-bottling.

Understanding differences among wine yeast strains in their ability to release volatile sulfur compounds (LMWSCs)

During fermentation, wine yeast not only produce ethanol and CO_2, but also a range of volatile compounds such as esters, higher alcohols or volatile sulfur compounds (LMWSCs). Different strains produce varying amounts of these flavour compounds, which means that wine yeast can be used as a tool by winemakers to modulate wine style. To assess this variability among different wine yeast strains, a series of triplicated small-scale fermentations were carried out in a synthetic grape juice (SGJ), and quantitative data on the production of LMWSCs was obtained for 100 yeast strains (Figure 9).

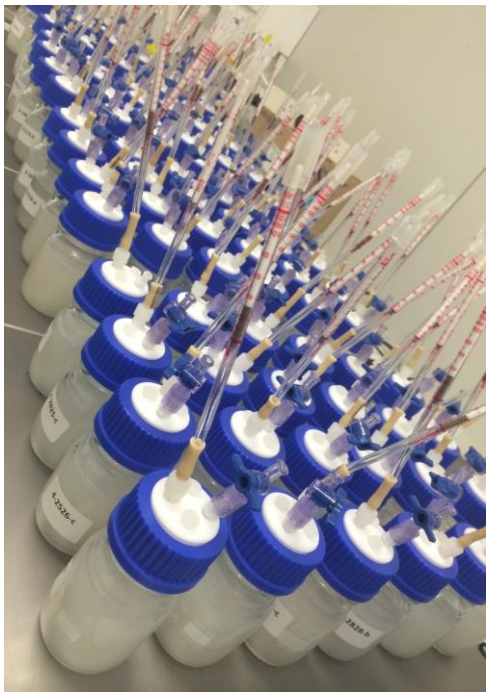


Figure 9. Picture showing the small-scale ferments (90 mL) in synthetic grape juice (SGJ).

SGJ was supplemented with grape-like concentrations of the cysteinylated and glutathionylated precursors of the volatile thiols 3-mercaptohexan-1-ol (3MH) and 4-mercapto-4-methylpentan-2-one (4MMP), which are responsible for the 'tropical fruit' characters of Sauvignon Blanc wines (Figure 10).

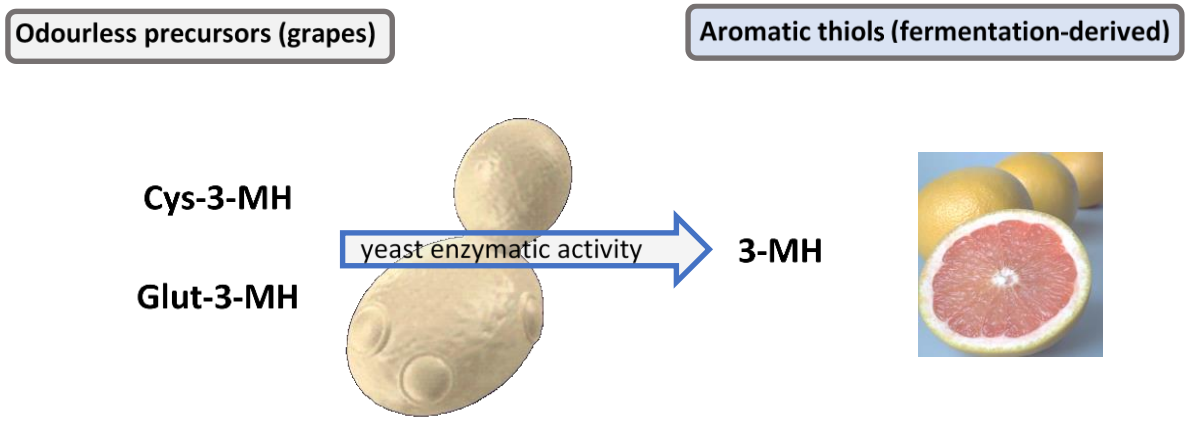


Figure 10. The odourless precursors of 3MH, cysteine-3-MH (cys-3MH) and glutathione-3-MH (glut-3-MH) are found in grapes. During alcoholic fermentation, the yeast converts glut-3MH in a series of enzymatic steps into cys-3MH. Then cys-3MH is cleaved by different yeast enzymes to release the thiol 3-MH, with aroma descriptors of 'grapefruit' and 'passionfruit'.

A large range of concentrations among the yeast was observed for the negative LMWSCs H_2S, methyl thioacetate (MeSAc), and ethyl thioacetate (EtSAc). In addition, a 20-fold and 35-fold difference in their ability to release the 'tropical' thiols 3-MH and 4-MMP, respectively, was observed among yeast strains. Interestingly, about 70% of the strains released low amounts of both 3-MH and 4-MMP; about 20% were classified as moderate releasers, and only 10% produced high amounts of these thiols. No trends were observed between commercial wine strains and natural isolates in their ability to release 4-MMP and 3-MH; however, strains recommended by yeast manufacturers for white winemaking tended to produce higher levels of both thiols than strains recommended for red wine. Finally, a strong positive correlation was observed in the formation of 3-MH and 4-MMP by the different wine yeast, suggesting that their formation might share common genetic determinants.

These studies were then reproduced in a red must, and analysis indicated a strong correlation between LMWSC results from the model study in SGJ and fermentations with real grape juice. Unfortunately, no data on 'tropical' thiol release was obtained on these red ferments.

To further explore whether the information obtained from fermenting SGJ could be used to predict the ability of a particular wine strain to release 'tropical' thiols in real grape juice, a subset of 26 of the 100 strains was used to ferment a small volume of a Chardonnay juice (200 mL). Again, a great variability in LMWSC and 'tropical' thiol formation was observed, and wine yeast strains were classified into different groups according to their sulfur production profile (Figure 11).

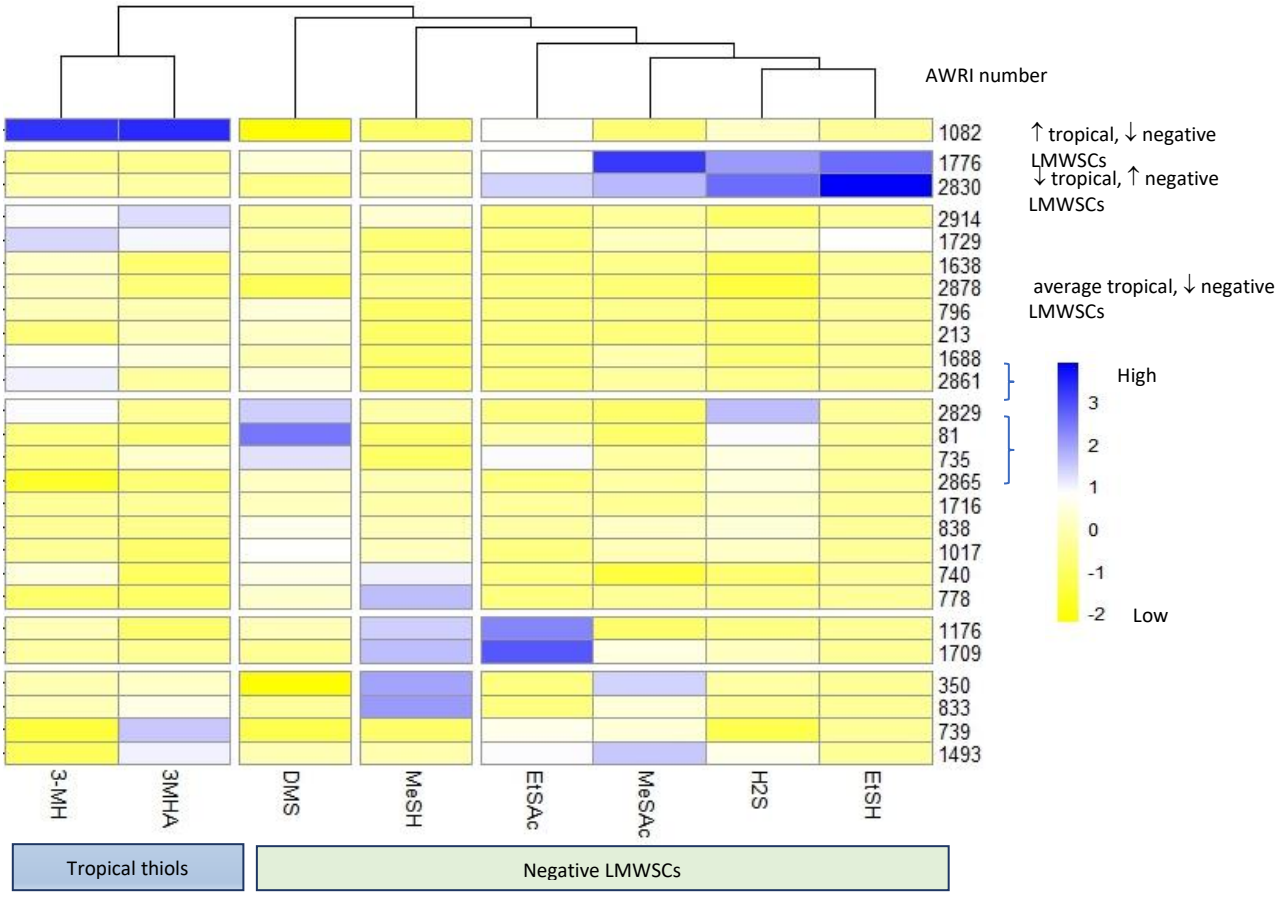


Figure 11. Heatmap showing the levels of different sulfur compounds ('tropical' thiols: 3-MH and 3-MHA, and negative LMWSCs: DMS, MeSH, EtSAc, MeSAc, H_2S and EtSH) after fermentation of a Chardonnay juice (200 mL) using 26 wine strains. Blue and yellow colours depict high and low levels of these compounds, respectively. The AWRI number identifier of each of the wine yeast strain is also shown.

A strong correlation was found between the concentration of 3-MH produced in the Chardonnay wine and those previously found in the SGJ (Figure 12). These results confirmed that model ferments using SGJ were good predictors of a strain's capacity to release 'tropical' thiols under winemaking conditions. A strong positive correlation was also observed between the levels of H_2S, ethanethiol (EtSH) and MeSAc in the Chardonnay wines, suggesting that the formation of these negative LMWSCs is to some extent dependant on yeast enzymatic activity.

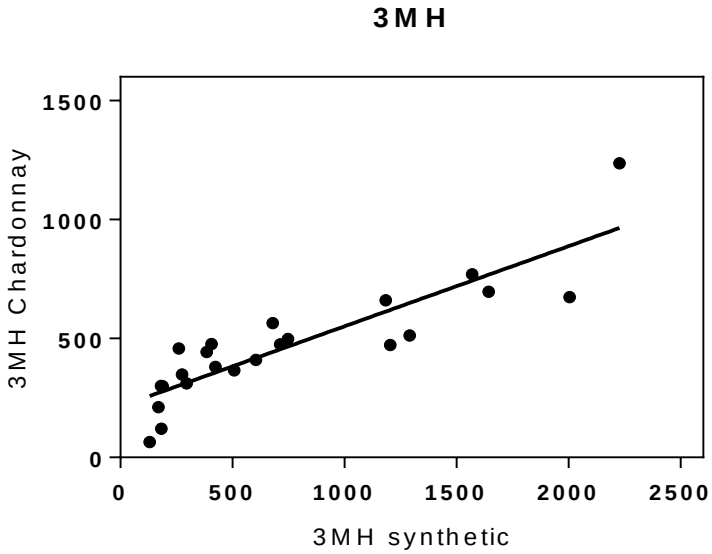


Figure 12. Relationship between 3-MH concentrations (ng/L) in fermentations of a synthetic grape juice and a Chardonnay juice with 26 different wine strains. The goodness of the fit between both data sets is expressed as $R^2 = 0.765$ ($P<0.0001$).

Based on these results, a high and a low-LMWSC producer were selected for a pilot-scale ferment in Grenache grapes (50kg) for the 2017 vintage. Preliminary chemical analysis of the wines confirmed the same trends observed in these small-scale ferments. These two strains could be used to investigate the genetic determinants involved in the formation of different LMWSCs by yeast.

Formation of 'tropical' thiols 3-MH and 4-MMP from grape odourless precursors: glutathionylated precursors of 3-MH and 4-MMP.

The 'tropical fruit' characters of Sauvignon Blanc wines are attributed to the presence of the aromatic thiols 3-MH, 3-MHA and 4-MMP. These thiols are not detectable in grape juice to any significant extent but are released by yeast during alcoholic fermentation. While the processes involved in the release of 3-MH and 4-MMP from their cysteinylated precursors have been studied extensively, degradation pathways for glutathione-*S*-conjugates (GSH-3-MH and GSH-4-MMP) have not. A candidate-gene approach was taken, focusing on genes known to play a role in glutathione and glutathione-*S*-conjugate turnover in *Saccharomyces cerevisiae*. Several of these genes were deleted in a laboratory strain background, and the ability of the single deletants to release 3-MH and 4-MMP was assessed in SGJ spiked with their respective glutathionylated precursors (Cordente et al. 2015). Results confirmed several yeast genes as playing a critical role in thiol release: Opt1p was found as the major transporter responsible for uptake of GSH-3-MH and GSH-4-MMP inside the yeast cell for their cleavage, and vacuolar Ecm38p as a key determinant of 3-MH release from GSH-3-MH. These results were in agreement with a previous genome-wide screening of the release of H_2S from the amino acid cysteine (Winter et al. 2014), in which the vacuolar function was found to be critical for H_2S formation.

Formation of 'tropical' thiols 3-MH and 4-MMP from grape odourless precursors: cysteinylated precursors of 3-MH and 4-MMP.

As described previously, fermentations conducted using 100 yeast strains in both a synthetic grape juice and a red must, as part of the AWRI's yeast research, were analysed for LMWSCs. The analytical results, combined with genomic data on these yeast strains, revealed that the potential of a particular strain to release the 'tropical' thiols 3-MH and 4-MMP from cysteine-bound precursors correlated with certain alleles (versions) of the gene *IRC7*. The majority (70%) of yeast strains analysed were classified as low thiol releasers, and it was established that the cause for this phenotype was the presence of inactivating mutations in the yeast *IRC7* gene. Therefore, DNA sequence of *IRC7* can be used as a molecular marker to determine the likely potential of any given strain to release volatile thiols. Identification of this marker opens the door for the breeding of wine strains with enhanced thiol-producing capabilities, enabling the release of untapped 'tropical' thiol aroma in different white wine varieties, such as Chardonnay.

Formation of H_2S and other negative LMWSCs from cysteine

To identify genes important to H_2S release from the amino acid cysteine, a series of strains were constructed expressing different candidate enzymes at high levels. Six of these yeast genetic determinants were found to regulate the formation of H_2S from cysteine during fermentation of a SGJ. In particular, overexpression of *CYS3* and *STR3* led to significant increases in the levels of H_2S, while overexpression of *CYS4* had the opposite effect. Finally, overexpression of *MET17* only led to an increase in the levels of MeSAc, suggesting that this gene might play an important role in the formation of this negative LMWSC. Interestingly, among all these genes, *IRC7* was found to play the most critical role in the formation of H_2S and other negative LMWSCs, as observed previously with the 'tropical' thiols 3-MH and 4-MMP.

To further study the role of *IRC7,* three versions of this gene with different LMWSC-releasing abilities were assessed in a SGJ at three different concentrations of cysteine. A strong positive correlation was observed between the amount of H_2S released during fermentation and the initial concentration of cysteine in the media, for each of the three *IRC7* alleles (Figure 13). In addition, the amount of H_2S released by yeast also strongly correlated with the levels of EtSH, EtSAc, MeSAc, and carbon disulfide (CS_2). These results confirm the important role that yeast (and in particular the *IRC7* gene) play in the formation of H_2S and other negative LMWSCs from organic sulfur compounds such as cysteine, and possibly glutathione (GSH).

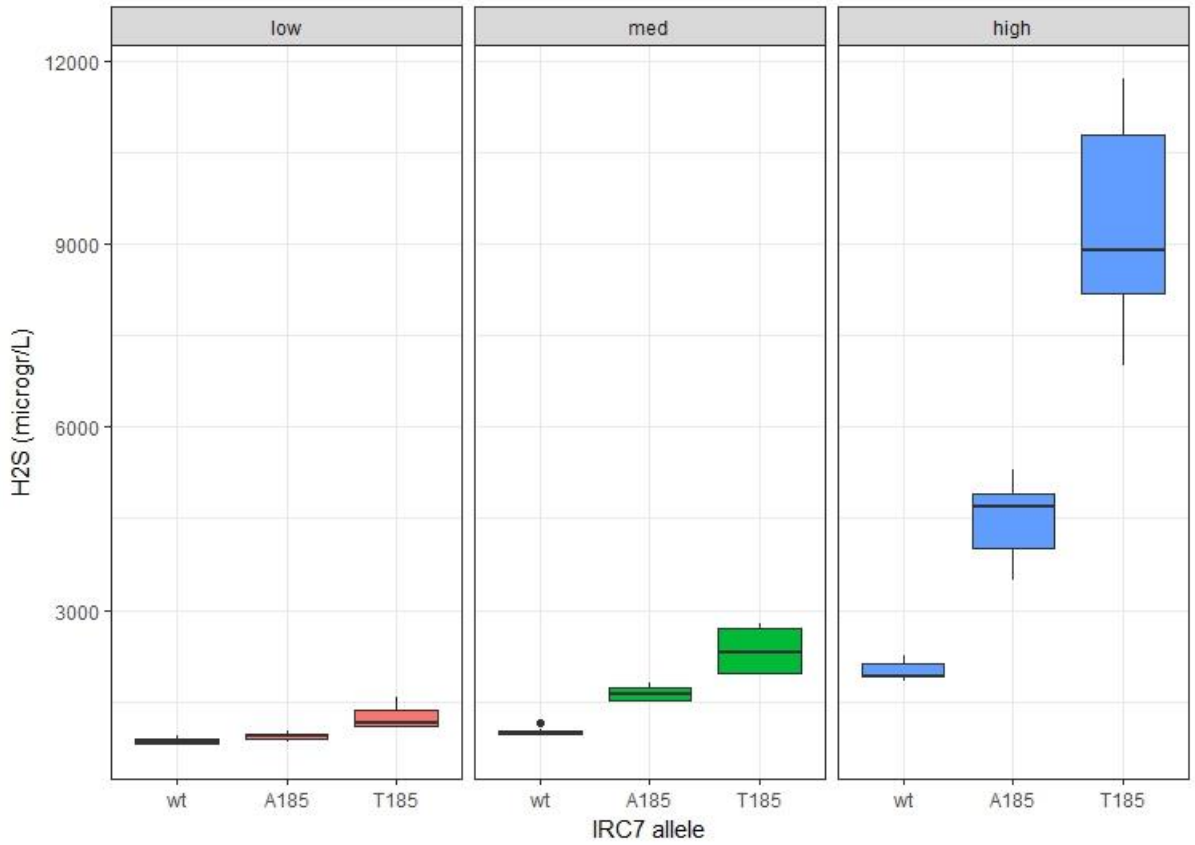


Figure 13. Production of H_2S during fermentation of a SGJ supplemented with different concentrations of cysteine (low, med and high). Three yeast strains expressing different versions (alleles) of the gene *IRC7* were used in this fermentation experiment (wt, A185, and T185)

Effect of copper on yeast performance and biochemistry

The effect of copper on yeast performance and biochemistry has been a focus of research in Project 3.2.2 – 'Fit for purpose yeast' and the final report for that project should be referred to for more details of this work.

Outcome and conclusion

The objectives of this project have been achieved to a high standard. New methods were developed to compliment the current technologies and expand the AWRI's ability to quantify a wider range of LMWSC and precursor compounds. Precursor compounds associated with increased LMWSC concentrations in wines post-bottling were thoroughly investigated and the risk that these compounds pose to 'reductive aroma' formation were determined. Factors that affect the formation and fate of LWMSCs, such as wine pH, residual metal concentrations and SO_2 treatment were also thoroughly investigated and the effects of these compounds on H_2S and MeSH formation in wine post-bottling were determined. Differences among wine yeast in their ability to release both positive and negative LMWSC were also determined.

Practical implications for the Australian grape and wine industry include:

- The impacts of residual copper, aluminium, iron, manganese, and zinc on 'reductive aroma' formation in wines post-bottling have clearly been defined. Winemakers can assess the risks and rewards of using a metal such as copper as a fining agent during wine production.
- Management strategies for using copper as a fining agent and removing it to reduce the risk of 'reductive aromas' have been proposed.
- Precursor compounds responsible for H_2S, MeSH, and EtSH formation in finished wines have been assessed and winemakers can measure the concentrations of these specific precursors in their wines to determine their risk for 'reductive aroma' formation in wines post-bottling.
- Several yeast markers have been identified that modulate the formation of important LMWSCs, such as the 'tropical' thiols 3-MH and 4-MMP and H2S, from their precursors. This information will help winemakers to make an informed yeast strain choice to suit their wine style, and potentially reduce the formation of negative LMWSCs in a winery environment.

Recommendations

Based on results from this project, the sulfur-containing amino acids Cys, Met and GSH are not be considered to pose a direct or significant risk as precursors to H_2S or MeSH formation through direct desulfurisation in wines post-bottling. It should be noted that their interactions as antioxidants or in other roles may have an effect on LMWSCs. However, the concentration of the sulfur-containing amino acid SMM is related to DMS formation, as previously shown by Segurel et al. (2005), and researchers and producers interested in understanding the sensory characters associated with DMS could consider monitoring SMM.

The presence of thioacetates and disulfides does appear to represent a direct risk as precursors to their associated thiols such as MeSH and EtSH. As such, it is recommended that thioacetates and disulfides are monitored to assess potential risk of post-bottling LMWSC release. In addition, it is recommended that further research into the role of yeast in modulating thioacetate production is performed, as well as additional research to better characterise disulfides and polysulfanes as potentially significant precursors.

This project has shown that metals can act singly or in combination to greatly influence evolution of undesirable 'reduced' aromas. Winemakers that wish to lower the risk of such characters should therefore take steps to minimise metal concentrations in wine. Oxygen concentration in wine also significantly interacts with metals in the evolution of LMWSCs. As such, it is recommended that winemakers ensure they know the 'total packaged oxygen' (TPO) concentration of their products and its likely progression post-bottling. 'Macro-oxygenation' during fermentation can also be considered as a tool for decreasing the residual metal concentrations in wines. An awareness of the impact of additives (for example bentonite) on metal concentrations in wine should also be developed by winemakers.

Copper has been noted for its role in accelerating post-bottling formation of LMWSCs. As such, winemakers should ensure they understand the risk profile of copper addition processes and should be aware of the timing options for copper additions. This research indicates that the ideal time for such additions is late in fermentation in the presence of fermentation solids. The risk from the presence of copper in juice (potentially from vineyard sprays etc.) on certain copper-sensitive yeast strains should also be considered prior to fermentation. The differential behaviour of LMWSC evolution in reds compared to whites in relation to copper should be noted. The predominant form of copper remaining in wine is usually copper sulfide and research into the mechanisms of copper sulfide reactions during wine aging is recommended.

It is recommended that winemakers consider the effects of pH and SO_2 timing in relation to their modulation of LMWSC development as it relates to copper and precursors such as thioacetates.

Several markers have been identified that can be used to predict a yeast's potential to release 'tropical' volatile thiols and H_2S from their precursors. Little is known about the role of wine yeast in the production of other important LMWSCs such as MeSH or thioacetates. Therefore, it is recommended that research be directed toward the characterisation of yeast markers responsible for the metabolism of sulfur compounds and formation of relevant precursors and negative volatile compounds. Identification of these markers will help winemakers to choose the best strain to modulate their wine style, and will open the door for the breeding of novel wine strains with reduced production of negative LMWSCs.

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Project 2.2.1– Collecting and disseminating information regarding agrochemicals registered for use and maximum residue limits in Australian viticulture

Executive summary

The aim of this project was to enable grape and wine producers to manage agrochemical residue levels in wine. This was achieved by collating and providing accurate and timely information on regulatory and technical aspects of chemicals registered for use in Australian viticulture, and the maximum residue limit (MRL) requirements of those chemicals in domestic and key export markets.

The main method of conveying information to industry and other stakeholders was through the booklet *Agrochemicals registered for use in Australian viticulture* (commonly known as the 'Dog book'). Over the course of the project, a new edition of the 'Dog book' was published and distributed each year, and significant changes were made to the size and structure of the publication in response to the changing demands of industry practitioners.

In addition to a hardcopy version of the 'Dog book', growers and winemakers can also access agrochemical information via a pdf version available from the AWRI website, or by interrogating the online agrochemical database. A mobile app was also introduced for the 2013/2014 growing season, permitting users to use their mobile devices to access relevant information and make decisions in the field.

The project team monitors potential pest, disease and agrochemical issues through regular communications with key industry and government stakeholder networks. Changes to product information and agrochemical use options were managed in consultation with chemical suppliers, and in accordance with the fortnightly gazettes issued by the Australian Pesticides and Veterinary Medicines Authority (APVMA). In response *eBulletins* were distributed to a database of ~3,300 stakeholders, in a timely manner, to allow industry to respond to regulatory changes.

Changes to established market MRLs, or the addition of new markets, required existing agrochemical practices to be frequently re-evaluated and reviewed. Any changes were discussed with and endorsed by the industry Agrochemical Reference Group, which includes representatives of regional-based grower organisations and major wine companies.

Highlights

- 850 sanitary and phytosanitary (SPS) notifications and 110 APVMA gazettes were reviewed for viticulture-relevant information, and the information was used to update the 'Dog book' and associated online resources.
- Over 30,000 'Dog books' were distributed to the Australian grape and wine community.
- As part of ongoing efforts to have an MRL established for phosphorous acid, a major dossier was submitted to the Food and Agriculture Organization/World Health Organization Joint Meeting on Pesticide Residues (JMPR).
- Following changes to the residue definition of captan in the European Union (EU), timely advice on its use and possible alternatives was provided, which averted potential trade issues.
- A 'Dog book' app was developed for Apple and Android devices. Since the launch of the app there have been more than 5,400 downloads.
- The following active constituents were reviewed and evaluated: amisulbrom; boscalid; captan; clothianidin; cyflufenamid; diuron; fenamifos; fenpyrazamine; fluazinam; flumioxazin; fluopyram; phosphorous acid; proquinazid.

Objectives

The objectives of this project are to:

- Develop and produce the annual publication *Agrochemicals registered for use in Australian viticulture* ('Dog book') for distribution to wine sector stakeholders.
- Develop and maintain up-to-date content concerning agrochemicals that is extended through email bulletins and web and mobile-based extension tools.
- Provide assistance to the Australian wine industry to achieve full compliance with agrochemical MRLs and compositional standards in grapes and wine for major export markets.
- Assist the Australian wine industry to use agrochemicals responsibly, in a cost effective, socially responsible manner and with minimal impact on the environment.
- Develop and maintain a skills base that can support the wine industry in the identification and resolution of agrochemical issues.
- Maintain and develop a database of information about APVMA registered products (insecticides, fungicides and herbicides) for viticulture.
- Maintain and develop a database of export market MRLs for the chemicals registered for use in Australian viticulture.
- Expand web and mobile extension tools to provide greater detail about active constituents and product information to stakeholders.

Outcome and conclusion

All objectives of the project have been met. Brief details are provided for each objective below.

- *Develop and produce the annual publication Agrochemicals registered for use in Australian viticulture ('Dog book') for distribution to wine sector stakeholders.*

A total of 850 SPS notifications and 110 APVMA gazettes were reviewed for viticulture-relevant information, which was used to update the 'Dog book' and associated online resources. The 'Dog book' was produced annually from 2013 to 2017. Table 1 summarises how the 'Dog book' was distributed to stakeholders over the investment period.

Table 1. Distribution of agrochemical information to stakeholders via different hard copy and electronic versions of the 'Dog book' over the four-year investment period

Agrochemical information source	Distribution
'Dog book' hard copy	>30,000 hard copies distributed
Online pdf	>8,300 downloads
Online search facility	>11,300 times accessed

- *Develop and maintain up-to-date content concerning agrochemicals that is extended through email bulletins and web and mobile-based extension tools.*

  Over the course of the project, 15 agrochemical *eBulletins* were issued to a distribution list of more than 3,000 email addresses and 13 active constituents were reviewed. All changes were reflected in updates to the 'Dog book' and associated electronic information sources.

- *Provide assistance to the Australian wine industry to achieve full compliance with agrochemical MRLs and compositional standards in grapes and wine for major export markets.*

  Approximately 450 specific enquiries about agrochemical and MRLs were addressed. In addition, the MRL webpage received around 13,000 queries. When the use of a particular chemical could pose a risk when exporting wine, advice was provided to mitigate that risk.

- *Assist the Australian wine industry to use agrochemicals responsibly, in a cost effective, socially responsible manner and with minimal impact on the environment.*

  The environmental and health and safety impacts of registered active constituents were considered and reflected in the recommendations section of the 'Dog book'. This resulted in the addition of re-entry-period information to the booklet, and the exclusion of broad-spectrum insecticides from the recommendations section.

- *Develop and maintain a skills base that can support the wine industry in the identification and resolution of agrochemical issues.*

  The number of staff members involved in the project was expanded, to ensure a suitably qualified staff cohort would always be available to respond to industry demand. Staff involved in the project included Mardi Longbottom, Creina Stockley, Anne Lord and Randell Taylor.

- *Maintain and develop a database of information about APVMA registered products (insecticides, fungicides and herbicides) for viticulture.*

  The MRL and Agrochemical databases were updated as required throughout the investment period to reflect information gathered from chemical suppliers, the APVMA and the Australian Government Department of Agriculture and Water Resources.

- *Expand web and mobile extension tools to provide greater detail about active constituents and product information to stakeholders.*

  The 'Dog book' was expanded from 2015/16 to include information about re-entry periods. Additionally, information about biosecurity was added in 2016/17 to make it easier for growers to identify and report exotic vineyard pests. Plans to include information about effects of active constituents on beneficial insects and livestock grazing were not able to be realised due to lack of sufficiently reliable information on which to base advice.

This project assists stakeholders to meet regulatory requirements, through the collation, distribution, and regular updating of MRL and other regulatory information and recommendations, related to agrochemical use. Information is disseminated chiefly via hard copy and electronic versions of the 'Dog book', online databases and associated extension materials, which include the

regulatory requirements of key export markets. The centralisation of these industry-wide activities under this project avoids duplication, and ensures that consistent and up-to-date information is available to all grapegrowers and wine producers.

The project team also works to avoid potential trade issues, such as those posed by changes in the EU to the residue definition for captan. Advice was provided to growers not to use this agrochemical on grapes destined for export to the EU, and advice on possible alternatives was provided.

Work by the team to establish an MRL for phosphorous acid continued, and included preparation of a submission to the Food and Agriculture Organization/World Health Organization Joint Meeting of Pesticide Residues in 2016. The submission is due to be assessed in late 2017.

Project 2.2.3– Informing Australia's wine consumers through understanding issues of wine consumption, health and nutrition

Executive summary

This project was funded by Wine Australia from January 2015 to June 2017, and followed on from National Wine Foundation (NWF) projects funded between July 2013 and December 2014. The aim of this 30-month project was to generate and disseminate evidence-based and scientifically sound information regarding wine and health/nutrition to facilitate informed decision-making by the wine and associated industries, policy makers and consumers. Alcohol's place in society is being robustly debated, particularly in Australia. That debate has the potential to lead to major changes to the wine sector's trading environment, through increased regulation in areas such as taxation, pricing, advertising, dietary guidelines and associated public health policies and strategies.

In June 2016, all project activities were reviewed. Proposed outputs and activities for 2016/2017 were generated through consultation and feedback from stakeholders including: the Winemakers' Federation of Australia (WFA); the Health and Nutrition Steering Committee; members of the National Wine Foundation; Alcohol Beverages Australia (ABA) (the pan-industry body with board representation from WFA, Accolade Wines, Pernod Ricard Winemakers and Treasury Wine Estates); and DrinkWise Australia (which has board representation from WFA, as well as representation by Accolade Wines, Pernod Ricard Winemakers and Treasury Wine Estates). A stop/go report incorporating a Project Variation was submitted to Wine Australia for review, and was approved.

Work undertaken in this project was required by the Australian wine industry to inform and support Wine Australia's *Strategic Plan 2015–2020*, and the WFA *Health & Social Responsibility Strategy 2011-2014*, and related *Responsible Winery Initiative* and *Action Plan*. The objective was to provide information about wine's role in a healthy diet and lifestyle, in accordance with the growing population and clinical data.

The project's approach to inform debate and decision-making was three-fold:

- generation and provision of scientific information such as reviews, reports, critiques and briefings;
- communication to stakeholders via publication of peer-review papers and industry articles; and
- provision of submissions to government on behalf of the WFA and ABA.

Additional project activities included the generation of content for consumers which can be accessed from the AWRI, ABA and WFA websites, and the provision of lectures and workshops.

Highlights

- Nine peer-reviewed papers, two book chapters and six articles were published on a range of wine and health/nutrition related issues.

- In May 2016, a paper authored by the AWRI and Italian collaborators titled 'The case for anthocyanin consumption to promote human health: a review' won a Tanner Award as the most-cited paper published in *Comprehensive Reviews in Food Science and Food Safety* in 2013, with 41 citations.

- Five industry fact sheets were produced on wine and cardiovascular diseases, cancers, cognitive decline/dementia, diabetes and obesity; and twelve complementary AWRI wine and health fact sheets and frequently asked questions were revised and disseminated via the AWRI website.

- A workshop entitled 'A comprehensive review of the wine and health landscape' was successfully staged at the 16th Australian Wine Industry Technical Conference on 24 July 2016.

Objective

This project will provide evidence-based material to enable the Australian wine sector and government stakeholders to make informed decisions on wine and health issues.

Outcomes and conclusions

The objective of the project has been met through delivery of the following activities and outcomes.

Submissions

Project staff participated in multiple workshops organised by the Australian Government Department of Health and subsequently prepared comprehensive scientific submissions to inform:

- the Intergovernmental Committee on Drugs responsible for drafting the *National Drug Strategy 2016-2025*
- the *National Alcohol Strategy 2016-2021*; the Senate Standing Committee on Economics (References Committee) on personal choice and community impacts;
- the Public Call for Evidence on the Health Effects of Alcohol Consumption, as part of the review of the National Health and Medical Research Council's (NHMRC) *Australian Guidelines to Reduce Health Risks from Drinking Alcohol* (2009).

Provision of scientific information

Critiques of a number of publications were prepared including:

- the Australian Bureau of Statistics alcohol consumption data and its alignment with Australian Institute of Health and Welfare data;
- the Foundation for Alcohol Research and Education's publication *Risky business–The alcohol industry's dependence on Australia's heaviest drinkers*;
- St Vincent's Health Australia's publication *Alcohol-related harm and violence policy*;
- the Legal and Constitutional Affairs References Committee's *Interim Report–Need for a nationally-consistent approach to alcohol-fuelled violence*;
- the Health Council of The Netherlands' *Guidelines for Healthy Eating* of 2015;
- the UK Chief Medical Officers' *Low Risk Drinking Guidelines* of 2016;
- the *Huffington Post's* article 'What your moderate drinking habit is doing to your health' (7 June 2017) and the *Australian Financial Review* article 'The silent damage from drinking moderately down the decades' (7 June 2017).

In addition, health policy papers on alcohol's impact on cardiovascular disease, cancer, cognitive dysfunction/dementia, diabetes, immunological disorders and obesity were drafted for WFA and ABA. Complementary to these policy papers, 12 AWRI wine and health fact sheets and frequently asked questions were revised and disseminated via the AWRI website.

In preparation for the May 2016 review of the NHMRC's *Australian Guidelines to Reduce Health Risks from Drinking Alcohol*, a summary and comparative review of international alcohol drinking guidelines and associated public health policy changes and directions was undertaken. A comprehensive literature review was also undertaken on aspects of wine consumption and human health, including wine consumption compared with other risk factors associated with death from chronic diseases. This was an extension of the project entitled *Analysis of the health and social benefits of wine in moderation* funded by the NWF from July 2013 to December 2014.

Because alcohol drinking guidelines form the basis for the development of government policies and community materials on the health effects of alcohol consumption, it is important that they are based on sound scientific evidence. When overall or all-cause mortality is considered, there is solid

evidence that the risks of moderate consumption of wine (equivalent to no more than 20 g of alcohol per day, or two standard drinks) are lower than the risk of heavier consumption for the general population. There is also some evidence that moderate consumption of wine is lower risk than abstinence for the general population.

Peer-reviewed and industry papers
To ensure that the research community and the wine and associated industries and consumers were also fully informed about the evidence base for alcohol drinking guidelines, nine position papers and two book chapters were published after peer review, and another six articles were published in industry publications (see list below).

Committee
A Health and Nutrition Steering Committee was established in consultation with WFA, which is comprised of Australian clinical practitioners and university researchers. This committee is available to be called on if advice is required on alcohol and health research.

Additional project activities included the generation of content for consumers, which can be accessed from the AWRI, ABA and WFA websites, and the provision of lectures and workshops. For example, a workshop was held at the 16th Australian Wine Industry Technical Conference in July 2016, where project staff provided presentations entitled 'Evolving evidence for the role of wine in reducing the risk of four primary causes of death in Australia' and 'Things you need to know.'

Project staff also presented a keynote address entitled 'Changing the upper limits of moderate alcohol consumption - the need for a world-wide politic on alcohol consumption' at the WineHealth 2017 conference held in Logroño, Spain, and formed part of the moderating panel for a session titled 'Round table innovation and future'. Presentations on responsible alcohol consumption were also provided to Treasury Wine Estates personnel in Adelaide and Melbourne, as part of their global focus week on responsible alcohol consumption.

The collation and generation of evidence-based and scientifically sound information on the impact of wine consumption on human health outcomes has provided timely information to the Australian wine industry, government, consumers and other stakeholders, to enable informed decision-making.

The provision of balanced wine and health information has, for example:

- been used to fill identified knowledge-gaps such as the impact of the moderate consumption of alcoholic beverages such as wine on the risk of non-communicable diseases such as cardiovascular disease, diabetes, cognitive dysfunction/dementia and cancers;

- had a tangible impact on government guidelines, policy and strategies that continue to incorporate that health and social harms from alcohol consumption only arise from drinking risky amounts in risky patterns; and

- informed the *WFA Health & Social Responsibility Strategy 2011-2014*, and related *Responsible Winery Initiative and Action Plan* with its core policy and support strategies, communication and activities that position moderate wine consumption as an integral part of a healthy diet and lifestyle.

This information has enabled industry to continue to operate without significant societal, regulatory and economic imposts, and to demonstrate its duty of care to its consumers in enabling them to make informed decisions regarding their alcohol consumption and health.

Overall, the project and its associated communication activities has provided a strong foundation for the industry to continue to confidently interact with government and key stakeholders on alcohol and health issues.

The well-resourced 'anti-alcohol lobby' is increasing its campaign to impose the restrictive public health policies and strategies, similar to those which were effective in reducing tobacco use in Australia, in order to reduce per capita alcohol (and hence wine) consumption. Therefore, it is imperative that the industry continues to provide evidence-based recommendations of alternative, effective, tailored practices and strategies to reduce harmful alcohol consumption, while continuing to provide information about the potential health benefits from regular and moderate alcohol consumption for the majority of the adult Australian population. This can best be achieved by effectively communicating the key research findings of alcohol and health research to stakeholders, in order to forge and maintain good working relationships with government and allied government organisations, such as the National Health and Medical Research Council.

Articles published during the project

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Project 2.2.4– Increasing Australia's influence in market access, safety, regulatory and technical trade issues

Executive summary

Maintaining market access or opening markets for Australian wine, nationally and internationally, is facilitated by managing and reducing current and potential barriers to trade. Accordingly, the Australian wine industry needs to anticipate, facilitate and influence regulation of wine composition, production, labelling and marketing. The purpose of this project was to identify and mitigate any impediments to market access. The approach taken has been two-fold:

- Provision of readily-accessible regulatory-related scientific and technical advice and assistance for the activities of key industry stakeholders; and

- Representation at national and international industry forums, such as the Organisation International de la Vigne et du Vin (OIV) and the APEC Wine Regulatory Forum (APEC WRF), International Wine Technical Summit (IWTS), Federation International des Vins Spiritueux (FIVS), and participation on relevant working groups and committees. The timely identification and management of scientific and technical trade issues, via the collection, evaluation, generation and dissemination of data on these issues, has helped maintain market access for Australian wine, facilitating the trade of Australian wine nationally and internationally.

Highlights

- Tentative provisions for additives metatartaric acid and yeast mannoproteins have been developed by the WHO/FAO Joint Expert Committee on Food Additives (JECFA) based on submissions prepared by the project team and submitted on behalf of the WFA, WA, DAWR and OIV. Together with the toxicological evaluation, they will be presented next to the Codex Committee on Food Additives (CCFA) for its consideration and adoption.

- Following an AWRI submission, FSANZ provided permission for the use of specific enzyme processing aids, Aspergillopepsin I and II, in Australian winemaking.

- Potassium carbonate has been included in the *International Code of Oenological Practices* of the OIV, authorised for the reduction of the titratable acidity and the actual acidity in winemaking.

- Processing aids, dimethylpolysiloxane and *Aspergillopepsin* I and II (Proctase), have advanced to Step 5 in the 8-step resolution process of the OIV prior to inclusion in the *International Code of Oenological Practices.*

- Analytical and other supporting scientific data were provided which prevented the potential for establishment of an OIV maximum limit for phthalates in wine.

- Analytical and other supporting scientific data were provided which promoted the revoking of a maximum limit for manganese in wine internationally.

- The AWRI's online databases were expanded to contain information on *Analytical requirements for the export of Australian wine* specific to 44 individual countries as well as trading blocs, and

on *Permitted additives and Processing aids for winemaking and wine importing countries* for 28 individual countries.

- A workshop entitled 'The changing regulatory environment of Australian wine' was held at the 16th AWITC in conjunction with Wine Australia. Project staff provided a presentation at the workshop entitled 'Wine labelling - the growing influence of the anti-alcohol lobby on health warning labels'.

Objectives

- Wine Australia (WA), the Department of Agriculture and Water Resources (DAWR), the Winemakers' Federation of Australia (WFA) and Australian Vignerons (AV) receive regular reports on OIV deliberations and resolutions.

- WA, DAWR, WFA and AV receive the technical advice required to support policy development.

- Australian delegation to OIV able to participate in an informed manner on oenology practices, method of analysis, food safety and nutrition-related discussions at OIV meetings.

- Support communications to industry on market access and regulatory developments.

Outcomes and conclusions

The objectives of the project have been met through provision of the following outcomes and activities.

Scientific and technical support

Scientific and technical advice was provided to both national and international regulatory forums for numerous issues. An example is the occurrence of heavy metals such as arsenic, cadmium, copper, lead and manganese in Australian wine. In 2014, Wine Australia issued a warning to wine exporters regarding increased scrutiny of manganese, iron and copper levels in wine by Chinese authorities. Maximum regulatory levels of 2 mg/L for manganese, 1 mg/L for copper and 8 mg/L for iron were then being enforced in wines imported into China. While the regulatory levels set for copper and iron were consistent with other trading nations, the limit for manganese in wine caused concern across all wine-exporting countries. The AWRI analysed the manganese levels of more than 1,500 wines, where a significant number were found to exceed the 2 mg/L limit, but residual manganese was well below any regulatory limits established by other countries. In 2016, China revoked the maximum limit for manganese in wine.

The presence of phthalates in wine was investigated and the AWRI participated in OIV-led inter-laboratory ring tests that evaluated methods for the analysis of phthalates in wines. Phthalates were not detectable in any of the Australian wines analysed and this data was provided to Food Standards Australia New Zealand (FSANZ) following a call for submissions for *Proposal P1034 – Chemical Migration from Packaging into Food*.

Other market access issues investigated included adverse allergenic reactions to wine, and the analysis of proteinaceous and other allergens in wine and associated labelling in different export markets. The project team also presented an evaluation on the health and safety of consumers from phthalate concentrations observed in wine to the OIV's Commission IV Safety and Health, demonstrating that there is no toxicological justification for the maximum limits for phthalates in wine proposed by certain importing countries.

In addition, the project team also participated in a review of the Codex Alimentarius Commission's Proposals for New and/or Revision of Food Additive Provisions; the preparation of a product information declaration for wine industry suppliers; revisions of the Australian wine industry's policy and position statement on genetically modified organisms; the Australian wine industry crisis management plan; and a cost-benefit analysis for energy labelling on alcoholic beverages as well as an assessment of appropriate energy content calculations for the Wine Industry Technical Advisory Committee (WITAC).

A comprehensive report was prepared on the relevant definitions and legislation in place for OIV member countries in relation to nutrition, labelling and ingredient listing in preparation for pending legislative changes.

Submissions
An application to enable use of protease enzymes such as Aspergillopepsin I & II in Australian winemaking was successfully lodged with Food Standards Australia New Zealand and these enzymes are now approved processing aids for Australian wine.

Countries that do not have regulations for wine and winemaking (or only have limited regulations) often defer to the provisions of the Codex Alimentarius Commission (CAC). Metatartaric acid, oenological tannins and yeast mannoproteins have not been permitted for wine by the CAC. Comprehensive dossiers on these compounds were prepared by the project team for the Winemakers Federation of Australia (WFA), Wine Australia (WA), Department of Agriculture and Water Resources (DAWR) and OIV, and submitted to the Joint Expert Committee on Food Additives (JECFA) in 2016 for review in 2017. Additional information and assistance were also provided to JECFA during the six-month review period prior to evaluation at their 84th meeting in June 2017, when the safety assessment and draft specifications were provided. Tentative specifications for metatartaric acid and yeast mannoproteins were developed by JECFA at the evaluation and these evaluations will be presented next to the Codex Committee of Food Additives for its consideration and adoption and finally to the CAC.

In addition, a comprehensive technical and toxicological dossier on metatartaric acid was subsequently prepared for submission to the European Food Safety Authority (EFSA) by the OIV, and for the Japanese Ministry of Health, Labour and Welfare by the European Commission and Oenoppia.

AWRI databases
The AWRI's online databases have been continuously updated and complement Wine Australia's Export Market Grids. In addition to information for regional trading blocs, these databases have been expanded to contain information on *Analytical requirements for the export of Australian wine* specific to 44 individual countries, and information on *Permitted additives and Processing aids for winemaking and wine importing countries* in 28 individual countries.

International forums
Two of the four outputs and associated activities of this project related specifically to the OIV. The project team continued to participate at the OIV as members of the DAWR-led Australian delegation, together with representatives of the Winemakers' Federation of Australia (WFA).

The project team contributed to the OIV 'Taskforce on allergens' which coordinates analytical and clinical research on the potential for residual protein in protein-fined wine and its significance for human health, in Australia, France, Germany and Italy. The code *Good fining practice for wine to be applied after the use of proteinaceous [allergenic] wine fining agents [casein and egg white]* was adopted at the 2014 General Assembly and incorporated into European Commission regulations. This code supports the good winemaking practices of the Australian wine industry that advocate

further fining and filtration post protein-fining to reduce the risk of an allergic reaction to wine from residual egg, fish and milk protein.

The project team supported the passage of Australian-initiated draft resolutions and dossiers on agar, dimethylpolysiloxane, protease enzymes and potassium carbonate in the OIV expert groups as although these processing aids are permitted in Australia they were not yet permitted for winemaking under OIV regulations. Potassium carbonate was adopted at the 2017 General Assembly and has been added to the list of products authorised for the reduction of the titratable acidity and actual acidity, while the other resolutions have progressed to step 5 in the 8-step resolution process. In addition, numerous draft OIV proposals were reviewed through participation in the Microbiology, Technology, Specifications of Oenological Products, Food Safety, and Consumption and Nutrition and Health expert groups and the Methods of Analysis Sub-commission. Furthermore, the project team provided analytical and other data on manganese and other heavy metal levels in Australian and international wine to demonstrate their natural variability and that they are below any toxicologically relevant levels, and also provided a toxicological justification for not establishing maximum limits for phthalates in wine.

The project team was also represented at the IWTS where APEC wine-importing nations workshopped the standardisation of analytical testing for wine-importing countries. The objective was to promote standardised testing of wine protocols for those countries importing (Australian) wines. It is expected that this will assist to reduce trade barriers for Australian wine producers and limit the impact of incorrect testing on wine trade. One significant vehicle for this has been the APEC WRF and associated Enhanced Risk Controls working group, which is chaired by the AWRI. A major initiative has been an international ring test program for international regulatory laboratories to monitor their performance in testing wine analytes, which is now in its third year. Since its initiation, the correlation of results from laboratories has improved significantly, helping to reduce disputes between export and import markets. Another initiative has been the development of a compendium of the standard analytical methods used for wine testing in different countries as well as initiating a database of typical levels of wine components, which will be curated by the AWRI. Besides chairing this working group, the project team is also represented on APEC WRF working groups looking at standardised export certificates, pesticide MRLs and good regulatory practice, all contributing to ease of trade in the Asia Pacific region.

Through the IWTS, the project team has been a major contributor to papers on the low risk nature of methanol in wine, the need for consistent definitions of sugar and methods for it measurement, issues with the use of sugar free extract in the assessment of wine for adulteration and the inherently low food safety risk of wine from a microbiological standpoint.

The project team also has membership of FIVS and serves on its Scientific and Technical and Sustainability Committees, where work tabled includes papers outlining the lack of correlation between data required for analytical export certificates and actual food safety risks, and the need for international acceptance of results from laboratories accredited to the ISO 17025 standard.

The timely identification and management of scientific and technical trade issues, via the collection, evaluation, generation and dissemination of data on these issues, has helped maintain market access for Australian wine, facilitating the trade of Australian wine nationally and internationally.

Examples include enabling the:

- export of wine which is fully compliant with international provisions for manganese (and other heavy metal) maximum levels in wine (where 25% of all Australian wines contain manganese in excess of the arbitrary 2 mg/L maximum limit previously petitioned by China);

- export of wine (bottled and bulk transported) which is fully compliant with international provisions for phthalate maximum levels in wine; and

- export of Australian wine which is fully compliant with the changing provisions and regulations of its export markets.

The regular involvement of the AWRI with the OIV Expert Groups has ensured that OIV provisions and regulations for wine and winemaking, which are reflected in the complementary European Union provisions and regulations for wine and winemaking, are consistent with, and not more restrictive than, the provisions and regulations to which Australian wine must comply.

A proactive approach is required to continue to maintain market access or open markets for Australian wine. Changes to the regulatory environment of wine composition and production, labelling and marketing need to be anticipated, facilitated and influenced in national and international forums to maintain market access and reduce the potential for regulatory and economic imposts on industry. This can best be facilitated by maintaining and expanding the established effective relationships with key regulatory stakeholders in export markets, such as the OIV, FIVS, Asia-Pacific Economic Cooperation (APEC) Regulatory Forum, International Wine Technical Summit (IWTS), World Wine Trade Group (WWTG), and working with these organisations to provide scientific and technical advice and assistance on relevant issues.

Overall, the project and its associated activities has provided a strong foundation for the industry to continue to confidently export wine which is compliant with the provisions and regulations of its domestic and export markets.

Project 3.2.5– Safeguarding and realising the potential of the Australian wine microbial germplasm collection

Executive summary

The AWRI wine microorganism culture collection (AWMCC) is the largest repository of wine-associated yeast and bacteria in the southern hemisphere. It provides the Australian wine industry with novel, non-commercially available yeast and bacterial winemaking strains for efficient and reliable fermentations and as a means to shape and diversify wine style. The AWMCC is also essential for capturing the value of Australia's investment in microbial strain isolation and development, especially given the current focus on bioprospecting and generating uniquely Australian isolates. It is also fundamental to the success of Wine Australia-funded biological research projects, which depend on ready access to correctly identified strains.

Newly isolated and generated microbial strains are continuously being identified and added to the AWMCC, together with relevant supporting information. From 2013 to 2017, 1,301 new strains sourced from researchers and industry were added to the collection. The AWMCC now contains more than 10,000 wine-related microorganisms with some isolates dating back almost 80 years.

The collection contains:

- more than 3,000 natural yeast isolates and laboratory-modified yeast strains for research
- a wine yeast genome deletion library of more than 1,700 strains
- a laboratory yeast genome deletion library of around 4,800 strains
- more than 1,100 bacterial strains, the majority of which are malolactic bacteria.

The AWMCC is a member of the World Federation of Culture Collections and is part of the Atlas of Living Australia.

Highlights

- During the investment period, the AWMCC received 1,301 microorganisms from industry and researchers (1,012 yeast and 289 bacterial strains) and 2,423 microorganisms were supplied to industry and researchers (1,804 yeast and 619 bacterial strains).
- A back-up collection was established at a secure offsite facility to ensure the ongoing integrity of the collection in the event of a catastrophic failure of the storage facilities at AWRI.
- A total of 1,763 deletion strains from the wine yeast deletion library were provided to researchers at the University of Adelaide for a Wine Australia-funded project.
- A total of 83 previously difficult-to-classify yeast strains were identified, and more than 400 microbial isolates were provided for genome sequencing projects.

Objectives

The objectives of this project were to:

- following OECD guidelines, maintain and further develop a secure state-of-art world-class collection of Australian wine-associated yeast and bacterial strains
- provide quality assurance, to internationally recognised standards (OECD guidelines), of all strains held within the AWMCC
- develop a secure back-up, off-site duplicate microbial collection
- maintain and develop a collection of yeast and bacteria which have been identified to genus and species levels using molecular and/or biochemical techniques
- undertake quality assurance on existing, and new additions of, yeast and bacterial strains in the AWMCC
- provide for additional Australian wine-associated microorganisms, including strains generated and/or isolated in Wine Australia-funded research, being deposited in the AWMCC.

Outcome and conclusion

The AWMCC provides access to the genetic diversity of microorganisms collected over many regions and many decades and this has been crucial for several successful Wine Australia-funded projects. Many strains in the collection pre-date the currently available commercial strains and thus provide a depth and complexity for genetic comparisons that would have been otherwise unavailable.

- More than 200 strains of *Saccharomyces* yeasts from the collection, including 106 commercially available strains gathered over more than 50 years, were genetically sequenced and formed the basis of a study that provided the most comprehensive understanding to date of the genetic diversity of commercially available wine yeast (Borneman et al. 2016).
- Sequencing data enabled the selection of appropriately diverse yeast strains for use in the AWRI's fermentation nutrients project. This project better defined yeast strain-specific performance parameters for a large group of commercially available wine strains, improving the matching of strain to must. The sequence data showed that many commercial strains were genetically very similar, if not identical. This enabled a targeted design incorporating diverse candidates from the newly sequenced strains, rather than choosing randomly from commercial strains which might have provided redundant data and significantly reduced the value of the new knowledge generated.
- Of the collection's more than 360 *Oenococcus oeni* isolates, 200 were sequenced. A subset of these was used for identifying *O. oeni* strains that tolerate malolactic fermentation-limiting wine parameters, grow rapidly and efficiently metabolise malic acid (Costello et al. 2017).
- More than 1,000 of the yeast strains in the collection are non-*Saccharomyces* strains. These were accessed for a project that compared 50 genetically diverse strains to investigate the possibility of using non-conventional yeast to produce wine with reduced ethanol content. An outcome of this project was the identification of a non-*Saccharomyces* strain that could result in lower ethanol when used in sequential inoculations of Shiraz ferments (Contreras et al. 2014).
- The collection contains more than 350 *Brettanomyces* isolates, a genus responsible for wine spoilage and subject to ongoing concern in the wine industry. More than 60 of these isolates were screened as part of a project aimed at developing a monitoring regime to detect the emergence of sulfite-tolerant *Brettanomyces* strains. This project highlighted the importance of having available 'ready-to-go' candidate strains previously deposited over many years, because the number of suitable isolates that become available during any particular vintage can vary widely.
- As part of a study seeking to better understand the importance of timing and strength of yeast flocculation in wine production, a candidate non-flocculating yeast was identified during a screen of 99 *Saccharomyces* strains, with the help of a high-throughput sedimentation assay.

These examples clearly illustrate the importance and value of having instant access to the greatest possible diversity of well-characterised wine-related microorganisms and the AWMCC is the most comprehensive source of such material.

In summary, through this project the AWMCC has been able to grow into a substantially larger, more genetically diverse and operationally secure microorganism collection for the Australian wine industry. Future research projects, particularly those involving bioprospecting and metagenomics approaches for the study of microbial diversity, are expected to deliver several thousand more isolates to the collection in the coming years. To ensure efficient operation and enable support for complex experimental designs involving hundreds of strains, new high-throughput storage, handling

and culturing tools, combined with latest generation sequencing and data mining technologies, will be incorporated. This will improve handling of large numbers of isolates and help capture the full value of this resource for the benefit of the Australian wine industry.

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Project 4.1.1– The staging and conduct of extension programs

Executive summary

The objective of this project was to provide a multifaceted and centralised extension platform to deliver research outcomes, innovations and practical solutions to Australian grape and wine producers. Face-to-face events were delivered through the long-standing Grape and Wine Roadshow program. Roadshow seminars delivered content prepared by researchers from the AWRI, affiliated WIC partners and other organisations. Relevant content was chosen by the local regional association on each occasion, giving an indication of which topics were most relevant in their region at that time. Similar content was also delivered electronically via a webinar platform. Roadshow workshops delivered tailored practical solutions to prevailing wine sector challenges with content prepared by AWRI viticulturists and winemakers. Written extension included preparation of *Technical Notes* and collation of articles for the bi-monthly AWRI *Technical Review,* preparation of articles for AWRI *eNews*, Wine Australia *R&D@Work* and *RD&E news*, *Ask the AWRI* columns and other channels, as well as preparation of content for the AWRI website. *eBulletins* were also prepared when required to provide the wine sector with rapid, early warnings of emerging trends and associated recommendations, based on observations from the AWRI helpdesk.

A total of 117 Roadshow events were delivered over the four years reaching 3,132 attendees, averaging 27 attendees per event. A total of 90 webinars were delivered to 1,463 attendees, averaging 16 attendees per event. A total of 237 articles and webpages were prepared and published.

Evaluation forms were collected after each roadshow event to assess metrics such as reasons for attendance, overall event satisfaction and intention to adopt. Results showed that the Roadshow platform is valued for its delivery of information on the latest research and for the opportunity it gives participants to build on their knowledge base. More than 60% of respondents indicated they would re-evaluate their current practices in the light of information presented.

A total of 97,923 webpage views/year and 20,993 downloads of fact sheets and other packaged material were recorded for the winemaking and viticulture areas of the AWRI website since the new AWRI website structure was updated in 2014, demonstrating the wide reach of this platform. Non-peer-reviewed industry articles, such as those contributed by this project, continue to be the main publications requested from the John Fornachon Memorial Library. Abstracts were collated for each of 24 AWRI *Technical Review* issues published over four years, providing grape and wine producers access to the latest relevant current literature.

Future extension activities will build on the successes of this project and continue to support rapid awareness and adoption of new and improved production and management techniques and technologies. The next extension project will focus on delivering events such as seminars, workshops and webinars to encourage awareness of innovations. New content, including tastings of wines made from research projects, will be introduced so that producers can directly see the benefits of innovations trialled in practice.

The AWRI acknowledges the support of AWRI researchers, researchers from other organisation and individuals that have contributed their time in preparation of material delivered during roadshow seminars and webinars. Acknowledgement of the in-kind support from Australian grape and wine region associations to organise and publicise roadshow events has also been vital in delivering an effective face-to-face extension platform.

Highlights

- 118 Roadshow eventss were delivered over the four years reaching 3,212 attendees, averaging 27 attendees per event
- 90 webinars were delivered to 1,463 attendees, averaging 16 attendees per event
- 237 articles and webpages were prepared
- 97,923 webpage views/year were recorded for the winemaking and viticulture sections of the AWRI website and 20,993 downloads were recorded of fact sheets and other packaged material
- Three new workshops were prepared and delivered:
  - *Adapting to difficult vintages* – providing solutions to growing grapes and making wine in a warmer climate
  - *Addressing regional challenges* – providing solutions to regional issues in each region highlighted by AWRI helpdesk trends
  - *Pinot Noir winemaking trial tastings* – showcasing wines prepared using different winemaking practices.

Objectives

The objective of this project is to raise awareness and facilitate uptake of research outcomes, assist producers to understand the practical value of these outcomes and overcome any potential barriers for adoption. This is achieved by extending research outcomes in a form that encourages adoption by people employed in the Australian wine industry.

Outcome and conclusion

Figure 1 summarises the motivations for attendance at AWRI extension events, as reported in post-event surveys. The primary motives (for 93% of participants) for attendance at Grape and Wine Roadshows were to be regularly informed about current research or new practices and for participants to continually build on their existing knowledge base.

Figure 1. Motivation of participants attending AWRI Roadshows, sourced from attendee surveys.

Table 1 details the target number of extension events versus the number of events delivered for the four years, along with attendance figures and percentage of non-AWRI speakers. All targets were exceeded, both for number of events and for attendance.

Table 1. Number of extension events held, event attendance and percentage of non-AWRI presenters.

Extension event	**Target**	**Delivered**
Face to face presentations		
Roadshow Seminars – number of events	60	69
Roadshow Seminars – number of attendees	1,500	1,967
Roadshow Seminars – percentage of external presenters	10%	21%
Roadshow Workshops – number of events	40	48
Roadshow Workshop – number of attendees	1,000	1,165
Roadshow Workshop – percentage of external presenters	10%	19%
Electronic presentations		
Webinars – number of events	80	90
Webinars – number of attendees	-	1,463
Webinars – percentage of external presenters	40%	60%
Webinars – downloads from the AWRI's YouTube channel	-	10,430

Roadshow feedback forms were handed out at the conclusion of each event. Participants were asked to rate the roadshow overall, as well as the usefulness to their business. Feedback showed that 87% of participants rated the events as *good* or *excellent* and 76% found the information presented useful. This confirms the success of face-to-face communication through the Grape and Wine Roadshow platform.

Adoption and intention to adopt were captured by asking the following question:
As a result of what you have heard today, what actions will you take:

- I will make no changes
- I will reassess current practice/consider alternative choices
- I will seek extra information online
- I will discuss possibilities with my peers/consultants/retailers/AWRI/government organisation
- I will change my current practices

A total of 62% of participants indicated they would re-evaluate their current practices based on what they had heard, demonstrating that Australian grape and wine producers continue to demonstrate a continuous improvement mindset. In terms of intention to adopt, 48% of participants indicated they would seek extra information about the new practices mentioned or would discuss adoption with peers, business decision-makers or key influencers after the roadshow. Only 9% of participants said they would immediately adopt the new practices mentioned in the roadshows with no need to seek out any further information. This data is consistent with findings found in Hill et al. (2014) which demonstrated that Australian grape and wine producers primarily adopt innovations in a multi-tiered process; first they need to be made aware of the new innovations via several channels, they will then

discuss the innovation with trusted peers and local networks, and then they need to see the innovation trialled in practice, before adopting it themselves.

Table 2 details the targets and delivered numbers of written extension material for the four years. All targets were exceeded except for AWRI *eNews* articles, where one fewer article than the target number was produced. Written articles produced through this project regularly feature among the most popular requests to the AWRI library with one example, the 'Ask the AWRI' column on unmanned aerial vehicles (drones), the most requested publication during the 2016/17 financial year. Non-peer-reviewed publications continue to be the preferred platform to initially inform grape and wine producers of new innovations, changes or practical solutions, with material then reinforced during face-to-face extension events. Producing content to be delivered to the 3,600-strong distribution list for AWRI *eNews* and *eBulletins*, as well as material for Wine Australia subscribers and publications in a variety of trade magazines, gives a broad platform to ensure the content has the widest possible reach for grape and wine producers.

Table 2. Target and delivered number of written extension material for the four years

Extension event	**Target**	**Delivered**
Written material		
Technical Review Technical Notes	8	10
AWRI *eNews* articles	48	47
Wine Australia R+D@Work columns	12	17
Wine Australia eNewsletter articles	20	41
Ask the AWRI columns	24	47
AWRI *eBulletins*	8	25
Technical Review Abstract Reviews	24	24
AWRI website webpage additions	48	50

An example of the information reach and information reinforcement strategy is demonstrated by the multi-channeled approach used to inform producers about changes to water addition regulations that occurred in early 2017. An initial e*Bulletin* was produced to inform stakeholders of the changes to regulations about water addition to musts. An *Ask the AWRI* article was then produced highlighting the possible quality implications of adding water to musts. A water addition calculator was prepared and added to the AWRI website. An AWRI webinar was also prepared and presented by researchers from the University of Adelaide working in this area. A *Technical Review* article was then produced which demonstrated some more practical aspects of water additions. A Roadshow workshop presentation was prepared to deliver the information in face-to-face events. In addition, a new research project commenced during the year to evaluate the practice during the 2017 vintage.

Extension of material via the AWRI website had the widest reach of all written communications. An average of 97,923 webpage views per year occurred for the winemaking and viticulture areas of the website since the AWRI website structure was updated in 2014. Additionally, 20,993 fact sheets and other pieces of packaged material were downloaded since 2014, demonstrating that this channel should not be overlooked in extension delivery. Material communicated via the website has an advantage over face-to-face channels in that material is always accessible once uploaded, rather than just being available at a single point in time. An example of this is the 90 webinars that were delivered to 1,463 participants that attended the webinars over the four years of the agreement. Following those events, 10,430 views, or ten times the initial audience of the webinars, have occurred since webinar recordings were added to the AWRI YouTube channel on 6 November 2014. Communication of research outcomes, new innovations or practical solutions via video channels rather than text, or

demonstrations performed online or via YouTube, is thus a powerful and proven tool to communicate with grape and wine producers moving forward.

Looking to the future, the next AWRI extension project will build on the successes of this project and continue to support awareness and adoption of new and improved production and management techniques and technologies. The aspect of the current project that focuses on generation of content for written communication platforms will be incorporated into the communication project under the next investment agreement. The extension project will focus on delivering events such as seminars, workshops and webinars to increase awareness and understanding of available innovations. Tastings of wines made from research projects will be introduced so that producers can see the benefits of innovations trialled in practice, to encourage greater adoption of the innovations or practice changes. The combination of these channels will ensure grape and wine producers are aware of innovations, minimise barriers to adoption and reduce adoption timeframes for Australian producers.

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Project 4.1.2– Specialised technical troubleshooting and responsive helpdesk services for the Australian wine sector

Executive summary

The AWRI helpdesk provides timely and confidential support for technical problems encountered by Australian grapegrowers and winemakers. Services include identifying the root causes of problems, and providing research-based, practical, up-to-date remediation solutions, as well as future prevention strategies. The service also ensures that Australian grapegrowers and winemakers are competitive on the world stage, by having the latest technical information readily to hand. Investigative services, including analysis of problem grapes or wine, are conducted when a problem cannot be solved through discussions with the producer.

Activities in the helpdesk project over the investment period have been critical in identifying and resolving technical issues and have provided knowledge to industry personnel across areas including grapegrowing, winemaking, packaging, regulatory, trade and scientific matters. The adoption of advice and solutions provided by helpdesk also prevented sub-standard fruit or wine from entering the supply chain. In instances where grape or wine value was not recoverable, the technical reports provided by the services enabled producers to pursue insurance or legal claims, including claims arising from the supply of inferior processing aids, additives or packaging materials. The project also contributed to the resolution of issues which, if left unresolved or repeated by other wineries, could incur losses or unnecessary remediation costs, create inefficiencies or potentially damage brands and reputations.

The AWRI helpdesk also provided an early warning system for emerging technical issues, generating information which was applied to prioritisation of research and extension activities. A number of the trends observed in helpdesk enquires during the investment period were related to extreme weather events or climate change and included issues such as earlier harvest dates, higher sugar levels and stuck fermentations, vintage compression, agrochemical issues and smoke taint. This information was used to inform the development of the 'Adapting to difficult vintages' workshop delivered by the extension project.

During the investment period helpdesk staff responded to and confidentially answered 7,647 queries, and performed 860 winemaking investigations. The service was accessed by producers of varying size, from the smallest to the largest Australian grape and wine producers. On average, approximately 11% of enquiries turned into investigations where wines were requested and submitted for further analysis and investigation. Each client who used the investigation service was surveyed, and the results (shown in the Outcome and Conclusion section below) reflect the high value users put on this unique service.

Future directions for the project will see the helpdesk develop more of an online platform for industry, improving access, response times, image capture and reporting, while maintaining the personal contact and advice that is highly valued by industry personnel. It will also be involved in trials of new technologies and the making of wine to demonstrate issues or innovations.

Highlights

- Helpdesk staff responded to 7,647 requests for technical advice, covering grapegrowing, wine production and scientific and regulatory-related matters.
- More than 80% of enquiries were answered within 24 hours.
- Helpdesk staff performed 860 winemaking investigations
- A rapid response was provided to regions affected by smoke from significant fire events in 2014, 2015 and 2016 (South Australia, Victoria, Tasmania, Western Australia). This included face to face Q&A sessions, practical management techniques post-harvesting and detailed discussions and interpretation of analytical results.
- To assist with the interpretation of smoke taint analytical data, a database of background levels of smoke-related phenols and phenolic glycosides was established, initially with five common grape varieties, and later expanded to include a total of ten varieties, namely: Semillon, Chardonnay, Sauvignon Blanc, Riesling, Pinot Gris, Pinot Noir, Merlot, Cabernet Sauvignon, Shiraz, and Grenache.
- Assistance was provided to help wine exporters understand and adapt to regulatory changes regarding metal content (particularly manganese) in wine exported to China
- The helpdesk addressed a number of enquiries regarding sooty mould in vintage 2017. This has been identified as a priority issue for some regions, and has led to the commencement of new research activities

Objectives

To provide rapid support to grapegrowers and winery production staff on technical and scientific issues encountered during the production of wine.

The key objectives are to:

- provide the Australian wine sector with real-time access to experts from a range of disciplines to provide pre-competitive solutions to grape and wine production, composition and processing problems and scientific industry issues
- identify and solve technical problems
- provide Wine Australia, Winemakers' Federation of Australia and Australian Vignerons with an early warning system for industry-wide trends and emergencies, generating information that can be applied to the prioritisation of research activities.

Outcome and conclusion

The key objectives in this project were successfully met.

Over the investment period, helpdesk service staff responded to 7,643 requests for technical advice, covering grapegrowing, wine production and scientific and regulatory-related matters, with more than 80% of the enquiries answered within 24 hours. A breakdown of the queries received by major category and by year is shown in Table 1.

Table 1. Enquiries received by the AWRI helpdesk from 2013/2014 to 2016/2017

	2013/14	2014/15	2015/16	2016/17
Winemaking	1,309	1,265	1,336	1,117
Viticulture	466	487	657	483
Regulatory	178	150	99	96
Total	**1,953**	**1,902**	**2,092**	**1,696**

Queries received were classified further using a range of key words, enabling trends in technical issues to be monitored at a more specific level. Figure 1 shows the most commonly used key words for queries received over the four-year investment period, with smoke taint the most dominant issue, followed by taints and contaminations.

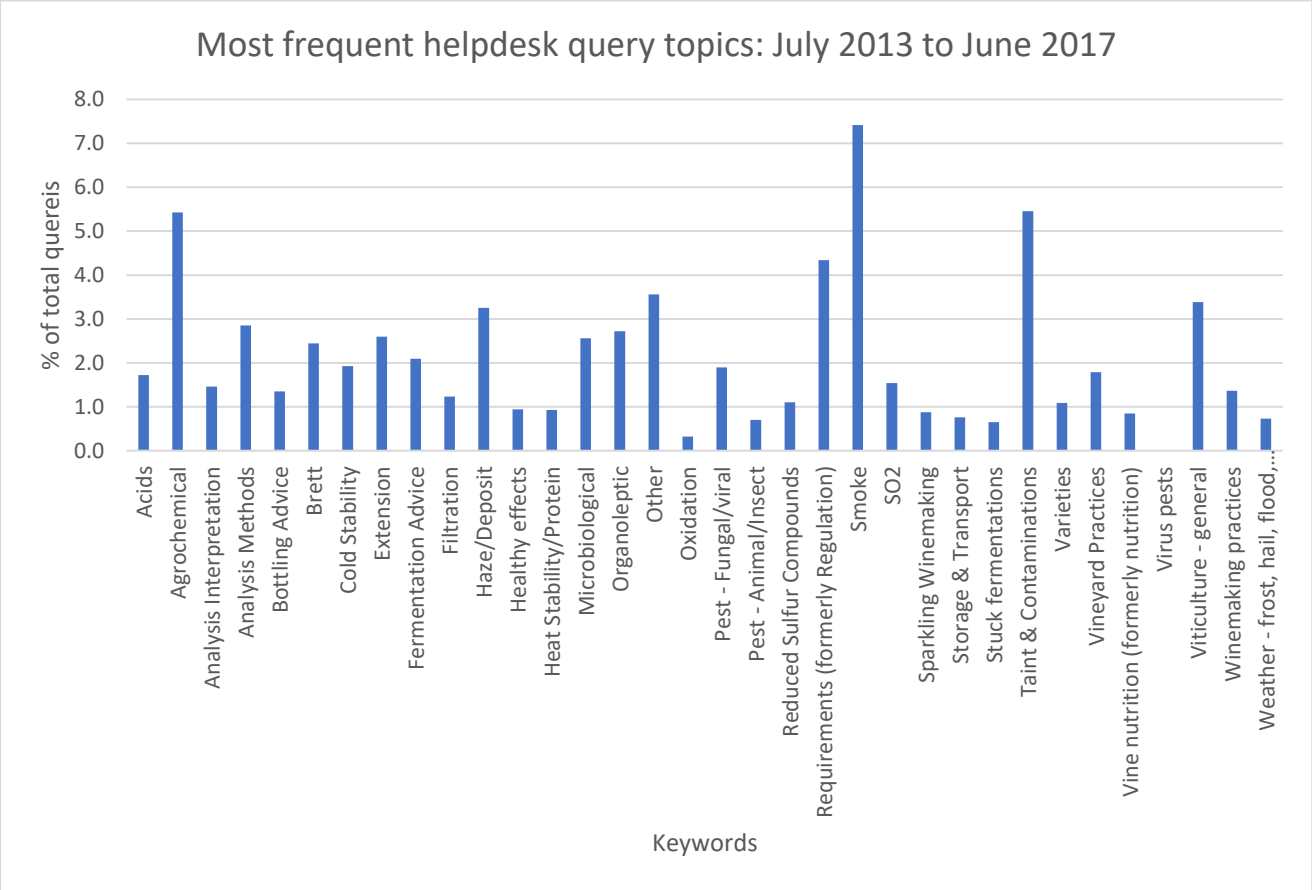

Figure 1. The most frequent helpdesk query topics over the period from July 2013 to June 2017

Helpdesk service staff performed 860 investigations, providing detailed understanding of the issue(s) and steps for remediation where possible. The types of investigations conducted are broken down in Table 2.

Table 2. Types of investigations conducted by the AWRI helpdesk during the investment period

Investigation type	Number of investigations
Haze and deposits	240
Sensory	213
Closure	19
Microbiological	102
Taints and contaminations	175
Other	111
TOTAL	**860**

Identification of hazes and deposits was the most common type of investigation, followed by sensory-related problems and taints and contaminations. Microbiological problems also made up a significant proportion of the issues investigated. Efforts have been made to address the types of problems that are seen most commonly through extension and communication activities.

Impact and measures of success
Each client who used the investigation service and submitted samples for investigation was surveyed. The survey was automatically sent out once the investigation had been completed, and included an email reminder after a period of seven days. The survey response rate was around 9%.

The survey results indicated:
- 86% of respondents rated the access to the helpdesk as easy, or very easy.
- 88% of respondents were satisfied or very satisfied that their problem had been resolved.
- 83% of respondents indicated that it was easy to understand the solution to their problem.
- 70% of respondents were able to avoid product loss through contacting the AWRI helpdesk.
- The potential value of wine involved in the 860 investigations conducted was estimated to be $69m.
- 97% of respondents indicated the suggestions provided by the helpdesk team were helpful.
- 72% of respondents indicated that they would change their practice based on recommendations provided by the helpdesk team to prevent a repeat of the problem.

Economic return on investment
Each client was asked to indicate how much wine was involved in the investigation conducted, and the quality grade of the wine involved. With these two figures, an approximate cost of the wine involved in the investigation could be estimated. The quality gradings were limited to unfinished, premium or ultra-premium. The dollar value attached to each of the quality gradings was based on export approval data (sourced from Wine Australia). Bulk wine was assumed to be $0.98 per litre, premium wine was assumed to be $5.94 per litre and ultra-premium wine $14.81 per litre. The figures used were an average of the price of white and red wine in these quality ranges.

The value of wine involved in the investigations (potential direct savings) ranged from $0 to $594,000 with a mean value of $81,029. Extrapolation of the data to the total number of investigations conducted (860) suggests the potential direct savings over the project was $69,685,083. This equates to a return of 25:1 on a $2,750,418 investment from Wine Australia over the investment period. It should be noted, however, that while this figure of 25:1 is based on the work of the helpdesk as a discreet project, in reality the success of the project is highly reliant on the

rest of the work at the AWRI and elsewhere which informs the investigations conducted and advice given.

Direct feedback
The impact of the helpdesk service can also be assessed based on direct feedback from users. A selection of quotes is reproduced below.

> "Changes in cellar operations were justified with AWRI's assistance"
>
> "Excellent service and recommendations"
>
> "Without doubt the AWRI are the pinnacle in terms of winemaking services worldwide. Over the past 8 years we have won many trophies and gold medals both in national and regional shows and I feel that this is a direct result in the professional advice we receive from the AWRI team. The regional workshops are invaluable and greatly in need of more throughout the industry to improve the quality across the board. Without the AWRI we have no industry, out of 10 I give them 11."
>
> "Always very helpful and efficient"
>
> "Thanks so much. Thanks again, such an amazing resource."

Future directions for the AWRI helpdesk
The next AWRI helpdesk project will continue to deliver the technical support and investigatory services that are so valued by the Australian grape and wine community. At the same time, it will work towards developing a more efficient online platform that will improve access and response times. In addition, the helpdesk will conduct trials of new equipment/additives of potential benefit to the Australian wine industry, and each year will make a series of wines to illustrate technical issues or innovations which will be presented to industry via the AWRI roadshow program.

Project 4.1.3– Library service

Executive summary

The John Fornachon Memorial Library (JFML) holds the largest knowledge base of grape and wine resources in the southern hemisphere and offers specialised information services and resources to the Australian grape and wine sector. Since 2013, most of the items sourced have been in digital format and the search tools that are offered are available online at all times via the AWRI website.

The library collection comprises 87,800 items covering 'vines to wines' and this valuable industry-owned resource is the backbone of a suite of information services available to the Australian grape and wine sector. More than 90% of the 9,569 articles delivered by the project over the investment term were provided from the library's own collection and the remaining requests were fulfilled via inter-library loans. Library services are used by levy payers as well as government, research organisations, students, suppliers and other participants working across the Australian wine sector.

Requests for technical information about oenology and viticulture remain popular. Resources on consumer and marketing studies, climate and the environment, sustainability and grape varieties are expanding due to a growing user demand. The library will continue to monitor this demand and other emerging trends to ensure acquisition activities align with current and future user needs.

The provision of user-friendly online tools is also a focus for the library as more users choose online platforms to find information. The eBooks platforms first introduced in 2013 has been expanded and now includes over 120 titles, providing another way for industry members to access grape and wine publications at any time and from any location.

Looking forward, the next Library project will continue to seek innovative ways to deliver technical and scientific content, and facilitate the use of that information and knowledge, through the use of digital technologies.

Highlights

Project highlights over the investment period include:

- More than 16,000 items were added to the library collection and many of the total 87,800 items are now in digital formats.
- Over 5,900 requests were responded to, with 9,569 articles delivered in that period. Over 80% of requests were completed within one business day.
- A version of the library catalogue optimised for viewing on mobile phones and tablets was launched, enabling users to search and order items using mobile devices.
- An eBook collection was launched and then expanded to two eBook platforms.
- Online information packs were added to the AWRI website in 2013 and usage has steadily increased in the last three years.
- Library resources and services were showcased at the Australian Wine Industry Trade Exhibition in 2013 and 2016.

Objectives

The key objectives of this project were to:

- maintain a comprehensive collection of technical and scientific resources covering 'vine to wine'
- ensure easy access to the library collection for Australian wine sector participants, government and other research institutions.

Outcome and conclusion

Maintain a comprehensive collection of technical and scientific resources covering 'vine to wine'

Over the last five years, the library has added over 16,000 records to its library catalogue. Many of its 87,800 items are now in digital format. A Collection Development Plan is reviewed on a regular basis to ensure there is a strategic approach to sourcing and maintaining this industry-owned collection of grape and wine publications and resources. Items are acquired as a result from user requests and monitoring the latest materials published on grape and wine production. Information request trends suggest an increasing demand for information and resources relating to consumer and marketing studies, climate and the environment, sustainability and varieties. Investing in resources and topic areas experiencing strong demand is a priority.

All items in the library collection are catalogued in a database, which is available for levy payer access via the AWRI website. Where possible, permissions to include abstracts of books and articles are sourced from the copyright owners and abstracts are added to the catalogue to help library users to find relevant information efficiently.

Since a 2014 launch, the library's eBook collection has seen steady growth. To ensure growth and a diversity of titles, a second platform was added in 2016. As it currently sits, the collection holds 120 titles across a broad range of industry relevant topics. All eBooks are catalogued within the library database and access is integrated via the AWRI website.

Ensure easy access to the library collection for Australian wine sector participants, government and other research institutions.

The library catalogue is the primary search tool and is available on the AWRI website. A version optimised for viewing on mobile phones and tablets was launched in 2013/14, making the catalogue more accessible via mobile devices. Specialised online databases, maintained by this project, are also available on the website:

- The staff publications database contains citations of over 1,900 publications authored by AWRI staff. The AWRI publications section of the website received over 27,000 hits from July 2013 to June 2017 and over 3,330 staff publications were requested in that time.
- Online information packs are topic-based and developed to help users easily locate information on common topics such as oenology, viticulture, sustainability, and wine and health. There are now 17 information packs available online and the use of these packs is steadily increasing over the last two years, averaging 1,765 hits per year.

The library also offers literature search services for in-depth research and retrieval of information.

Overall, the library received over an average of 1,480 document delivery requests per year and an average of 2,340 articles were dispatched each year.

Brettanomyces is the most requested topic for AWRI staff publications, followed by articles relating to smoke taint. The top five requested articles between July 2013 to June 2017 are listed in Table 1. Both *Brettanomyces* and Smoke taint were topical issues during the investment agreement period as reported in the AWRI helpdesk project.

Table 1. The top five requested articles from the JFML during the investment period.

Article
Coulter, A. Post-bottling spoilage - who invited Brett? *Aust. N.Z. Grapegrower Winemaker* (559): 78-86; 2010.
Coulter, A., Robinson, E., Cowey, G., Francis, I. L., Lattey, K., Capone, D., Gishen, M., Godden, P. Dekkera/*Brettanomyces* yeast - an overview of recent AWRI investigations and some recommendations for its control. Bell, S. M. (ed.). *Impacts on wine flavour: proceedings of a seminar;* 10-11 July 2003, Barossa Convention Centre, Tanunda, S.A. Australian Society of Viticulture and Oenology: Adelaide, S.A.: 41-50; 2003.
Curtin, C., Bramley, B., Cowey, G., Holdstock, M., Kennedy, E., Lattey, K., Coulter, A., Henschke, P., Francis, L., Godden, P. Sensory perceptions of 'Brett' and relationship to consumer preference. Blair, R.J. (ed.). *Proceedings of the thirteenth Australian wine industry technical conference,* 29 July-2 August 2007, Adelaide, S.A. Australian Wine Industry Technical Conference Inc.: Adelaide, S.A.: 207-211; 2008.
Curtin, C. D., Borneman, A. R., Henschke, P. A., Godden, P. W., Chambers, P. J., Pretorius, I.S. Advancing the frontline against Brett: AWRI breakthrough offers potential to transform the battle against Brett. *Wine Vitic. J.* 26 (6): 18-25; 2011.
Ristic, R., Osidacz, P., Pinchbeck, K. A., Hayasaka, Y., Fudge, A. L., Wilkinson, K. L., The effect of winemaking techniques on the intensity of smoke taint in wine. *Aust. J. Grape Wine Res.* 17(2): 29-40; 2011.

The key communication channels used to promote the library's resources and services include:

- A dedicated 'Information Services' section on the AWRI website
- Newly published AWRI publications listed in every issue of AWRI *eNews* and each year in the AWRI's Annual Report
- *eBulletins* and *eNews*
- The Australian Wine Industry Trade Exhibition, held every three years in conjunction with the Australian Wine Industry Technical Conference.

Library and information services contribute to advancing the knowledge and capabilities of the whole industry. Without these services, grape and wine producers would need to individually invest in setting up their own libraries and spending additional time researching and accessing information. The ability to access an industry-owned suite of library resources and services means producers can focus on their core business of growing grapes and making wine. By having easy access to the latest published research and technical information, they can build their understanding of innovations and learn how new technologies and processes can be implemented in their vineyards and wineries.

Over the last five years, the methods by which grape and wine producers contact the library have changed. More than 80% of library requests are now received via the AWRI website or email, reflecting an increasingly online audience. This change is being taken into account in future plans for the JFML, which will have an increased focus on digital resources and platforms, so as to maintain the library's relevance and continue to provide efficient access to relevant and valuable information for the benefit of grapegrowers and winemakers and other library users.

Project 4.1.4– Communication with stakeholders

Executive summary

This project's primary purpose was to deliver timely and accurate communications to Australia's grape and wine producers in order to enhance awareness and adoption of R&D outcomes. It was designed to work closely with, and complement, the activities in Projects 4.1.1. (Extension), 4.1.2 (Helpdesk) and Project 4.3.1 (Regional nodes).

Activities in this project included production of a large range of electronic and hard-copy industry publications such as the AWRI website, Annual Report, *eNews* and *eBulletins*, *Technical Review*, the AWRI Report, 'Ask the AWRI' column, 'Alternative varieties' column and other trade articles. An active presence in social media, management of the AWRI's interactions with traditional media and promotion of the AWRI webinar program were also included.

Over the investment period the project delivered edited content for publication in a wide range of different outlets (see Table 1 in the 'Outcome and conclusion' section below).

Success of the project in reaching its target audience was demonstrated in a number of ways including: website hits, growth in subscriber numbers for the AWRI's electronic newsletters and bulletins, growth in social media following, requests for articles from readers of *Technical Review,* media interest in AWRI stories, and feedback from editors of trade journals and content consumers.

Communication with stakeholders via electronic and hard copy media will continue under the new investment agreement with Wine Australia, including an exploration of possible new formats for delivery of information, informed by research about how people access information.

Highlights

- The major update of the AWRI website in January 2016, which gave the site a fresh new look, made navigation easier and was accompanied by a major review and update of content. This, along with the removal of password protection from some areas of the site, greatly improved usability and resulted in increased web traffic.
- Delivery of more than double the target number of industry articles over the term of the agreement– ensuring AWRI research was regularly being translated into formats tailored to an industry audience
- Coordination of AWRI contributions to a special issue of the *Australian Journal of Grape and Wine Research* commemorating the AWRI's 60th birthday that featured 18 review articles authored by AWRI staff.
- Use of the *eBulletin* format to respond quickly to industry issues such as floods, frosts, heatwaves, bushfires, pest/disease outbreaks and changes to agrochemical recommendations.
- Addition of webinar recordings to the AWRI YouTube channel – greatly increasing the reach of this important communication mechanism.
- Close cooperation with the helpdesk and extension projects that ensured consistent messaging and relevant up-to-date content.

Objective

- Enhance the awareness and adoption of R&D outcomes in the Australian wine sector through timely and accurate communication.

Outcome and conclusion

Table 1 summarises achievement against publication targets during the project term. Cumulative targets for the four years were all met or exceeded, with those for industry articles and *eBulletins* significantly exceeded.

Table 1. Achievement of project targets

Outcome	Number achieved	Target
AWRI Reports in *Wine & Viticulture Journal*	24	24
Alternative varieties columns in *Wine & Viticulture Journal*	24	24
Ask the AWRI column in *Australian and New Zealand Grapegrower and Winemaker*	48	48
Articles in non-peer-reviewed industry journals	53	24
Issues of *AWRI Technical Review*	24	24
Issues of *AWRI eNews*	24	24
AWRI *eBulletins*	72	48
Media releases	19	As required
Articles in GWRDC Innovators' Network eNewsletter/Wine Australia RD&E news	54	48
Articles in GWRDC/Wine Australia R&D@work	29	24
AWRI Annual Reports	4	4
Webinars promoted	92	80

Articles and regular columns in industry journals were a valued platform for communicating with grape and wine producers about latest research results and providing practical advice on specific technical issues. As an example, the 'Ask the AWRI' column about unmanned aerial vehicles (drones) published in *The Australian & New Zealand Grapegrower & Winemaker* was the most requested publication from the AWRI's John Fornachon Memorial Library during the 2016/17 financial year, demonstrating the relevance of this style of communication.

eBulletins were also an essential vehicle for reaching grape and wine producers quickly, particularly in relation to weather events or emerging technical issues. One example where an *eBulletin* assisted practitioners to adapt quickly to a weather-related event was the *eBulletin* issued in January 2014, which warned of an impending heatwave across southern Australia. By drawing on the latest forecasting tools from the Bureau of Meteorology, the AWRI helpdesk was able to issue the warning before the heatwave's arrival and provide advice about irrigation practices to adopt before, during and after the heatwave to minimise damage to fruit and vines. Some of the key advisory *eBulletins* issued during the project term are listed in Table 2.

Topics initially addressed via *eBulletins* were also often followed up by other communication channels. For example, soon after the *eBulletin* on frost issued in November 2013, a webinar was held, and this proved to be one of the most attended webinars during that year. This approach of combining different communication channels has been used across the project to ensure messages reach the widest audience possible.

Table 2. A selection of key *eBulletins* issued during the project term

Title	Date
Support available in response to smoke taint and frost	15 November 2013
Timely reminder about disease risk	13 December 2013
Two current issues: heatwave warning and impact of elemental sulfur residues	9 January 2014
How should I deal with split fruit and disease pressure as harvest approaches?	18 February 2014
Vineyard sprays – clarifying label directions	24 February 2014
Support available on two recent viticultural issues – hail damage and restricted spring growth	8 December 2014
Dealing with fire damage and smoke taint	12 January 2015
Increased risk of bunch rots following heavy rain	16 January 2015
Managing late season wet weather	20 January 2016
Stuck ferments need a rapid response	19 April 2016
Managing vineyards after a wet winter and spring	23 September 2016
Weather update, managing waterlogged vineyards and additional re-entry period information	5 October 2016
Change to Food Standards Code regarding addition of water to high sugar must/juice	16 February 2017

Tables 3 and 4 presents some metrics of audience reach over the four years of the project term. Key points to note are the increases in audience of the AWRI website, *eBulletin* and *eNews* and social media channels. These reflect trends seen in the broader community of improvements in internet access and growth of smart phone use. This increasing importance of electronic media will be taken into account in the planning of future communication activities, without forgetting the importance of more traditional platforms.

Over the project term, social media was used as a way to promote other communication channels and draw attention to key messages and online resources. The AWRI's YouTube channel proved to be a popular platform for viewing of AWRI webinars and other video resources.

The AWRI Annual Report was distributed by mail to all Australian levy payers, ensuring all levy payers were reached at least annually, even if they were not engaged with other AWRI communication channels.

AWRI *Technical Review* is now predominantly an online publication, with just a small number of hard copies printed. Each new issue of Technical Review was promoted via an *eBulletin* and through social media. Library requests for articles included in the 'Current Literature' section averaged at over 800 per year, indicating that this format is still valued by readers as a way to access the most relevant grape and wine related literature.

Wine Australia's monthly electronic newsletter and *R&D@work* column were other important avenues for communicating with the grape and wine community. The 'researcher profiles' included in *R&D@work* were a great way for readers to get to know some of the AWRI's scientists and put a human face to research outcomes.

Table 3. Metrics of audience growth between 2013 and 2017

	2013	**2017**
Growth in annual website visitor numbers	~50,000	~78,000
Growth in annual website page views	~255,000	~336,000
Growth in *eNews* subscribers (distributed every second month)	~2,600	~3,600
Growth in *eBulletin* subscribers	~2,600	~3,250
Growth in Twitter following	~1,800	~3,300
Growth in Facebook 'likes'	~185	~750

Table 4. Additional metrics of audience reach

YouTube views of AWRI webinars since November 2014	10,430
Annual report distribution	~3,700 hard copies distributed to Australian levy payers annually and electronic version available online
Articles requested following their inclusion in the 'Current Literature' section of *Technical Review*	3,304 over four years
GWRDC Innovators' Network eNewsletter/Wine Australia RD&E news (distributed every month)	~1,700 subscribers as at March 2016
GWRDC/Wine Australia R&D@work	Printed in every second issue of *Australian New Zealand Grapegrower and Winemaker*

The next steps for the AWRI's communications with stakeholders over the term of the new investment agreement with Wine Australia will include:

- Creation of new and relevant content for internal and external AWRI communication platforms. This process is led by the AWRI helpdesk team and draws on their regular contact with industry and detailed understanding of emerging industry issues.
- Maintenance and update of digital tools (e.g. apps and calculators).
- Liaison with AWRI researchers to assist with translation and packaging of research outcomes into formats suitable for different communication platforms.
- Coordination, editing and delivery of a wide range of electronic and hard copy publications for an industry audience.
- Management of the AWRI's corporate communications and branding, ensuring that all AWRI communications are of a high standard. This includes editing of reports, grant applications and other corporate documents, and management of the AWRI's media presence including promotion of outcomes from Wine Australia-funded research, development and extension activities.
- Exploration of new formats for delivery of information, drawing on social science research related to how people access information.

Project staff will also continue to work closely with Wine Australia to ensure harmonised RD&E communications for the Australian grape and wine community.

Project 5.1.3– Efficient management and administration

Executive summary

The AWRI's research, development and extension activities are underpinned and enabled by leadership and essential services provided by the Corporate Services group. The group works closely with the AWRI Board to provide an appropriate mix of strategic, commercial and scientific leadership, guidance and support to all AWRI staff, and increases the efficiency of all Wine Australia investments at the AWRI by allowing operational staff to focus on their core activities with minimal administrative and commercial demands.

While predominantly supported by Wine Australia, the costs of delivering the Corporate Services function over the four-year agreement term were significantly defrayed by contributions made by the AWRI's Commercial Services group, projects not funded by Wine Australia, or otherwise underwritten by the AWRI, which together contributed more than $3.3 million.

These services will continue to be delivered in a broadly equivalent manner from July 2017 under renewed funding arrangements between the AWRI and Wine Australia.

Highlights

Most activities of the Corporate Services group are routine in nature, supporting the broader achievements of the research, development, extension and commercial teams across the AWRI. The following achievements of the group are however worthy of specific recognition:

Progress towards RDE reform

Towards the later stages of the agreement the AWRI worked closely with Wine Australia on reforming the Australian grape and wine research, development and extension (RDE) system to improve the productivity of the system for its investors and better integrate RDE outcomes with marketing activities. Some outcomes of this work (for example, the AWRI playing a more active role in Wine Australia's market development activities) were implemented during the term of the agreement, others (such as the AWRI playing a direct role in the administration of the Regional Program) are expected to become effective from July 2017 as part of the replacement investment agreement, while further elements remain prospects for reform over the longer term.

Charitable registration and concessional tax status

The AWRI was granted charitable exemptions under payroll taxation legislation in South Australia, Victoria and Queensland (all states in which it has employees). These exemptions, together with registration as a charity with the Australian Charities and Not-for-profit Commission, and preferential status with suppliers such as Microsoft, have allowed the AWRI to further lower its cost base and deliver even greater value for its industry stakeholders.

Supporting other wine industry organisations

The AWRI assisted numerous smaller wine industry organisations through the provision of secretariat and other support services, including to the Australian Wine Industry Technical Conference Incorporated, Wine Innovation Cluster (WIC), WIC Winemaking Services, the Interwinery Analysis Group, and Bioplatforms Australia, as well as discrete events including Crush symposia, the VALO program, International Wine and Health Conference and the Yeast: Products and Discovery meeting of the Australasian Yeast Group.

Diversified investment strategy
A new investment policy was developed in order to address risks associated with concentration of investments within a single class of assts. This resulted in the appointment of an external investment manager, and the approval and implementation of a conservative investment strategy which saw a significant component of the AWRI's reserves invested across a range of new investments (all of which are quoted and actively traded on the Australian Securities Exchange), in addition to a component retained in fixed interest instruments. The enhanced returns expected to be generated by this portfolio over the longer term will enable the AWRI to further invest in critical capabilities and activities to support the Australian grape and wine industry.

Development and implementation of an IT Strategic Plan
In 2015 an IT Strategic Plan was developed, setting out a roadmap for step-changes in IT infrastructure, service delivery and knowledge management within the AWRI over the subsequent three years in the first instance. The implementation of that plan is substantially progressed but remains ongoing, financially supported by the Strategic IT reserve previously created by the AWRI Board. As a knowledge organisation, the enhancements to the AWRI's IT environment achieved through this plan add considerable value to almost every aspect of the organisation's operations.

New Director election process
Following extensive negotiations with the Department of Agriculture and Water Resources and amendments to the AWRI's constitution, an electronic ballot process was developed and used for the first time in October 2014 to elect Directors to the AWRI Board. This development simplifies the voting process for levy payers, encourages greater participation in voting, and greatly improves the efficiency of administering elections.

Strengthening of workplace health and safety (WHS) systems
Partly in response to shifts in legislative requirements, the AWRI reviewed and strengthened various aspects of its WHS management processes, resulting in the renewal of the AWRI's WHS policy, education sessions conducted for the AWRI's employees and Directors, and enhanced regular reporting to the AWRI Board and senior management. An external WHS review was completed by an industry expert, leading to a range specific WHS initiatives including a contractor management framework, regular defensive driver training sessions, improved training record management and a greater focus on chemical management. More recent efforts have focused on the promotion of mental well-being.

Renewed focus on the AWRI's 'Employer of Choice' program
The AWRI's 'Employer of Choice' program was substantially refreshed and now features an increased focus on health and well-being, with specific initiatives including 'healthy heart' assessments, skin cancer checks, influenza vaccinations, subsidised Pilates/yoga classes, and access to fresh fruit and freshly ground coffee, with other new benefits including a workplace banking program, Microsoft products for home use, and access to preferential wine programs in conjunction with industry partners. It became evident that sufficient access to professional development opportunities represents an important element in ensuring the AWRI's status as an employer of choice, and such access was greatly enhanced through availability of a broader range of financial support, most significantly from the AWRI's Directors who throughout the agreement elected for the organisation to retain many of their directorship fees to support such activities. These changes were significantly informed by annual Human Resources surveys, introduced to monitor employee satisfaction, cultural metrics and various aspects of the AWRI's employer of choice status, as well as capturing employee input in developing and refining workplace conditions and benefits.

Strengthened risk management framework
The AWRI revised and strengthened its risk management framework, encompassing a substantial overhaul of its Risk Oversight and Management Policy as well as the establishment and maintenance of an operational risk register and strategic risk register. Both registers are subject to periodic rounds of review by the AWRI's management team and Board, and risk is now included as a standing agenda item in all senior management meetings.

Objectives
This project will support the activities of the AWRI operational staff through the efficient and effective provision of services including:

- Leadership and administration
- Business development
- Contract management
- Finance and accounting
- Human resources
- Information technology
- Operations management
- Risk management, including workplace health and safety
- Corporate governance.

Outcome and conclusion
The Corporate Services group now operates with a staffing complement around a third less compared to a decade prior, despite a modest increase to the number of operational staff and substantial growth in total revenue over this time. Both internal benchmarking and external feedback indicate that the cost base of the AWRI's Corporate Services group is reflective of Australian and international best practice. Despite constrained resourcing, the level of service provided by Corporate Services across the AWRI and to other wine industry organisations is highly regarded – the positive working environment (naturally, owing to a range of factors) is well reflected in the results of annual staff surveys, which routinely show well over 90% of respondents confirming that 'all things considered, the AWRI is a great place to work'.

Appendix 1: Presentations delivered by AWRI staff between 1 July 2013 and 30 June 2017

Staff	Title of presentation	Presented to and where	Date
C.S. Stockley	Wine, health and social responsibility	South Australian Wine Industry Council, Adelaide, SA	5 Jul 13
M.P. Krstic	Welcome, introduction and setting the scene for the Pinot Noir workshop	15th Australian Wine Industry Technical Conference, Workshop Program, Sydney, NSW	13 Jul 13
	Summary and wrap up Pinot Noir workshop		
D.L. Capone	Aroma compounds important to Pinot Noir – an overview of recent eucalyptus/mint research		
	Varietal thiols and green characters		
	Eucalyptol		
R.G. Dambergs	Manipulating Pinot Noir expression in the winery through novel winemaking practices		
M.L. Longbottom	Organic vs conventional N2O emissions		
I.L. Francis	Key wine aroma compounds: their origin, aroma properties and how to dial them up or down		
	Fermentation-derived aroma compounds and grape-derived monoterpenes		
	Pepper and spice in Shiraz: what influences rotundone levels in wines?		
C.M. Mayr	Reductive and oxidised characters		
C.A. Black	Managing wine faults and taints		
E. Wilkes	Copper adds never do any harm		

C.D. Curtin	Can 'Brett' be a good thing?		
P.R. Dry	Terroir, the deciding factor		
H.E. Holt	Small berries, good wine!		
P.A. Henschke	Wine: from the academic to the artisan		14 Jul 13
J.R. Bellon	Interspecific hybrids: new yeast for the New World		
C.A. Varela	Winemaking approaches to lower ethanol concentration in wine		
K.A. Bindon	Sequential harvest trial		
I.L. Francis	Tasting of commercially available low alcohol wines		
M.E. Smith	Reductive aromas – a review of fate and formation		
M.P. Day	Impact of oxygen during winemaking on wine style – introduction		
	Impact of oxygen during winemaking on wine style – results and implications		
M.Z. Viviers	Influence of metals on post bottling reductive aroma formation		
P.A. Smith	Factors influencing wine style evolution in-bottle – Oxygen Transfer Rate (OTR) and closure selection		
N. Scrimgeour	Welcome and introduction to rapid analytical measurement tools		
	BevScan: non-invasive spectroscopic screening		
W.U. Cynkar	Measuring YAN for optimal juice composition		
R.A. Muhlack	The Ferment Simulator		

R.G. Dambergs	The Wine Portal: applications for tannin measurement	15th Australian Wine Industry Technical Conference, Workshop Program, Sydney, NSW	
N. Scrimgeour	Future developments and trends		
M.L. Longbottom	Greenhouse gas abatement in viticulture		
K.K. Forsyth	Carbon accounting in the grape and wine sector		
C.D. Curtin	Brettanomyces research and practical management of 'Brett' in the winery		
H.E. Holt	Introduction to sensory evaluation		15 Jul 13
P.O. Williamson	Using sensory science to develop successful wines		
	Consumer sensory testing		
E. Wilkes	CMCs and other crystallisation inhibitors		
	Testing for protein stability, tips and downfalls		
	Testing for tartrate stability		
S. Nordestgaard	Refrigeration management		
K.K. Forsyth	Continuous stabilisation methods		
M. Marangon	Heat stability, what is it?		
	Proctase and other alternatives		
R.A. Muhlack	Bentonites aren't bentonites		
P.R. Dry	Update on emerging varieties in Australia, New Zealand and California		

	White wine varieties: description and tasting		
	Red wine varieties: description and tasting		
C.D. Curtin	Shaping wine style through choice of yeast		
A.D. Coulter	Improved tools: laccase assay		
M. Marangon	Proctase – a viable alternative to bentonite for protein stabilisation of white wines		
S.A. Schmidt	Chardonnay clonal variation – a comparative genomic and phenotypic evaluation		
K.A. Bindon	From grape to consumer: relationships between grape maturity, wine composition and wine sensory properties in Cabernet Sauvignon	15th Australian Wine Industry Technical Conference, Fresh Research Session, Sydney, NSW	
A. Contreras	Can non-conventional yeast be used for the production of wines with lower alcohol concentration?		
M.Z. Viviers	The effects of metals on the evolution of volatile sulfur compounds during wine maturation		
R.A. Muhlack	Creating value from by-products – an industry review and insights into practical case studies	15th Australian Wine Industry Technical Conference, Main Program, Sydney, NSW	16 Jul 13
S. Connew	The AWRI's Semillon project	15th Australian Wine Industry Technical Conference, Workshop Program, Sydney, NSW	
P.J. Costello	Expanding the Chardonnay sensory profile through malolactic fermentation		
E.J. Bartowsky, P.J. Costello	MLF options for flavour management		
M.P. Day	White wine phenolics – introduction		
	White wine phenolics – results and implications for winemaking		
P.A. Smith	Polysaccharides – what do we know about their effects on wine style?		

	Winemaking – what factors influence tannin in red wines?		
K.A. Bindon	What factors influence tannin extractability?		
J.M. McRae	Tannins and their effects on sensory		
C.S. Stockley	Where is allergen labelling at?	15th Australian Wine Industry Technical Conference, Workshop Program, Sydney, NSW	16 Jul 13
L.M. Hoxey	Lean laboratory systems		
	QA systems, making sure it all works		
E. Wilkes	LIMS, it doesn't have to be complicated		
	Practical problem solving, how do we do it?		
E. Wilkes, L.M. Hoxey	Common troubleshooting, some hard-won notes		
V.T. O'Brien	The need for Australian industry to sell on value not price		
P.A. Henschke, A.R. Borneman, C.D. Curtin	Meet the wild yeasts		
C.D. Curtin	Harnessing genomics to ensure a 'Brett'-free future for Australian wine	15th Australian Wine Industry Technical Conference, Main Program, Sydney, NSW	17 Jul 13
A.R. Borneman	Next-generation DNA sequencing and its application by the wine industry		
G.D. Cowey	Introduction to the world of tasting	15th Australian Wine Industry Technical Conference, Workshop Program, Sydney, NSW	18 Jul 13
	Basic flavours, taints and faults and thresholds		
C.A. Simos	The Advanced Wine Assessment Course and masterclasses		
S. Connew	The Australian wine show system		
M. Essling	Welcome and introduction to objective measures of quality workshop		

	Common vineyard measures – the theory		
R.G. Dambergs	Towards the prediction of wine outcomes from grape compositional measures		
C.S. Stockley	Translation of science into public health policy	WineHealth 2013 International Wine and Health Conference, Sydney, NSW	19 Jul 13
C.A. Varela	Strategies for reducing alcohol levels in wine	AWRI webinar	30 Jul 13
P.R. Dry	How can irrigation management strategies be used to manipulate wine quality?	Farmer Johns Viticulture Update, Barossa, SA	30 Jul 13
M.P. Krstic	Smoke taint update	State Government Victoria, Department of Environment and Primary Industries, Melbourne, Vic	31 Jul 13
E. Wilkes	The latest on CMCs	AWRI webinar	6 Aug 13
D.L. Johnson	The AWRI – introduction and 5-year RD&E Plan overview	AWRI roadshow seminar, Rutherglen, Vic	13 Aug 13
M.P. Krstic	Why do varieties respond differently to drought and heat stress and what does this mean for your irrigation management?		
	Vine balance – how does it affect yield and quality?		
	Features of the AWRI website		
	How can irrigation management strategies be used to manipulate wine quality?		
P.A. Henschke	Practical strategies for reducing alcohol levels in wine		
	Increasing red and white wine complexity with the AWRI's Bayanus yeast		
I.L. Francis	Pepper and spice in Shiraz: what influences rotundone levels in wine?		
R.A. Muhlack	Energy for the future: moving towards on-site renewable biomass and solar technology		

D.L. Johnson	The AWRI – introduction and 5-year RD&E Plan overview	AWRI roadshow seminar, Bendigo, Vic	14 Aug 13
M.P. Krstic	How can irrigation management strategies be used to manipulate wine quality?		
	Does soil and vine nutrient status affect wine quality?		
	Features of the AWRI website		
I.L. Francis	Pepper and spice in Shiraz: what influences rotundone levels in wine?		
E.J. Bartowsky	Using MLF to accentuate wine aroma and flavour		
	Using the timing of MLF inoculation to optimise your winemaking		
E. Wilkes	Carboxymethylcellulose – an important tool for white wine tartrate stabilisation		
P.A. Henschke	Causes and management of slow and stuck fermentations		
D.L. Johnson	The AWRI – introduction and 5-year RD&E Plan overview	AWRI roadshow seminar, Avoca, Vic	15 Aug 13
P.A. Henschke	Did you know that DAP can strongly affect the flavour profile and style of wine?		
	Causes and management of slow and stuck fermentations		
E. Wilkes	Practical management of 'Brett' in the winery		
	Saving time and money: automated methods for juice and wine analysis		
E.J. Bartowsky	Strategies for a successful MLF		
	Using MLF to accentuate wine aroma and flavour		
I.L. Francis	Rotten egg, cabbage and rubber: compounds responsible for reductive off-flavours in wines		

M.P. Krstic	Features of the AWRI website		
S. Connew	The Hunter Valley Semillon project	Hunter Valley Wine Show Trade Familiarisation, Brokenwood Wines, Pokolbin, NSW	16 Aug 13
Hixson, K. Forsyth	Grape marc tannin – review of scientific rigour	Meat and Livestock Australia, External Science Review Panel, Brisbane, Qld	20 Aug 13
M.L. Longbottom	Building resilience and sustainability in the grape and wine sector	Western Australia Wine Industry Technical Committee, Curtin University, Margaret River, WA	22 Aug 13
M.P. Krstic	Smoke taint update	Avoca Information Centre, Avoca, Vic	28 Aug 13
M. Essling	Does soil and vine nutrient status affect wine quality?	AWRI roadshow seminar, McLaren Vale, SA	3 Sep 13
	Berry sensory assessment in the vineyard for fruit grading – does it work?		
M.P. Krstic	The AWRI – introduction and 5-year RD&E Plan overview		
	Vine balance – how does it affect yield and quality?		
	Importance of sampling for quality parameters in the vineyard		
	Features of the AWRI website		
T.E. Siebert	Spicing up Shiraz: viticultural and winemaking influences on the peppery aroma compound rotundone		
D.L. Capone	The origin of eucalyptus flavour in red wine		
J.R. Bellon	Hybrid yeast: new yeast for the New World	AWRI webinar	

C.A. Simos	The AWRI – introduction and 5-year RD&E Plan overview	AWRI roadshow workshop, McLaren Vale, SA	10 Sep 13
P.R. Dry	Why is harvest getting earlier and what can we do about it?		
	Hotter and drier in the vineyard		
	Salinity and sodicity in the vineyard		
	Growing grapes in wet seasons		
	Practical vineyard and winery group exercise		
	New varieties for a changing climate tasting		
A.D. Coulter	Hotter and drier – processing ripe fruit		
	Bushfires and smoke taint tasting		
	Efficiencies in the winery		
	Energy use and winery wastewater		
M.G. Holdstock	Salty juice and wine		
	Winemaking in wet seasons		
D.L. Johnson	'Mythbusting' session	Savour Australia 2013, Adelaide Convention Centre, Adelaide, SA	16 Sep 13
M.J. Herderich	Tannins in wine: development and application of methods for their quantitative analysis and the characterisation of physico-chemical properties.	42. Deutscher Lebensmittelchemikertag, Braunschweig, Germany	17 Sep 13

C.A. Simos	The AWRI – introduction and 5-year RD&E Plan overview	AWRI roadshow seminar, Clare, SA	25 Sep 13
	Features of the AWRI website		
P.R. Dry	Terroir – separating fact from fiction		
	It's getting hotter – what does this mean for our vineyard management strategies?		
M. Essling	Does soil and vine nutrient status affect wine quality?		
C.S. Stockley	Health, nutrition and other warning labels		
D.L. Capone	The origin of eucalyptus flavour in red wine		
P.J. Costello	Using the timing of MLF inoculation to optimise your winemaking		
C.A. Simos	Welcome and introduction/overview of the AWRI	AWRI roadshow seminar, Barossa Valley, SA	3 Oct 13
	Features of the AWRI website and close		
M.L. Longbottom	Vine balance – how does it affect yield and quality?		
	Great wines from grafted vines		
	Improving water use efficiency with rootstocks		
M. Essling	How can irrigation management strategies be used to manipulate wine quality?		
	Does soil and vine nutrient status affect wine quality?		
N. Scrimgeour	Winery cost reduction strategies		
	How to significantly reduce your carbon footprint without spending any money		

P.A. Henschke	Causes and management of slow and stuck fermentations		
G.D. Cowey	A tasting of wine produced using AWRI innovations	National Livestock Methane Program (NLMP), AWRI, Urrbrae, SA	
J.M. McRae	Good wine texture starts in the grape	Wine Tasmania 2013 Field Day, Rosevears, Tas	
D.L. Johnson (presented by L.E. Rose)	2013 overview of the AWRI and alignment with SA Government strategic priority	South Australian Wine Industry Council, Adelaide, SA	
M.Z. Viviers	The effects of metals on the evolution of volatile sulfur compounds during wine maturation	AWRI webinar	8 Oct 13
E.J. Bartowsky	Waiter, there are bacteria in my wine! How bacteria help shape wine style	School of Molecular and Biomedical Science, University of Adelaide, Adelaide, SA	21 Oct 13
M.P. Krstic	Why is harvest getting earlier and what can we do about it?	AWRI roadshow workshop, Gippsland, Vic	29 Oct 13
	Hotter and drier in the vineyard		
	Salinity and sodicity in the vineyard		
	Growing grapes in wet seasons		
	Practical vineyard and winery group exercise		
	New varieties for a changing climate tasting		
A.D. Coulter	Hotter and drier – processing ripe fruit		
	Bushfires and smoke taint tasting		
	Efficiencies in the winery		
	Energy use and winery wastewater		
M.G. Holdstock	Salty juice and wine		

	Winemaking in wet seasons		
M.P. Krstic	Why is harvest getting earlier and what can we do about it?		30 Oct 13
	Hotter and drier in the vineyard		
M.P. Krstic	Salinity and sodicity in the vineyard	AWRI roadshow workshop, Mornington, Vic	30 Oct 13
	Growing grapes in wet seasons		
	Practical vineyard and winery group exercise		30 Oct 13
	New varieties for a changing climate tasting		
	N2O – Greenhouse gas abatement in viticulture		
A.D. Coulter	Hotter and drier – processing ripe fruit		
	Bushfires and smoke taint tasting		
	Efficiencies in the winery		
	Energy use and winery wastewater		
M.G. Holdstock	Salty juice and wine		
	Winemaking in wet seasons		
M.P. Krstic	Why is harvest getting earlier and what can we do about it?	AWRI roadshow workshop, Yarra Valley, Vic	31 Oct 13
	Hotter and drier in the vineyard		
	Salinity and sodicity in the vineyard		

	Growing grapes in wet seasons		
	Practical vineyard and winery group exercise		
A.D. Coulter	Hotter and drier – processing ripe fruit		
	Bushfires and smoke taint tasting		
	Efficiencies in the winery		
	Energy use and winery wastewater		
M.G. Holdstock	Salty juice and wine		
	Winemaking in wet seasons		
V.T. O'Brien	Consumer oriented design	McLaren Vale Grape Wine and Tourism Association Board, McLaren Vale, SA	
C.A. Simos	Welcome and introduction/overview of the AWRI	AWRI roadshow seminar, Griffith, NSW	5 Nov 13
	Features of the AWRI website and close		
K.A. Bindon	Tannin from grape to wine: new insights on a complex system		
	Grape ripeness and wine composition (Cabernet Sauvignon)		
C.D. Curtin	Choose the right yeast to achieve the red wine style you want		
	Wild ferments – what are the alternatives?		
	Did you know that DAP can strongly affect the flavour profile and style of wine?		
	Complex yeast nutrients – how do they fit into your fermentation management strategy?		

R.A. Muhlack	An update on the Griffith node activities		
C.A. Simos	Welcome and introduction/overview of the AWRI	AWRI roadshow seminar, Orange, NSW	6 Nov 13
	Why is managing dissolved oxygen at bottling so important?		
	Features of the AWRI website and close		
P.R. Dry	Why do we need new clones?		
	How can cultural practices be used to improve fruit set?		
K.A. Bindon	Viticultural management of grape and wine phenolics		
	Grape ripeness and wine composition (Cabernet Sauvignon)		
C.D. Curtin	Wild ferments – what are the alternatives?		
C.A. Simos	Welcome and introduction/overview of the AWRI	AWRI roadshow seminar, Mudgee, NSW	7 Nov 13
	Managing stuck fermentation and rescue procedures		
	Features of the AWRI website and close		
P.R. Dry	How can irrigation management strategies be used to manipulate wine quality?		
R.A. Muhlack	Doing more with less: sustainable process solutions for profitability		
K.A. Bindon	Viticultural management of grape and wine phenolics		
C.D. Curtin	Managing H2S during fermentation – latest research		
	Practical management of ‘Brett’ in the winery		

C.A. Simos	Welcome and introduction/overview of the AWRI	AWRI roadshow seminar, Canberra, ACT	8 Nov 13
	Features of the AWRI website and close		
P.R. Dry	Does soil and vine nutrient status affect wine quality?		
	Terroir – separating fact from fiction		
K.A. Bindon	Viticultural management of grape and wine phenolics		
J.R. Bellon	Wild ferments – what are the alternatives?		
E. Wilkes	Pepper and spice in Shiraz: what influences rotundone levels in wines?		
	Copper in winemaking, the good and the bad		
M.P. Day	Post-bottling effects of early oxygen exposure during red winemaking	AWRI webinar	12 Nov 13
V.T. O'Brien	Consumer oriented design briefing presentation	AWRI/SA State Government Department for Manufacturing, Innovation, Trade, Resources and Energy joint briefing, Adelaide, SA	14 Nov 13
G.D. Cowey	Sensory evaluation wine aromas, flavours, faults and taints: Australian wine show tasting	Premium Wine Brands, Barossa Valley, SA	
P.O. Williamson	Relationship between chemical composition and preferences of Western and Asian consumers	Beijing International Wine-Tech Forum, Beijing, China	16 Nov 13
D.L. Capone	Recent work on the impact of 1,8-cineole on Pinot Noir	Pinot Massif, Hepburn Springs, Vic	18 Nov 13
E. Wilkes	Optimising your laboratory for the best results	AWRI webinar	19 Nov 13
P.R. Dry	Terroir and wine typicity	Soil and Wine Symposium, Urrbrae, SA	21 Nov 13
P.J. Dawson	2013 AWRI annual report	Winemakers' Federation of Australia AGM, Adelaide, SA	26 Nov 13

C.S. Stockley	The evolution of Australia's alcohol drinking guidelines	Wine Information Council Second Conference – Wine in Moderation: From Science to Art de Vivre, Brussels, Belgium	27 Nov 13
J.R. Bellon	New yeast for the New World	Wine Innovation Cluster 2013 research update and end of year networking event, Adelaide, SA	
J.M. McRae	What the Synchrotron can tell us about wine tannins		
C.M. Mayr	Shiraz reconstitution		
C.A. Simos	World Chardonnay and Pinot Noir tasting	World Chardonnay and Pinot Noir Masterclass, Adelaide, SA	28 Nov 13
			29 Nov 13
P.O. Williamson	Thinking outside the bottle: insights on how Chinese consumers choose wine	AWRI webinar	3 Dec 13
G.D. Cowey, M. Essling, A.D. Coulter	Adapting to difficult vintages – welcome and introduction	AWRI roadshow workshop, Mt Barker, WA	
M. Essling	Why is harvest getting earlier and what can we do about it?		
	Hotter and drier in the vineyard		
	Practical vineyard and winery group exercise		
	Growing grapes in wet seasons		
A.D. Coulter	Winemaking in wet seasons		
	Bushfires and smoke taint tasting		
	Sustainability and efficiencies in the winery		
G.D. Cowey	Hotter and drier – processing ripe fruit		

	New varieties for a changing climate tasting		
	Salty juice and wine		
	Salinity and sodicity in the vineyard		
G.D. Cowey	Adapting to difficult vintages – welcome and introduction	AWRI roadshow workshop, Pemberton, WA	4 Dec 13
	New varieties for a changing climate tasting		
	Salty juice and wine		
	Salinity and sodicity in the vineyard		
	Hotter and drier – processing ripe fruit		
M. Essling	Why is harvest getting earlier and what can we do about it?		
	Hotter and drier in the vineyard		
	Practical vineyard and winery group exercise		
	Growing grapes in wet seasons		
A.D. Coulter	Winemaking in wet seasons		
	Bushfires and smoke taint tasting		
	Sustainability and efficiencies in the winery		
G.D. Cowey	Adapting to difficult vintages – welcome and introduction	AWRI roadshow workshop, Margaret River, WA	5 Dec 13
	New varieties for a changing climate tasting		
	Salty juice and wine		
	Salinity and sodicity in the vineyard		
	Hotter and drier – processing ripe fruit		
M. Essling	Why is harvest getting earlier and what can we do about it?		
	Hotter and drier in the vineyard		
	Practical vineyard and winery group exercise		
	Growing grapes in wet seasons		
A.D. Coulter	Winemaking in wet seasons		
	Bushfires and smoke taint tasting		
	Sustainability and efficiencies in the winery		

G.D. Cowey	Adapting to difficult vintages – welcome and introduction	AWRI roadshow workshop, Swan Valley, WA	6 Dec 13
	New varieties for a changing climate tasting		
	Salty juice and wine		
	Salinity and sodicity in the vineyard		
	Hotter and drier – processing ripe fruit		
M. Essling	Why is harvest getting earlier and what can we do about it?		
	Hotter and drier in the vineyard		
	Practical vineyard and winery group exercise		
	Growing grapes in wet seasons		
A.D. Coulter	Winemaking in wet seasons		
	Bushfires and smoke taint tasting		
	Sustainability and efficiencies in the winery		
M.L. Longbottom	Greenhouse gas emissions in vineyards	AWRI webinar	10 Dec 13
W.U. Cynkar	Measuring YAN for optimal juice composition	2013 Interwinery Analysis Group Annual General Meeting, Clare, SA	13 Dec 13
D.L. Johnson	2013 AWRI annual report presentation and update	NSW Wine Industry Association, Sydney, NSW	20 Jan 14
R.G. Dambergs	Copper – the good, the bad and the ugly	Improved winemaking practices workshop, Richmond, Tas	21 Jan 14
S. Connew	Getting the most from your smartphone and tablet		

P.W. Godden	Spontaneous fermentations		
R.G. Dambergs	Copper – the good, the bad and the ugly	Improved winemaking practices workshop, Tamar Valley, Tas	22 Jan 14
S. Connew	Getting the most from your smartphone and tablet		
P.W. Godden	Spontaneous fermentations		
D.L. Johnson	2013 AWRI annual report presentation and update	Wines of WA Board meeting, Dardanup, WA	12 Feb 14
C.A. Simos	Welcome and introduction/overview of the AWRI	AWRI roadshow seminar, Coonawarra, SA	14 Feb 14
	Features of the AWRI website and close		
P.R. Dry	It's getting hotter – what does this mean for our vineyard management strategies?		
	Terroir – separating fact from fiction		
	How can cultural practices be used to improve fruit set?		
M.P. Krstic	Importance of sampling for quality parameters in the vineyard		
	Berry sensory assessment in the vineyard for fruit grading – does it work?		
C.D. Curtin	Complex yeast nutrients – how do they fit into your fermentation management strategy?		
E.J. Bartowsky	Strategies for a successful MLF		
C.S. Stockley	Health, nutrition and other warning labels		
G.D. Cowey	Trouble-free winemaking	Institute of Masters of Wine, Reading, UK	
D.L. Johnson	2013 AWRI annual report presentation and update	Wine Tasmania Board meeting, Campbell Town, Tas	20 Feb 14

P.W. Godden		SA Wine Industry Association, Adelaide, SA	
D.L. Johnson	Agriculture, Food, Water and Energy Nexus	Plant Genomics Centre, Urrbrae, SA	27 Mar 14
R.A. Muhlack, P.W. Godden	Refrigeration efficiency: a new tool to model refrigeration demand	Griffith, NSW	3 Apr 14
R.G. Dambergs	Understanding and manipulating red wine phenolics for quality		
M.J. Herderich	The Australian wine sector and the Australian Wine Research Institute	Joint Research Centre - Institute for Reference Materials and Measurements, Geel, Belgium	7 Apr 2014
P.J. Chambers	Improving wine through yeast strain development	5th European Yeast Flavour Conference: Biotechnology for Natural Flavours Production, Montreux, Switzerland	5 May 14
C.A. Simos	Welcome and introduction/overview of the AWRI	AWRI roadshow seminar, Renmark, SA	8 May 14
	Features of the AWRI website and close		
P.R. Dry	How can irrigation management strategies be used to manipulate wine quality?		
	Why do we need clones?		
	How can cultural practices be used to improve fruit set?		
M.P. Krstic	Does soil and vine nutrient status affect wine quality?		
	Why do varieties respond differently to drought and heat stress – and what does this mean for your irrigation management?		
K.A. Bindon	Managing phenolic quality in the vineyard		
M.L. Longbottom	Nitrogen management in viticulture	Margaret River grapegrowers, Curtin University, Margaret River, WA	13 May 14

P.W. Godden	The wine science horizon	Institute of Masters of Wine 8th Symposium, Florence, Italy	16 May 14
D.L. Johnson	Sc2.0 Synthetic Yeast genome project launch and wine tasting	Synthetic Biology Symposium, Macquarie University, Sydney, NSW	27 May 14
M.L. Longbottom	Greenhouse gas abatement in viticulture	Hunter Valley grapegrowers, Pokolbin, NSW	
I.L. Francis, W.P. Pearson	Descriptive terminology	AWRI Advanced Wine Assessment Course, Urrbrae, SA	2 Jun 14
M.P. Krstic	Insights into smoke taint	Royal Australian Chemical Institute – Food Nutrition and Analytical Chemistry Group, Moorabbin, Vic	4 Jun 14
P.W. Godden	Getting to know wild yeasts tasting	Refrigeration and improved winemaking practices workshop, Canberra, ACT	6 Jun 14
R.A. Muhlack	Refrigeration efficiency: a new tool to model refrigeration demand		
R.G. Dambergs	Understanding and manipulating red wine phenolics for quality		
P.R. Dry	International Shiraz production and performance	Shiraz Symposium, Melbourne, Vic	11 Jun 14
I.L. Francis	Capturing the pepper character in Shiraz		
D.L. Capone	Managing the eucalyptus character in Shiraz		
R.A. Muhlack	Refrigeration efficiency: a new tool to model refrigeration demand	Refrigeration and ferment simulation workshop, Rutherglen, Vic	13 Jun 14
	Automating fermentation control with computer simulation		
S. Connew	Working smarter, not harder in the vineyard: making the most of your smart phone and tablet	AWRI webinar	17 Jun 14
G.D. Cowey	Adapting to difficult vintages – welcome and introduction	AWRI roadshow workshop, Murgon, Qld	
	Hotter and drier – processing ripe fruit		

	Salinity, sodicity and salty wine		
	New varieties for a changing climate tasting		
M. Essling	Hotter and drier in the vineyard		
	Growing grapes in wet seasons		
	Q&A session and case studies		
A.D. Coulter	Bushfires and smoke taint tasting		
	Winemaking in wet seasons		
	Sustainability and efficiencies in the winery		
S. Nordestgaard	Lees: rheology, reverse racking and recovery	Winery Engineering Association Conference, McLaren Vale, SA	18 Jun 14
S.A. Schmidt	What's under the hood? Genomic differences powering variation in primary fermentation		
J.M. McRae	Recent developments in removing protein haze from white wines		
G.D. Cowey	Adapting to difficult vintages – welcome and introduction	AWRI roadshow workshop, Stanthorpe, Qld	19 Jun 14
	Hotter and drier – processing ripe fruit		
	Salinity, sodicity and salty wine		
	New varieties for a changing climate tasting		
M. Essling	Hotter and drier in the vineyard		
	Growing grapes in wet seasons		
	Q&A session and case studies		

A.D. Coulter	Bushfires and smoke taint tasting		
	Winemaking in wet seasons		
	Sustainability and efficiencies in the winery		
C.A. Simos	Welcome and introduction/overview of the AWRI	AWRI roadshow seminar, Murgon, Qld	24 Jun 14
	Features of the AWRI website and close		
P.R. Dry	How can irrigation management strategies be used to manipulate wine quality		
	Why do bunches get hot and what does this mean for wine quality?		
	Why do we need new varieties for the future?		
K.A. Bindon	Viticultural management of grape and wine phenolics		
E. Wilkes	Cold stabilisation		
C.A. Simos	Welcome and introduction/overview of the AWRI	AWRI roadshow seminar, Stanthorpe, Qld	25 Jun 14
	Winemaking management strategies for Botrytis and Powdery Mildew		
	Features of the AWRI website and close		
P.R. Dry	How can cultural practices be used to improve fruit set?		
	Is it possible to control bunch rot without fungicides?		
K.A. Bindon	Viticultural management of grape and wine phenolics		
	Crafting diverse wine styles through an understanding of how grape composition affects wine composition		

E. Wilkes	Cold stabilisation		
T.J. Abbott, P.W. Godden	Automating fermentation control with computer simulation	Refrigeration and ferment simulation workshop, Mornington Peninsula, Vic	26 Jun 14
	Refrigeration efficiency: a new tool to model refrigeration demand		
P.W. Godden	Working smarter not harder in the vineyard – making the most of your smart phone and tablet	Refrigeration and improved winemaking practices workshop, Orange, NSW	30 Jun 14
P.W. Godden, W.P. Pearson	Vintage benchmark tasting – challenging wines and trying new techniques		
R.A. Muhlack	Refrigeration efficiency: a new tool to model refrigeration demand		
M.L. Longbottom	Mitigation, adaptation or innovation?	Opportunities in a new climate workshop, Adelaide, SA	1 Jul 14
D.L. Johnson	Overview and launch 'Opportunities in a new climate'		
E.J. Bartowsky	Unravelling the mysteries of malolactic fermentation: from microbiology to frontier technology	Australian Society for Microbiology, Melbourne, Vic	7 Jul 14
N. Scrimgeour	Unravelling the relationship between grape and wine composition	US webinar, hosted by Enartis	10 Jul 14
E.J. Bartowsky	Chasing wine aroma: impact of different LAB and MLF scenarios	Malolactic Symposium, Toulouse, France	10 - 11 Jul 14
P.W. Godden	Perspectives on Nebbiolo in Australia	Negociants Australia Working with Wine seminar, Adelaide, SA	14 Jul 14
J. Hixson, G.D. Cowey	What is the current climate and adaptation/mitigation research agenda for the wine industry?	Climate change and the SA wine industry workshop, Adelaide, SA	31 Jul 14
T.M. Parker	Flavours in wine	Melbourne Writers Festival 'Flavours in Wine' event, Mount Langi Ghiran, Vic	10 Aug 14
M.J. Herderich	Terroir effects on grape and wine aroma compounds	Advances in Wine Research Symposium at the American Chemical Society National Meeting, San Francisco, USA	11 Aug 14

P.W. Godden	What style of PinotG are you? Helping consumers understand the styles of wine made from Pinot Grigio and Pinot Gris	New Zealand Society for Viticulture and Oenology 'To Gris or not to Gris' workshop, Blenheim, New Zealand	26 Aug 14
M.P. Krstic	Winegrowing with technology - an Australian perspective	Romeo Bragato conference, Blenheim, New Zealand	28 Aug 14
E.J. Bartowsky	Genomic analysis of 80 *Oenococcus oeni* strains and connecting the genome with winemaking properties	11th International Symposium on Lactic Acid Bacteria, Egmond aan Zee, The Netherlands	3 Sep 14
	Chasing wine aroma: impact of different LAB and MLF scenarios	Malolactic Symposium, Stellenbosch, South Africa	5 Sep 14
M.J. Herderich	Latest developments in analysing macromolecules and metabolites in grapes and wine: practical applications and potential pitfalls	MacroWine 2014 - Macromolecules and secondary metabolites of grape and wine, Stellenbosch, South Africa	8 Sep 14
M.Z. Viviers	Formation of 'reduced' odours in wine post-bottling: natural occurring precursors or additive effect?		9 Sep 14
I.L. Francis	Describing wine aromas and flavours	Advanced Wine Assessment Course, AWRI, Adelaide, SA	
P.W. Godden	Looking towards the wine science horizon	Nederburg 40th anniversary wine auction, Paarl, South Africa	13 Sep 14
W.P. Pearson	Describing wine aromas and flavours	Advanced Wine Assessment Course, AWRI, Adelaide, SA	15 Sep 14
N. Scrimgeour	Unravelling the relationship between grape and wine composition	Pernod Ricard Winemakers, Barossa Valley, SA	18 Sep 14
M.Z. Viviers	Copper, it never does any harm	Distell, Stellenbosch, South Africa	
C.A Simos	Welcome and introduction	AWRI roadshow seminar, Geelong, Vic	24 Sep 14
	What options do you have in cold stabilising your wine?		
	Features of the AWRI website		
M.P. Krstic	How can irrigation management strategies be used to manipulate wine quality?		

	It's getting hotter - what does this mean for vineyard management strategies?		
M. Essling	Salt management in vineyards		
K.A. Bindon	Managing phenolic quality in the vineyard		
C.D. Curtin	Winemaking with non-conventional and hybrid yeast		
	Did you know that DAP can strongly affect the flavour profile and style of wine?		
C.A. Simos	Welcome and introduction	AWRI roadshow seminar, Macedon Ranges, Vic	25 Sep 14
	What options do you have in cold stabilising your wine?		
	Features of the AWRI website		
M.P. Krstic	How can cultural practices be used to improve fruit set?		
	Does soil and vine nutrient status affect wine quality?		
K.A. Bindon	Managing phenolic quality in the vineyard		
M. Essling	Why do we need new varieties for the future?		
C.D. Curtin	Winemaking with non-conventional and hybrid yeast		
	Did you know that DAP can strongly affect the flavour profile and style of wine?		
E.J. Bartowsky	Genomic analysis of 80 *Oenococcus oeni* strains and connecting the genome with winemaking properties	Crush 2014, the grape and wine science symposium, Adelaide, SA	
M.Z. Viviers	The evolution of 'reduced' odours in wine post-bottling: additive effect?		
S.A. Schmidt	Genomic differences powering variation in primary fermentation		

T.M. Parker	Lingering flavours: sensory and analytical studies demonstrating retronasal aroma perception from in-mouth release of flavour glycoconjugates		26 Sep 14
M.Z. Viviers	The revolving door of stinky sulfurs	AWRI webinar	7 Oct 14
G.D. Cowey	Adapting to difficult vintages welcome and introduction	AWRI roadshow workshop, Glen Innes, NSW	15 Oct 14
	Salinity, sodicity and salty wine		
	Hotter and drier - processing ripe fruit		
	Sustainability and efficiencies in the winery		
	New varieties for a changing climate tasting		
C.A. Simos	Bushfires and smoke taint tasting		
	Winemaking in wet seasons		
M. Essling	Hotter and drier in the vineyard		
	Growing grapes in wet seasons		
	Q&A session and case studies		
C.A. Simos	Welcome and introduction	AWRI roadshow seminar, Great Southern, WA	21 Oct 14
	What options do you have in cold stabilising your wines?		
	Features of the AWRI website		
M. Essling	It's getting hotter – what does this mean for our vineyard management strategies?		

	Why do we need new varieties for the future?		
I.L. Francis	Pepper and spice in Shiraz: what influences rotundone levels in wines?		
	Thinking outside the bottle: insights on how Chinese consumers choose wine		
P.A. Henschke	Managing H_2S during fermentation		
	Winemaking at low pH: avoiding stuck fermentations in whites and sparkling wines		
M.G. Holdstock	Adapting to difficult vintages welcome and introduction	AWRI roadshow workshop, Griffith, NSW	22 Oct 14
	Hotter and drier - processing ripe fruit		
	Winemaking in wet seasons		
	New varieties for a changing climate tasting		
M.L. Longbottom	Hotter and drier in the vineyard		
	Salinity, sodicity and salty wine		
	Growing grapes in wet seasons		
	Q&A session and case studies		
A.D. Coulter	Sustainability and efficiencies in the winery		
	Bushfires and smoke taint tasting		
C.A. Simos	Welcome and introduction	AWRI roadshow seminar, Pemberton, WA	
	Features of the AWRI website		

M. Essling	Does soil and vine nutrient status affect wine quality?		
	Is it possible to control bunch rot without fungicides?		
K.A. Bindon	Crafting diverse wine styles through an understanding of how grape composition affects wine composition		
	The changing wine style of the ripening grape		
P.A. Henschke	Increasing red and white wine complexity with the AWRI's *bayanus* yeast		
	Putting the texture back into white wine – the role of white wine phenolics		
J.R. Bellon	Introducing a new breed of wine yeast	Institute of Research on Cancer and Aging **in** Nice, France	
P.W. Godden	Summary of the wines entered and the judging process	Royal Adelaide Wine Show, national schools' competition awards ceremony, Wayville, SA	
C.A. Simos	Welcome and introduction	AWRI roadshow seminar, Margaret River, WA	23 Oct 14
	Features of the AWRI website		
M. Essling	It's getting hotter – what does this mean for our vineyard management strategies?		
	Why do bunches get hot – and what does this mean for wine quality?		
P.A. Henschke	Winemaking with non-conventional and hybrid yeast		
K.A. Bindon	Predicting wine tannin and colour in the vineyard		
	The changing wine style of the ripening grape		
I.L. Francis	The origins of eucalyptol and minty flavours in red wine		

	Thinking outside the bottle: insights on how Chinese consumers choose wine		
C.A. Simos	Welcome and introduction	AWRI roadshow seminar, Swan Valley, WA	24 Oct 14
	Why is managing dissolved oxygen at bottling so important?		
C.A. Simos	Features of the AWRI website		
M. Essling	Does soil and vine nutrient status affect wine quality?		
I.L. Francis	Key wine flavour compounds		
	Pepper and spice in Shiraz: what influences rotundone levels in wines?		
P.A. Henschke	Winemaking with non-conventional and hybrid yeast		
E. Wilkes	SO_2, the misunderstood component	AWRI webinar	30 Oct 14
J.R. Bellon	Introducing a new breed of wine yeast	Biochemistry Department, Cambridge University, UK	7 Nov 14
C.S. Stockley	Wine and health - anything new?	Treasury Wine Estates' annual technical seminar, Magill, SA	
G.D. Cowey	The carbon farming initiative and emissions reduction fund: climate change policy and the impact on the grape and wine sector	AWRI webinar	11 Nov 14
P.R. Dry	Extreme and increasing temperatures – effects on grapes and wine	Opportunities in a new climate workshop, Penola, SA	
	Cultural practices to improve fruit set	AWRI webinar	13 Nov 14
M.P. Krstic	Adapting to difficult vintages welcome and introduction	AWRI roadshow workshop, Bendigo, Vic	17 Nov 14
	Hotter and drier in the vineyard		

	Salinity, sodicity and salty wine		
	Growing grapes in wet seasons		
	Q&A session and case studies		
M.G. Holdstock	Winemaking in wet seasons		
	New varieties for a changing climate tasting		
	Hotter and drier - processing ripe fruit		
A.D. Coulter	Sustainability and efficiencies in the winery		
	Bushfires and smoke taint tasting		
J.M. McRae	Alternatives to bentonite - what's on the horizon?	Australian Society of Viticulture and Oenology seminar, Adelaide, SA	
S. Nordestgaard	Grape destemming and sorting technology - developments in-winery and on-harvester		
M.P. Krstic	Adapting to difficult vintages welcome and introduction	AWRI roadshow workshop, Avoca, Vic	18 Nov 14
	Hotter and drier in the vineyard		
	Salinity, sodicity and salty wine		
	Growing grapes in wet seasons		
	Q&A session and case studies		
M.G. Holdstock	Hotter and drier - processing ripe fruit		
	Winemaking in wet seasons		

	New varieties for a changing climate tasting		
A.D. Coulter	Bushfires and smoke taint tasting		
	Sustainability and efficiencies in the winery		
C.A. Simos	Additives and processing aids: when things go wrong	Australian Society of Viticulture and Oenology seminar, Adelaide, SA	19 Nov 14
M.G. Holdstock	Welcome and introduction	AWRI roadshow seminar, Mornington Peninsula, Vic	20 Nov 14
	What options do you have in cold stabilising your wines?		
	Features of the AWRI website		
J.M. McRae	Predicting wine tannin and colour in the vineyard		
	Managing phenolic quality in the vineyard		
P.A. Henschke	Managing H_2S during fermentation - latest research		
	Managing stuck fermentations		
R. Gawel	Managing the risk of protein haze formation in white wines		
	Putting the texture back into white wine – the role of white wine phenolics		
D.L. Johnson	2014 AWRI annual report	New South Wales Wine Industry Association, Sydney, NSW	
M.P. Krstic	Welcome and introduction	AWRI roadshow seminar, Gippsland, Vic.	24 Nov 14
	Features of the AWRI website		
	Vine balance - how does it affect yield and quality?		

K.A. Bindon	Managing phenolic quality in the vineyard		
E.J. Bartowsky	Using MLF to accentuate wine aroma and flavour		
	Using the timing of MLF inoculation to optimise your winemaking		
P.A. Henschke	Managing H_2S during fermentation - latest research		
	Winemaking with non-conventional and hybrid yeast		
I.L. Francis	Thinking outside the bottle: insights on how Chinese consumers choose wine		
M.P. Krstic	Welcome and introduction	AWRI roadshow seminar, Yarra Valley, Vic	25 Nov 14
	How can cultural practices be used to improve fruit set?		
	How can irrigation management strategies be used to manipulate wine quality?		
	Features of the AWRI website		
K.A. Bindon	Managing phenolic quality in the vineyard		
I.L. Francis	Pepper and spice in Shiraz: what influences rotundone levels in wines?		
P.A. Henschke	Winemaking with non-conventional and hybrid yeast		
E.J. Bartowsky	Technologies to manage microbial spoilage or delay MLF		
J.R. Bellon	New yeast for the New World	Plumpton College, UK	26 Nov 14
M.P. Day	The effect of oxygen in pressing and juice handling	AWRI webinar	27 Nov 14
C.A. Simos	Welcome and introduction	AWRI roadshow seminar, Griffith, NSW	1 Dec 14

	Features of the AWRI website		
P.A. Henschke	Managing H_2S during fermentation - latest research		
E.J. Bartowsky	Using the timing of MLF inoculation to optimise your winemaking		
R. Gawel	Putting the texture back into white wine – the role of white wine phenolics		
N. Scrimgeour	Winery cost reduction strategies/Doing more with less: sustainable process solutions for profitability		
	Measuring phenolics to add value to your business		
M.L. Downie	eBooks – introducing the AWRI's brand new Grape and Wine eBook collection	AWRI webinar	2 Dec 14
M. Essling	Impacts on soil health from organic, biodynamic and conventional viticultural practices	Burnett Mary Regional Group workshop, South Burnett, Qld	
	Emissions reductions opportunities in the viticulture industry		
J.R. Bellon	Introducing a new breed of wine yeast	Department of Genetics, University of Leicester, UK	4 Dec 14
M. Essling	Fungal disease management and survey analysis	Research to practice workshop, Stanthorpe, Qld	
	Bird control		
D.L. Johnson	2014 AWRI annual report	Queensland Wine Industry Association AGM, Stanthorpe, Qld	
C.D. Curtin	Biosciences research at the AWRI	Department of Biology, Catholic University of Leuven, Belgium	5 Dec 14
E. Wilkes	Impact of temperature on pH, it's not automatic	Interwinery Analysis Group, Berri, SA	
	Preparing your lab for vintage		

	SO_2, the misunderstood component		
C.D. Curtin	Biosciences research at the AWRI	Wolfe Laboratory, Conway Institute, University College Dublin, Ireland	8 Dec 14
S.A. Schmidt	What do you need to know about rehydration nutrients and nutrient additives?	AWRI webinar	9 Dec 14
I.L Francis	Wine flavours	AWRI wine flavours workshop, Coonawarra, SA	
T.E. Siebert	Stone fruit flavours		
	Pepper flavour (rotundone) in red wine		
D.L. Capone	Thiols and tropical flavours in Chardonnay		
	Green flavours in Shiraz and Cabernet Sauvignon		
	Eucalyptus flavour (1,8–cineole) in red wine		
D.L. Johnson	2014 AWRI annual report	Wine Tasmania Board meeting, Relbia, Tas	
J.M. McRae	Recent advances in the development of alternatives to bentonite	AWRI webinar	16 Dec 14
M.L. Longbottom	Climate and the wine industry	Opportunities in a new climate workshop, Melbourne, Vic	18 Dec 14
G.D. Cowey	Where does the wine industry sit in terms of Australian climate policy and the global carbon market?		
M. Essling	What are the opportunities for the grape and wine sector?		
C.A. Simos	Wine flavours, faults and taints	Themed tasting, Odney, UK	8 Jan 15
	Trouble free winemaking		
M.L. Longbottom and E.A. Riley[1]	*Botrytis* – risk and control options	AWRI webinar	16 Jan 15

M.P. Krstic, M.J. Herderich, M.G. Holdstock	Smoke taint Q&A	AWRI smoke taint seminar, Adelaide Hills, SA	20 Jan 15
M.L. Longbottom and L.M. Bevin	NSW DPI weather station network	AWRI/NSW DPI webinar	22 Jan 15
C.A. Simos	Welcome and introduction	AWRI roadshow seminar, Launceston, Tas	3 Feb 15
M.P. Krstic	I have *Botrytis* bunch rot - what can I do about it?		
	Vine balance - how does it affect yield and quality?		
K.A. Bindon	Managing phenolic quality in the vineyard.		
C.A. Simos	Winemaking strategies for *Botrytis* and powdery mildew		
	Features of the AWRI website		
C.A. Simos	Welcome and introduction	AWRI roadshow seminar, Hobart, Tas	4 Feb 15
	Winemaking strategies for *Botrytis* and powdery mildew		
	Features of the AWRI website		
M.P. Krstic	I have *Botrytis* bunch rot - what can I do about it?		
	Vine balance - how does it affect yield and quality?		
K.A. Bindon	Managing phenolic quality in the vineyard		
M.G. Holdstock	Adapting to difficult vintages, welcome and introduction	AWRI roadshow workshop, Geelong, Vic	10 Feb 15
	Salinity, sodicity and salty wine		
	Winemaking in wet seasons		

	Q&A session and case studies		
	New varieties for a changing climate tasting		
A.D. Coulter	Hotter and drier - processing ripe fruit		
	Bushfires and smoke taint tasting		
	Sustainability and efficiencies in the winery		
M. Essling	Hotter and drier in the vineyard		
	Growing grapes in wet seasons		
G.D. Cowey	Simulated flavours, faults, taints and mouth-feel tasting	Taints and faults workshop, Tanunda, SA	
N. Scrimgeour	Use of the AWRI WineCloud for rapid determination of grape polyphenols (webinar)	ISVEA polyphenols and wine congress, 2nd edition, Florence, Italy	13 Feb 15
A.M. Barker	The use of time-intensity to measure the in-mouth flavour release of precursors present in Gewürztraminer wine: training protocols and application of data analysis techniques.	9th Annual Australia and New Zealand Sensory and Consumer Science Symposium, Waiheke Island, New Zealand	17 Feb 15
C.A. Simos, M.P. Krstic	Smoke taint Q&A	AWRI smoke taint seminar, Manjimup, WA	
C.A. Simos	Industry development and support	Organisation Internationale de la Vigne et du Vin (OIV) MSc in wine management, Adelaide, SA	5 Mar 15
J.C. Hack	AWRI metabolomics node update	Metabolomics Australia analytical meeting, Parkville, Vic	17 Mar 15
	Analysis of stinky sulfur compounds in wine		
	R-based molecular feature extraction workflow for GCMS and LCMS data		18 Mar 15

P.O.Williamson, I.L. Francis	Influence of sensory perceptions in wine tasting	VALO launch event, Adelaide, SA	1 Apr 15
P.R. Petrie	Impacts of climate change on agriculture (wine)	Governor's leadership foundation program, climate, water and energy, Urrbrae, SA	9 Apr 15
M.J. Herderich	Treatment of wine with agar as a fining agent	OIV Technology Expert Group, Paris, France	15 Apr 15
R.L. Taylor	Magic in the laboratory - turning samples into numbers	Peracto company conference, Adelaide, SA	21 Apr 15
M.J. Herderich	Potassium carbonate for de-acidification of wine	OIV specifications for oenological products expert group, Paris, France	23 Apr 15
D.L. Johnson	The AWRI's history	The AWRI's 60th birthday dinner, Adelaide, SA	30 Apr 15
E. Wilkes	AWRI and the WineCloud, phenolic measurements in grape and wine production	Enartis customer education centre, Sonoma, USA	1 May 15
M.L. Longbottom	Introduction to mulch and compost	NSW DPI skills development program mulch workshop, Griffith, NSW	6 May 15
	Results of mulch trials in other regions		
G.D. Cowey	Hotter and drier - processing ripe fruit and high sugar juices	AWRI roadshow workshop, Mildura, Vic	12 May 15
	Salinity, sodicity and salty wine		
	Winemaking in wet seasons		
	New varieties for a changing climate tasting		
M. Essling	Hotter and drier in the vineyard		
	Building resilience and sustainability in the grape and wine sector		
	Growing grapes in wet seasons		
A.D. Coulter	Bushfires and smoke taint tasting		

	Sustainability and efficiencies in the winery		
P.R. Petrie	Greenhouse gas emissions abatement in viticulture		
G.D. Cowey	Hotter and drier - processing ripe fruit and high sugar juices	AWRI roadshow workshop, Renmark, SA	13 May 15
	Salinity, sodicity and salty wine		
	Winemaking in wet seasons		
	New varieties for a changing climate		
M. Essling	Hotter and drier in the vineyard		
	Building resilience and sustainability in the grape and wine sector		
	Growing grapes in wet seasons		
A.D. Coulter	Bushfires and smoke taint tasting		
	Sustainability and efficiencies in the winery		
P.R. Petrie	Greenhouse gas emissions abatement in viticulture		
	Terroir – separating fact from fiction	AWRI roadshow seminar, Pokolbin, NSW	19 May 15
	I have *Botrytis* bunch rot - what can I do about it?		
C.D. Curtin	Did you know that DAP can strongly affect the flavour profile and style of wine?		
	Complex yeast nutrients – how do they fit into your fermentation management strategy?		
E.J. Bartowsky	Using the timing of MLF inoculation to optimise your winemaking		

E. Wilkes	Get the best out of your winery using 'lean production'		
	Energy for the future: moving towards on-site renewable biomass and solar technology		
C.A. Simos	Welcome and introduction		
	Features of the AWRI website		
T.E. Siebert	Common and interesting wine aromas	Blackwood winemakers and brewers club, Blackwood, SA	20 May 15
G.D. Cowey	Wine aromas, flavours, faults and taints Australian wine show	Pernod Ricard Winemakers, Tanunda, SA	21 May 15
P.R. Petrie	Terroir – separating fact from fiction	AWRI roadshow seminar, Langhorne Creek, SA	26 May 15
	Why do varieties respond differently to drought and heat stress – and what does this mean for your irrigation?		
M. Essling	Why do bunches get hot – and what does this mean for wine quality?		
	Greenhouse gas emissions abatement in viticulture		
K.A. Bindon	The changing wine style of the ripening grape		
T.J. Abbott	Making your production more environmentally sustainable		
C.A. Simos	Winemaking management strategies for *Botrytis* and powdery mildew		
	Welcome and introduction		
	Features of the AWRI website		
M.L. Longbottom	Greenhouse gas abatement in viticulture	Action on the Ground workshop, Margaret River, WA	

		Action on the Ground workshop, Swan Valley, WA	28 May 15
P.A. Smith	Factors influencing extractability, retention and modification of tannins during winemaking	9th World Congress on Polyphenols Applications, St. Julian's, Malta	3 Jun 15
C.S. Stockley	Celebrating the resveratrol clinical trial	Royal Melbourne Hospital, Melbourne, Vic	6 Jun 15
M.G. Holdstock	Adapting to difficult vintages	AWRI roadshow workshop, Coonawarra, SA	10 Jun 15
M.L. Longbottom	Hotter and drier in the vineyard		
A.D. Coulter	Hotter and drier - processing ripe fruit		
	Bushfires and smoke taint tasting		
M.G. Holdstock	Salinity, sodicity and salty wine		
	Q&A session and case studies		
	New varieties for a changing climate tasting		
M.L. Longbottom	Growing grapes in wet seasons		
A.D. Coulter	Winemaking in wet seasons		
	Sustainability and efficiencies in the winery		
M.L. Longbottom	Building resilience and sustainability in the grape and wine sector		
P.A. Henschke	Choose the right yeast to achieve the red style you want	AWRI roadshow seminar, Mildura, Vic	11 Jun 15
I.L. Francis	Pepper and spice in Shiraz: what influences rotundone levels in wines?		
	Thinking outside the bottle: insights on how Chinese consumers choose wine		

R. Gawel	Solids ferments: effect of juice clarity and clarification method on drivers of wine texture		
C.A. Simos	Welcome and introduction		
	Features of the AWRI website		
M.P. Krstic	Vine balance – how does it affect yield and quality?		
	Does soil and vine nutrient status affect wine quality?		
P.R. Petrie	Why do varieties respond differently to drought and heat stress – and what does this mean for your irrigation?		
	It's getting hotter – what does this mean for our vineyard management strategies?		
P.O. Williamson	Insights on how Chinese consumers choose wine and the influence of tasting on consumer preferences	Wine consumer and market insights symposium, Melbourne, Vic	18 Jun 15
I.L. Francis	Understanding wine consumers: the role of analytical sensory testing, consumer product acceptance and marketing research		
G.D. Cowey	Hotter and drier - processing ripe fruit	AWRI roadshow workshop, Launceston, Tas	23 Jun 15
	Salinity, sodicity and salty wine		
	Building resilience and sustainability in the grape and wine sector		
	New varieties for a changing climate tasting		
M.P. Krstic	Hotter and drier in the vineyard		
	Growing grapes in wet seasons		

A.D. Coulter	Bushfires and smoke taint tasting		
	Winemaking in wet seasons		
	Sustainability and efficiencies in the winery		
G.D. Cowey	Hotter and drier - processing ripe fruit	AWRI roadshow workshop, Hobart, Tas	25 Jun 15
	Salinity, sodicity and salty wine		
	Q&A session and case studies		
	Building resilience and sustainability in the grape and wine sector		
	New varieties for a changing climate tasting		
M.P. Krstic	Hotter and drier in the vineyard		
	Growing grapes in wet seasons		
A.D. Coulter	Bushfires and smoke taint tasting		
	Winemaking in wet seasons		
	Sustainability and efficiencies in the winery		
M.L. Longbottom	Building resilience and sustainability in the grape and wine sector	Opportunities in a new climate workshop, Willunga, SA	26 Jun 15
	Greenhouse gas abatement in viticulture		
	Climate and the wine industry		
M.G. Holdstock	Opportunities for the grape and wine sector		

	The emissions reduction fund - how does it work?		
J. Hixson, K. Hirlam	Maximising the potential of grape marc		
A. Mierczynska-Vasilev	Understanding the role of colloids in wines	French National Institute for Agricultural Research (INRA), Avignon, France	
M.J. Herderich	Terroir effects on grape and wine aroma compounds	Oeno 2015 Symposium, Bordeaux, France	29 Jun 15
A. Mierczynska-Vasilev	Red wine adsorption on functionalised surfaces	Oeno 2015 Symposium, Bordeaux, France	1 Jul 15
G.D. Cowey	Hotter and drier – processing ripe fruit	AWRI roadshow workshop, Langhorne Creek, SA	2 Jul 15
	Bushfires and smoke taint tasting		
	Sustainability and efficiencies in the winery		
P.R. Petrie	Hotter and drier in the vineyard		
	Growing grapes in wet seasons		
M.G. Holdstock	Salinity and sodicity in the vineyard		
	Winemaking in wet seasons		
	Q&A session and case studies		
	Building resilience and sustainability in the grape and wine sector		
	New varieties for a changing climate tasting		
C.S. Stockley	The evolution of 'drinking' guidelines and public health policy	38th World Congress of Vine and Wine, Mainz, Germany	8 Jul 15
K.A. Bindon	Managing phenolic quality in the vineyard	AWRI roadshow seminar, Rutherglen, Vic	14 Jul 15

M.P. Krstic	It's getting hotter – what does this mean for our vineyard management strategies?		
	Is it possible to control bunch rot without fungicides?		
E.N. Wilkes	Copper: the good, the bad and the ugly		
	Get the best out of your winery using 'lean production'		
P.A. Henschke	Managing stuck fermentations		
C.A. Simos	Winemaking management strategies for *Botrytis*		
K.A. Bindon	Managing phenolic quality in the vineyard	AWRI roadshow seminar, Bendigo, Vic	15 Jul 15
	The changing wine style of the ripening grape		
P.A. Henschke	Wild ferments – what are the alternatives?		
	Did you know that DAP can strongly affect the flavour profile and style of wine?		
E.N. Wilkes	Copper: the good, the bad and the ugly		
	Get the best out of your winery using 'lean production'		
M.P. Krstic	Terroir – separating fact from fiction		
	Importance of sampling for quality parameters in the vineyard		
	Terroir – separating fact from fiction	AWRI roadshow seminar, Avoca, Vic	16 Jul 15
P.A. Henschke	Wild ferments – what are the alternatives?		
	Choose the right yeast to achieve the red wine style you want		

E.N. Wilkes	Wine development in bottle – the role of oxygen		
	Get the best out of your winery using 'lean production'		
E.J. Bartowsky	Using MLF to accentuate wine aroma and flavour		
	Strategies for a successful MLF		
C.A. Simos	Introduction to the Advanced Wine Assessment Course	Advanced Wine Assessment Course (AWAC 37)	20 Jul 15
W.P. Pearson	Flavour, taints, faults and thresholds		
T.E. Siebert	Overview of aroma compound analyses and sensory methods at the AWRI and their application to 'black pepper' and 'stone fruit' aromas in wine	Staff and postgraduate students, Centre for Wine Research at DLR Rheinpfalz University, Neustadt, Germany	21 Jul 15
P.R. Petrie	Climate impacts and business adaptation	ASVO seminar, Mildura, Vic	22-23 Jul 15
C.A. Simos	Introduction to the Advanced Wine Assessment Course	Advanced Wine Assessment Course (AWAC 38)	27 Jul 15
W.P. Pearson	Flavour, taints, faults and thresholds		
G.D. Cowey	Hotter and drier – processing ripe fruit	AWRI roadshow workshop, Hunter Valley, NSW	28 Jul 15
	Salinity and sodicity in the vineyard		
	Q&A session and case studies		
	New varieties for a changing climate tasting		
M. Essling	Hotter and drier in the vineyard		
	Growing grapes in wet seasons		

	Building resilience and sustainability in the grape and wine sector		
A.D. Coulter	Bushfires and smoke taint tasting		
	Winemaking in wet seasons		
	Sustainability and efficiencies in the winery		
M.L. Longbottom	Entwine Australia – demonstrating environmental performance of Australian vineyards and wineries	Wines of Western Australia Board, Perth, WA	31 Jul 15
		Wine Grape Council of South Australia, Adelaide, SA	7 Aug 15
	Greenhouse gas abatement in viticulture	Opportunities in a new climate workshop, Adelaide Hills, SA	11 Aug 15
	Entwine Australia – new and improved program, carbon calculator and regional benchmarking		
	Climate and the wine industry		
J.L. Hixson	Maximising the potential of grape marc		
M.G. Holdstock	Opportunities for the grape and wine sector		
	The Emissions Reduction Fund – how does it work?		
P.R. Petrie	Why do varieties respond differently to drought and heat stress – and what does this mean for your irrigation management?	AWRI roadshow seminar, McLaren Vale, SA	12 Aug 15
	Terroir – separating fact from fiction		
M. Essling	How can irrigation management strategies be used to manipulate wine quality?		
	How can cultural practices be used to improve fruit set?		

K.A. Bindon	The changing wine style of the ripening grape		
J.R. Bellon	Winemaking with non-conventional and hybrid yeast		
C.D. Curtin	Complex yeast nutrients – how do they fit into your fermentation management strategy?		
T.T.M.T. Tran	Monitoring Brett, what are your options?	AWRI webinar	13 Aug 15
P.R. Petrie	Is it possible to control bunch rot without fungicides?	AWRI roadshow seminar, Canberra, ACT	19 Aug 15
K.A. Bindon	Managing phenolic quality in the vineyard		
R. Gawel	Putting the texture back into white wine – the role of white wine phenolics		
	Solids ferments: effect of juice clarity and clarification method on the drivers of white wine texture		
P.A. Henschke	Did you know that DAP can strongly affect the flavour profile and style of wine?		
M. Essling	Entwine Australia – new and improved program, carbon calculator and regional benchmarking	Opportunities in a new climate workshop, Mornington Peninsula, Vic	20 Aug 15
	Greenhouse gas abatement in viticulture		
	Opportunities for the grape and wine sector		
G.D. Cowey	Climate and the wine industry		
	The emissions reduction fund – how does it work?		
A.D. Coulter	Taints and faults	Lallemand National Conference, Glenelg, SA	
P.R. Petrie	Vine balance – how does it affect yield and quality?	AWRI roadshow seminar, Orange, NSW	
	Why do we need new clones?		

K.A. Bindon	Managing phenolic quality in the vineyard		
	Solids ferments: effect of juice clarity and clarification method on the drivers of white wine texture		
P.A. Henschke	Wild ferments – what are the alternatives?		
P.R. Petrie	Why do varieties respond differently to drought and heat stress – and what does this mean for your irrigation management?	AWRI roadshow seminar, Mudgee, NSW	21 Aug 15
	Vine balance – how does it affect yield and quality?		
K.A. Bindon	Solids ferments: effect of juice clarity and clarification method on drivers of wine texture		
E.N. Wilkes	Get the best out of your winery using 'lean production'		
M.L. Longbottom	It's getting hotter – what does this mean for our vineyard management strategies?	AWRI roadshow seminar, Clare Valley, SA	25 Aug 15
	Entwine Australia – new and improved program, carbon calculator and regional benchmarking		
T.E. Siebert	Pepper and spice in Shiraz: what influences rotundone levels in wines?		
R. Gawel	Solids ferments: effect of juice clarity and clarification method on the drivers of white wine texture		
M.Z. Bekker	Copper in winemaking: the good, the bad and the ugly		
S.A. Schmidt	Managing stuck fermentations		
I.L. Francis, P.O. Williamson, S. Mueller-Loose[1,2], L. Lockshin[2]	Determining the influence of sensory and non-sensory wine attributes on purchase intent: linking discrete choice and informed sensory testing	Pangborn 2015 Sensory Science Symposium, Gothenburg, Sweden	26 Aug 15
D.L. Johnson	Finding a better way		

M.G. Holdstock	Wine flavours, faults and taints	Winery Engineers Association National Conference, Tanunda, SA	27 Aug 15
S. Nordestgaard	Historical and future developments in grape pressing		
M.P. Day	The use and effects of oxygen during early stages of winemaking – research and industrial practice		
T.J. Abbott	Update on developments in environmental accounting for wineries		
A.D. Coulter	Smoke taint	SAWIA 2015 Bushfire workshop, Barossa Valley, SA	
M.L. Longbottom	Entwine Australia – new and improved program, carbon calculator and regional benchmarking	Opportunities in a new climate workshop, Langhorne Creek, SA	
	Greenhouse gas abatement in viticulture		
M. Essling	Climate and the wine industry		
G.D. Cowey	The Emissions Reduction Fund – how does it work?		
	Opportunities for the grape and wine sector		
K.C. Hirlam	Maximising the potential of grape marc		
P.W. Godden	Presentation and tasting of wines with links to AWRI research projects	SA Science Council, AWRI, Adelaide, SA	28 Aug 15
M.L. Longbottom	Entwine Australia – new and improved program, carbon calculator and regional benchmarking	Opportunities in a new climate workshop, Launceston, Tas	1 Sep 15
	Climate and the wine industry		
	Greenhouse gas abatement in viticulture		
M.G. Holdstock	The Emissions Reduction Fund – how does it work?		
	Opportunities for the grape and wine sector		

J.R. Bellon	Breeding new life into the ancient art of winemaking	University of South Australia, Adelaide, SA	
	The AWRI		
M.L. Longbottom	Entwine Australia – demonstrating environmental performance of Australian vineyards and wineries	Winemakers' Federation of Australia, Adelaide, SA	2 Sep 15
S. Nordestgaard	History of the wine press	Members of the Australian Institute of Food Science and Technology, Engineers Australia, Institution of Chemical Engineers, Royal Australian Chemical Institute, Adelaide, SA	3 Sep 15
P.A. Smith	What are the relationships between grape chemicals?	AWRI webinar	
C.A. Simos	Overview of smoke taint	Australia Fire and Emergency Service Authorities Council, Lenswood, SA	4 Sep 15
C.D. Curtin	Comparative genomics and transcriptomics of *Brettanomyces*	Department of Applied Microbiology, Lund University, Sweden	
M.L. Longbottom	Entwine Australia – demonstrating environmental performance of Australian vineyards and wineries	Wine Grape Growers Australia, Adelaide, SA	6 Sep 15
		Clare Region Winegrape Growers Association, Clare Valley, SA	7 Sep 15
C.D. Curtin	Wine research down under	Microwine EU Consortium, Copenhagen, Denmark	8 Sep 15
A.D. Coulter	Smoke taint	SAWIA 2015 Bushfire workshop, McLaren Vale, SA	
P.R. Petrie	Vine balance – how does it affect yield and quality?	AWRI roadshow seminar, Barossa Valley, SA	
	Why do bunches get hot – and what does this mean for wine quality?		
M. Essling	Does soil and vine nutrient status affect wine quality?		
	How can irrigation management strategies be used to manipulate wine quality?		

J.M. McRae	The changing wine style of the ripening grape		
T.E. Siebert	Pepper and spice in Shiraz: what influences rotundone levels in wines?		
M.L. Longbottom	Entwine Australia – demonstrating environmental performance of Australian vineyards and wineries		
P.R. Petrie	Climate change in viticulture	Accolade viticulture managers, AWRI, Adelaide, SA	9 Sep 15
C.D. Curtin	The good, the bad, and the ugly: volatile sulfur compound metabolism in *Saccharomyces cerevisiae*	Bioflavour Conference, Frankfurt, Germany	11 Sep 15
	Comparative genomics and transcriptomics of the industrial yeast species *Brettanomyces bruxellensis*	International Symposium on Specialised Yeast, Perugia, Italy	16 Sep 15
P.R. Petrie	Vineyard frost management strategies	AWRI webinar	17 Sep 15
P.W. Godden	Wine flavours, faults and taints	Westpac Group premium wine experience, Barossa Valley, SA	25 Sep 15
	Guided tasting		
C.S. Stockley	Wine and health research and related national and international issues	Future Leaders 2015, Loxton, SA	
C.A. Varela	Practical strategies for reducing alcohol levels in wine – generating wine yeasts that produce reduced levels of ethanol	AWRI webinar	1 Oct 15
T.E. Siebert	Volatile aroma compounds related to 'stone fruit' aroma in Viognier and Chardonnay wines	School of Pharmacy and Medical Science Symposium, UniSA, Adelaide, SA	2 Oct 15
C.A. Simos	Smoke taint: what lies beneath and other AWRI research projects	Anchor Yeast technical forum, South Africa	9 Oct 15
M.L. Longbottom	Entwine Australia	Adelaide Hills Wine Region Executive Committee, SA	12 Oct 15
M. Essling	Entwine Australia – new and improved program, carbon calculator and regional benchmarking	Opportunities in a new climate workshop, Riverland, SA	15 Oct 15
	Climate and the wine industry		

M.G. Holdstock	Opportunities for the grape and wine sector in the Emissions Reduction Fund		
J.L. Hixson	A novel use for grape marc – methane mitigation		
M. Essling	Greenhouse gas emissions abatement in viticulture – results of nitrogen use efficiency trials in vineyards		
I.L. Francis	Predicting allocation of grade from key grape compositional measures	2015 Australian Cabernet Symposium, Penola, SA	
M.L. Longbottom	Entwine Australia – environmental performance of Australian vineyards and wineries	AWRI webinar	
A.D. Coulter	Risk of unconventional gas exploration to wine quality	Natural Resources Committee on Fracking, Adelaide, SA	16 Oct 15
P.O. Williamson	Understanding wine consumers: linking sensory testing, consumer product acceptance and marketing research	Future Leaders visit to the AWRI, Adelaide, SA	20 Oct 15
C.A. Simos	An introduction to the AWRI		
G.D. Cowey	A changing environment	AWRI roadshow workshop, Kangaroo Island, SA	21 Oct 15
	Hotter and drier – processing ripe fruit		
	Bushfires		
	Salinity and sodicity in the vineyard		
M. Essling	Hotter and drier in the vineyard		
	Sustainability and efficiencies in the winery		
	Entwine Australia – new and improved program, carbon calculator and regional benchmarking	Opportunities in a new climate workshop, Kangaroo Island, SA	
G.D. Cowey	Climate and the wine industry		

	The Emissions Reduction Fund – how does it work?		
M. Essling	Greenhouse gas emissions abatement in viticulture – results of nitrogen use efficiency trials in vineyards		
C.D. Curtin	Comparative genomics and transcriptomics of *Brettanomyces*	School of Biology, University of Auckland, NZ	
M.P. Krstic	Oak volatiles 101 and trial results	Oak workshop, Healesville, Vic	22 Oct 15
C.A. Varela	AWRI biosciences wine research	SupAgro INRA, Montpellier, France	23 Oct 15
	Using non-conventional yeast for the production of reduced alcohol wines	4th International Symposium, Oenoviti International Network, Conegliano, Italy	26 Oct 15
M.L. Longbottom	Managing nutrition in a changing climate	Research to practice workshop, Riverland, SA	
	Principles of grapevine nutrition		
	Nutrients and grapevine growth		
	Nutrient analyses for grapevines		
K.A. DeGaris	Key grapevine nutrients		
	Alternative methods of grapevine nutrition		
M.L. Longbottom	Entwine Australia	McLaren Vale Grape Wine and Tourism Association Executive Committee, SA	27 Oct 15
G.D. Cowey	Hotter and drier – processing ripe fruit	AWRI roadshow workshop, Mudgee, NSW	
	Q&A session and case studies		
	Building resilience and sustainability in the grape and wine sector		
	New varieties for a changing climate tasting		

P.R. Petrie	Hotter and drier in the vineyard		
	Growing grapes in wet seasons		
A.D. Coulter	Bushfires and smoke taint tasting		
	Winemaking in wet seasons		
	Sustainability and efficiencies in the winery		
G.D. Cowey	Hotter and drier – processing ripe fruit	AWRI roadshow workshop, Orange, NSW	28 Oct 15
	Q&A session and case studies		
	Building resilience and sustainability in the grape and wine sector		
	New varieties for a changing climate tasting		
P.R. Petrie	Hotter and drier in the vineyard		
	Growing grapes in wet seasons		
A.D. Coulter	Bushfires and smoke taint tasting		
	Winemaking in wet seasons		
	Sustainability and efficiencies in the winery		
G.D. Cowey	Hotter and drier – processing ripe fruit	AWRI roadshow workshop, Canberra, ACT	30 Oct 15
	Q&A session and case studies		
	Building resilience and sustainability in the grape and wine sector		

	New varieties for a changing climate tasting		
P.R. Petrie	Hotter and drier in the vineyard		
	Growing grapes in wet seasons		
A.D. Coulter	Bushfires and smoke taint tasting		
	Sustainability and efficiencies in the winery		
	Winemaking in wet seasons		
D.L. Johnson	2015 AWRI annual report	Wine Tasmania Board (by teleconference)	3 Nov 15
M.L. Longbottom	Entwine Australia – new and improved program, carbon calculator and regional benchmarking	Opportunities in a new climate workshop, Padthaway, SA	4 Nov 15
	Climate and the wine industry		
	Greenhouse gas emissions abatement in viticulture – results of nitrogen use efficiency trials in vineyards		
G.D. Cowey	Opportunities for the grape and wine sector in the Emissions Reduction Fund		
	A novel use for grape marc – methane mitigation		
R. Gawel	The murky side of winemaking: solids ferments, wine composition and mouth-feel	AWRI webinar	5 Nov 15
C.A. Simos	Technical quality assurance of Australian wine	APEC regulators visit, AWRI, Adelaide, SA	13 Nov 15
M.J. Herderich	Wine authentication		
C.A. Simos	Introduction to the Advanced Wine Assessment Course	Advanced Wine Assessment Course (AWAC 39)	16 Nov 15
W.P. Pearson	Flavour, taints, faults and thresholds		

P.R. Petrie	Earlier, shorter, hotter? Is it really happening?	ASVO seminar, Adelaide, SA	19 Nov 15
M.G. Holdstock	Vintage 2015 – what we saw, what we learnt		
N. Scrimgeour	Using technology to keep on top of ferments		
K.C. Hirlam	Grape marc: maximising the potential of a winery by-product	Crush 2015, the grape and wine science symposium, Adelaide, SA	20 Nov 15
A. Mierczynska-Vasilev	Effects of surface functionalisation on the adsorption of red wine		
K.A. Bindon	The secret life of wine macromolecules		
R. Gawel	Murky winemaking: how juice solids affect the macromolecular composition and mouthfeel of white wine		
P.O. Williamson	Chinese consumer preferences of Australian, Chinese and French red wines assessed both blind and informed		
	Introduction to wine sensory evaluation	VALO exclusive at Chester's, McLaren Vale, SA	21 Nov 15
T.J. Abbott	The Australian wine industry's environmental assurance program: towards streamlined individual LCAs and a national model	AgriFoodLCA Conference, Melbourne, Vic	23 Nov 15
A.D. Coulter	Smoke taint, cold stability and copper	International Masters of Wine, National Wine Centre, Adelaide, SA	24 Nov 16
P.R. Petrie	Delayed pruning to help manage vintage logistics	AWRI roadshow seminar, Griffith, NSW	26 Nov 15
	Sampling for maturity and fruit grading		
R. Gawel	Phenolics and the interactive effects of pH, acidity and alcohol on bitterness and mouth-feel of white wine		
	Solids ferments: effect of juice clarity and clarification method on the drivers of white wine texture		

M. Essling	Integrated strategies for managing *Botrytis*		
	Why do varieties respond differently to drought and heat stress?		
E.N. Wilkes	Cold stability, hitting a moving target		
	Get the best out of your winery using 'lean production'		
C.A. Simos	Practical management of 'Brett' in the winery		
	Interactive session		
P.J. Chambers	Genetically modified foods: the mythology and science	Treasury Wine Estates technical conference, Magill, SA	
A.D. Coulter	Lingering smoky flavours: measurement and sensory aspects of smoke taint		
W.P. Pearson	Defining green flavour in Cabernet Sauvignon		27 Nov 15
M.P. Krstic	Wine sector R&D needs	Goulburn Valley Food R&D roundtable, Maroopna, Vic	2 Dec 15
S.A. Schmidt	Yeast competitive fitness in wine-like fermentation environments	Yeast Products and Discovery Conference, Adelaide, SA	
A.G. Cordente	Generation of novel *Saccharomyces cerevisiae* wine yeast mutants and *Saccharomyces* interspecies hybrids that overproduce 'rose' aroma compounds 2-phenylethanol and 2-phenylethyl acetate		2-4 Dec 15
J.R. Bellon	Breeding new life into the ancient art of winemaking		
C.A. Varela	Systems biology: a new approach to industrial yeast strain development		
C.D. Curtin	Comparative genomics of the industrial yeast species *Brettanomyces bruxellensis*		

N. Scrimgeour	Using in-line sensors to monitor sugar levels during fermentation	AWRI webinar	3 Dec 15
M.J. Herderich	Towards a scientific interpretation of the terroir concept: plasticity of the grape berry metabolome	Australia-Italy Grape and Wine Symposium, Adelaide, SA	7 Dec 15
C.A. Simos	Smoke taint Q&A	AWRI roadshow seminar - smoke taint, Frankland River, WA	8 Dec 15
S. Nordestgaard	Recent advances in destemming and sorting technology	AWRI webinar	
M. Essling	Entwine Australia – new and improved program, carbon calculator and regional benchmarking	Opportunities in a new climate workshop, Mildura, Vic	
	Climate and the wine industry		
J.L. Hixson	Opportunities for the grape and wine sector in the Emissions Reduction Fund		
	A novel use for grape marc – methane mitigation		
M. Essling	Greenhouse gas emissions abatement in viticulture – results of nitrogen use efficiency trials in vineyards		
M.P. Krstic	Smoke taint update – improved risk assessment	Drought, fire and smoke management seminar, Pyrenees, Vic	
M.L. Longbottom	Entwine Australia – new and improved program, carbon calculator and regional benchmarking	Opportunities in a new climate workshop, Barossa Valley, SA	10 Dec 15
	Climate and the wine industry		
	Greenhouse gas emissions abatement in viticulture – results of nitrogen use efficiency trials in vineyards		
M.G. Holdstock	Opportunities for the grape and wine sector in the Emissions Reduction Fund		
K.C. Hirlam	A novel use for grape marc – methane mitigation		
J.M. McRae	Hot news in heat stability!	Interwinery Analysis Group, Hahndorf, SA	10 Dec 15

M.J. Herderich	Defining the grape metabolome and its relationship with wine composition through comprehensive HPLC-MS/MS profiling of non-volatile grape and wine compounds	Pacifichem Congress, Honolulu, Hawaii, USA	17 Dec 15
M.L. Longbottom	Entwine Australia – new and improved program, carbon calculator and regional benchmarking	Opportunities in a new climate workshop, Margaret River, WA	13 Jan 16
	Climate and the wine industry		
	Greenhouse gas emissions abatement in viticulture – results of nitrogen use efficiency trials in vineyards		
M.G. Holdstock	Opportunities for the grape and wine sector in the Emissions Reduction Fund		
	A novel use for grape marc – methane mitigation		
M.L. Longbottom	Entwine Australia	Wines of Western Australia technical committee, Perth, WA	14 Jan 16
E.N. Wilkes	Why doesn't 14°Bé give 14% alcohol?	AWRI webinar	21 Jan 16
C.A. Simos	Smoke taint Q&A	AWRI roadshow seminar - smoke taint, Geographe, WA	22 Jan 16
I.L. Francis	Cloves, kerosene or capsicum? The science of wine sensory evaluation	Rotary Club of Mitcham, Mitcham, SA	27 Jan 16
M.L. Longbottom	Entwine Australia – new and improved program, carbon calculator and regional benchmarking	Opportunities in a new climate workshop, Hobart, Tas	28 Jan 16
	Climate and the wine industry		
	Greenhouse gas emissions abatement in viticulture – results of nitrogen use efficiency trials in vineyards		
G.D. Cowey	Opportunities for the grape and wine sector in the Emissions Reduction Fund		
	A novel use for grape marc – methane mitigation		

S. Nordestgaard	Destemming and sorting technology in the vineyard and winery	Limestone Coast Grape and Wine Council berry sensory/sorting workshop, Robe, SA	

A.D. Coulter	Cold stability and CMC	International Masters of Wine, Bordeaux, France	17 Feb 16
	Copper: advantages and disadvantages		
	Smoke taint		
	Cold stability and CMC	International Masters of Wine, UK	18-19 Feb 16
	Copper: advantages and disadvantages		
	Q&A session and case studies		
	Smoke taint		
P.O. Williamson	Influencing consumer choice: Short and medium-term effect of country of origin information on wine choice	Ehrenberg-Bass Institute of Marketing, University of South Australia, SA	25 Feb 16
C.A. Simos	Smoke taint Q&A	AWRI roadshow seminar - smoke taint, Launceston, Tas	4 Mar 16
P.O. Williamson, I.L. Francis	The latest advances in wine flavour research	Wine Australia Pan Asia and Trade group, AWRI, Adelaide, SA	10 Mar 16
E.N. Wilkes	Impact of closures on wine		
V.T. O'Brien	Luxury through innovation		
D.L. Capone	Determining the role of polyfunctional thiols in Chardonnay wine aroma	251st American Chemical Society National Meeting – Symposium on Flavour Chemistry of Alcoholic Beverages, San Diego, USA	14 Mar 16
M.Z. Bekker	Synergistic effects of copper and pH – winemaking variables that significantly impact reductive aromas in wines		

C.S. Stockley	Potassium carbonate as an additive in winemaking – safety aspects	OIV Food Safety Expert group meeting, Bordeaux, France	12 Apr 16
	PDMS as a processing aid in winemaking – safety aspects		
	Proctase health and safety evaluation		
C.A. Simos, M.P. Krstic	Stuck ferments Q&A	AWRI roadshow seminar, Yarra Valley, Vic	22 Apr 16
M.P. Krstic	Regional demonstration trials and findings	Trunk disease workshop, Mornington Peninsula, Vic	26 Apr 16
		Trunk disease workshop, Milawa, Vic	27 Apr 16
G.D. Cowey	Why is climate important in wine-grape production?	Opportunities in a new climate workshop, Great Western, Vic	28 Apr 16
	Trading carbon – what is the future for the wine industry?		
M. Essling	Entwine Australia – promoting a sustainable grape and wine community		
	How does the environmental performance of the Grampians stack up?		
M.P. Krstic	Regional demonstration trials and findings	Trunk disease workshop, Great Western, Vic	
V.T. O'Brien, G.A.P. Patacq	Design thinking	Design thinking/ideation workshop, Kingston-on-Murray, SA	2 May 16
G.D. Cowey	Why is climate important in wine-grape production?	Opportunities in a new climate workshop, Mount Barker, WA	4 May 16
	Where is all my energy going? Sources and sinks in the vineyard and winery		
	Keeping cool in the winery		
	Trading carbon – what is the future for the wine industry?		

M. Essling	Entwine Australia – promoting a sustainable grape and wine community		
	How does the environmental performance of Mt Barker stack up?		
	Fuel, fertiliser and electricity: the multi-pronged approach in the vineyard		
	Harnessing the sun at the winery		
	Grazing sheep in vineyards – saving fuel and money		
C.S. Stockley	Adverse food reactions, wine and winemaking	Oenoviti International Symposium and General Assembly, Bordeaux, France	10-11 May 16
N. Scrimgeour	The influence of different closure technologies and oxygen management techniques on wine shelf life	AWRI webinar	12 May 16
M.L. Longbottom	Why is climate important in wine-grape production?	Opportunities in a new climate workshop, McLaren Vale, SA	
	Entwine Australia – promoting a sustainable grape and wine community		
	How does the environmental performance of McLaren Vale stack up?		
	Grazing sheep in vineyards – saving fuel and money		
	Fuel, fertiliser and electricity: the multi-pronged approach in the vineyard		
G.D. Cowey	Where is all my energy going? Sources and sinks in the vineyard and winery		
	Keeping cool in the winery		
	Trading carbon – what is the future for the wine industry?		
	Harnessing the sun at the winery		

P.A. Smith	Opportunities for creating value in wine production: Impacts of proteins, polyphenols, polysaccharides, oxygen and sulfur compounds.	Marlborough Wine Research Centre, Blenheim, NZ	16 May 16
		Lincoln University, Nelson, NZ	
M. Essling	Why is climate important in wine-grape production?	Opportunities in a new climate workshop, Orange, NSW	18 May 16
	Practical ways to reduce emissions in the vineyard		
	Entwine Australia – promoting a sustainable grape and wine community		
	How does the environmental performance of Orange stack up?		
T.J Abbott	What is the world doing? What is Australia doing? What is the future for the wine industry?		
	Trading carbon – what is the future for the wine industry?		
	Practical ways to reduce emissions in the winery		
M. Essling	Trading carbon – what is the future for the wine industry?	Opportunities in a new climate workshop, Hunter Valley, NSW	19 May 16
	Practical ways to reduce emissions in the winery		
	Practical ways to reduce emissions in the vineyard		
	Why is climate important in wine-grape production?		
	Entwine Australia – promoting a sustainable grape and wine community		
	How does the environmental performance of the Hunter Valley stack up?		
E.N. Wilkes	Phenolic measurements using the WineCloud	Enartis, Windsor, California, USA	20 May 16

		Enartis, Paso Robles, California, USA	
M.L. Longbottom	Why is climate important in wine-grape production?	Opportunities in a new climate workshop, Adelaide Hills, SA	
	Practical ways to reduce emissions in the vineyard		
	Entwine Australia – promoting a sustainable grape and wine community		
	How does the environmental performance of the Adelaide Hills stack up?		
M.G. Holdstock	Practical ways to reduce emissions in the winery		
	Trading carbon – what is the future for the wine industry?		
	Smoke taint update		
E.N. Wilkes	Workshop on the practical analysis of SO_2, Alcohol, TA and sugar	International Wine Technical Summit, San Lusi Obispo, California, USA	23 May 16
C.A. Simos, P.O. Williamson	How spice character makes Australian Shiraz unique	Vinexpo 2016, Hong Kong	26 May 16
E.N. Wilkes	Brett, accepting the problem is the first step	Invisible Sentinel Seminar, Napa, California, USA	27 May 16
T.J Abbott	Where is all my energy going? Sources and sinks in the vineyard and winery	Opportunities in a new climate workshop, Clare Valley, SA	
	Trading carbon – what is the future for the wine industry?		
	Harnessing the sun at the winery		
	Opportunities for the grape and wine sector in the Emissions Reduction Fund		
M.L. Longbottom	Why is climate important in wine-grape production?		

	Entwine Australia – promoting a sustainable grape and wine community		
	How does the environmental performance of Clare stack up?		
	Grazing sheep in vineyards – saving fuel and money		
	Fuel, fertiliser and electricity: the multi-pronged approach in the vineyard		
W.P. Pearson	Aromas and flavours	Wine Australia sensory evaluation dinner, Sydney, NSW	
K.A. DeGaris	How can irrigation management strategies be used to manipulate wine quality?	AWRI roadshow seminar, Coonawarra, SA	31 May 16
	Why do varieties respond differently to drought and heat stress?		
M.L. Longbottom	Does soil and vine nutrient status affect wine quality?		
	Why do bunches get hot – and what does this mean for wine quality?		
P.A. Smith	What are the relationships between grape chemical composition, grape allocation grade and final wine style?		
R. Gawel	Putting the texture back into white wine – the role of white wine phenolics		
	Phenolics and the interactive effects of pH, acidity and alcohol on bitterness and mouth-feel of white wine		
C.A. Simos	Features of the AWRI website		
M.L. Longbottom	Why is climate important in wine-grape production?	Opportunities in a new climate workshop, Tumbarumba, ACT	2 Jun 16
G.D. Cowey	Trading carbon – what is the future for the wine industry?		
	Practical ways to reduce emissions in the winery		

M. Essling	Entwine Australia – promoting a sustainable grape and wine community		
	How does the environmental performance of Canberra District stack up?		
	Practical ways to reduce emissions in the vineyard		
G.D. Cowey	Why is climate important in wine-grape production?	Opportunities in a new climate workshop, Canberra, ACT	3 Jun 16
	Trading carbon – what is the future for the wine industry?		
	Practical ways to reduce emissions in the winery		
M. Essling	Entwine Australia – promoting a sustainable grape and wine community		
	How does the environmental performance of Canberra District stack up?		
	Practical ways to reduce emissions in the vineyard		
P.A. Smith	The changing wine style of the ripening grape	AWRI roadshow seminar, Riverland, SA	8 Jun 16
	What are the relationships between grape chemical composition, grape allocation grade and final wine style?		
S. Nordestgaard	Maximising quality during bulk wine transportation		
M. Essling	Agrochemical update		
	Is it possible to control bunch rot without fungicides?		
E.N. Wilkes	Copper in winemaking, the good and the bad		
P.R. Petrie	Vine balance – how does it affect yield and quality?		

	Great wine from grafted vines		
I.L. Francis	Thinking outside the bottle: insights on how Chinese consumers choose wine		
M.P. Krstic	Do you ignore your vineyard after harvest?		
E.N. Wilkes	Get the best out of your winery using 'lean production'		
S. Nordestgaard	Maximising quality during bulk wine transportation	AWRI roadshow seminar, Irymple, Vic	9 Jun 16
M. Essling	Agrochemical update		
E.N. Wilkes	The truth about sulfur dioxide		
M.P. Krstic	Do you ignore your vineyard after harvest?		
P.A. Smith	What are the relationships between grape chemical composition, grape allocation grade and final wine style?		
E.N. Wilkes	Get the best out of your winery using 'lean production'		
P.J. Chambers	Robot room tour and presentation	Wine Australia USA retail buyers group, Adelaide, SA	
P.A. Smith, A. Mierczynska Vasilev	Nanoparticle tracking analysis		
P.W. Godden	Screwcaps vs cork		
W.P. Pearson	Sensory perception of faults and positive flavour compounds		
M.P. Krstic	Welcome and setting the Chardonnay scene	Cooler climate Chardonnay symposium, Yarra Valley, Vic	16 Jun 16
I.L. Francis	Insights into flavour and aroma compounds in Chardonnay		

M.P. Krstic	International Chardonnay production and performance		
M.L. Longbottom	Entwine Australia – promoting a sustainable grape and wine community	Padthaway Grapegrowers Association, Padthaway, SA	17 Jun 16
J.M. McRae	Filtration effects on red wines	Treasury Wine Estates winemakers, Nuriootpa, SA	21 Jun 16
K.A. Bindon	Predicting wine tannin and colour in the vineyard	AWRI roadshow seminar, Murgon, Qld	22 Jun 16
	Managing the risk of protein haze formation in white wines		
M.L. Longbottom	Why do varieties respond differently to drought and heat stress?		
	Why do bunches get hot – and what does this mean for wine quality?		
P.J. Costello	Microbial spoilage of wine: a refresher on how to prevent it		
J.L. Hixson	Phenolics and the interactive effects of pH, acidity and alcohol on bitterness and mouth-feel of white wine		
C.A. Simos	Features of the AWRI website		
M.P. Krstic	Introduction – new vineyard management innovations	Future vineyard management practices workshop, Milawa, Vic	
S. Nordestgaard	Latest grape harvesting and grape sorting technologies		
M. Essling	Latest updates and insights into agrochemical use in viticulture		
M.J. Herderich	Old friends in new bottles: Aroma precursors in grapes and wine and the complexities of wine flavour	Wartburg symposium on flavour chemistry and biology, Germany	
M.L. Longbottom	Vine balance – how does it affect yield and quality?	AWRI roadshow seminar, Stanthorpe, Qld	23 Jun 16
	Does soil and vine nutrient status affect wine quality?		

	Entwine Australia – promoting a sustainable grape and wine community		
K.A. Bindon	Manipulation of Pinot Noir red wine phenolic profiles during winemaking		
P.J. Costello	Microbial spoilage of wine: a refresher on how to prevent it		
	Strategies for a successful MLF		
J.L. Hixson	Putting the texture back into white wine – the role of white wine phenolics		
C.A. Simos	Features of the AWRI website		
E.N. Wilkes	Practical analysis of SO_2 and alcohol	Interwinery Analysis Group seminar, Adelaide, SA	
	SO_2, what are we really measuring?		24 Jun 16
R. Gawel	Opportunities for creating value in wine production: impacts of proteins, polyphenols, polysaccharides, oxygen and sulfur compounds.	'Gold Standard' California Wine Laboratory Technical Group, Napa, California, USA	
G. D. Cowey	Q&A session and case studies	AWRI roadshow workshop, Rutherglen, Vic	28 Jun 16
	New varieties for a changing climate tasting		
	Hotter and drier – processing ripe fruit		
	Salinity, sodicity and salty wine		
A.D. Coulter	Bushfires and smoke taint tasting		
	Winemaking in wet seasons		
P.R. Petrie	Hotter and drier in the vineyard		

	Growing grapes in wet seasons		
	Building resilience and sustainability in the grape and wine sector		
A.D. Coulter, G.D. Cowey	Stuck fermentation		
M. Essling	Entwine Australia – promoting a sustainable grape and wine community	Sustainable Australia winegrowing launch, Adelaide Hills, SA	
V.T. O'Brien, G.A.P. Patacq	Building emotive appeal in wine offerings	Barossa Grape and Wine Association, Barossa Valley, SA	28 Jun 16
R. Gawel	White juice solids: effects on phenolics, polysaccharides, and mouth-feel of white wine	American Society for Enology and Viticulture 67th National Conference, Monterey, California, USA	29 Jun 16
P.R. Petrie	Hotter and drier in the vineyard	AWRI roadshow workshop, Goulburn Valley, Vic	
	Growing grapes in wet seasons		
	Building resilience and sustainability in the grape and wine sector		
A.D. Coulter	Winemaking in wet seasons		
	Bushfires and smoke taint tasting		
G.D. Cowey	Salinity, sodicity and salty wine		
	Hotter and drier – processing ripe fruit		
	New varieties for a changing climate tasting		
A.D. Coulter, G.D. Cowey	Stuck fermentation		

P.A. Smith	Defining the mechanisms and impact of winemaking treatments on tannin and polysaccharides in red wine: recent progress in creating diverse styles	Macrowine Conference, Nyon, Switzerland	30 Jun 16
M.J. Herderich	Grape metabolites, aroma precursors and the complexities of wine flavour		

M.L. Longbottom	How does the environmental performance of Langhorne Creek stack up?	Opportunities in a new climate workshop, Langhorne Creek, SA	5 Jul 16
	Entwine Australia - demonstrating environmental performance of Australian vineyards and wineries		
P.R. Petrie	Smarter farming on Adelaide's doorstep	National Climate Change Adaptation Research Facility (NCCARF) Climate Adaptation 2016, Adelaide, SA	6 Jul 16
M.L. Longbottom	Time to renew – how to enter and interpret your Entwine data	AWRI webinar	13 Jul 16
M.Z. Bekker, M.P. Day, M.E. Smith, P.A. Smith, E.N. Wilkes	Effectiveness of Cu-remediation. Can you get your freshness back?	16th Australian Wine Industry Technical Conference, Workshop Program, Adelaide, SA	24 Jul 16
	Factors influencing volatile sulfur compound formation in wines post-bottling		
I.L. Francis	Undesirable flavour in Shiraz wines from high vigour rootstocks		
	Introduction/closing 'Recent advances in flavour'		
	Exogenous sources of flavour/ 'green' flavour in red wines		
S. Nordestgaard	History and developments in heating of red grapes for extraction		
C.S. Stockley	Things you need to know		
	Evolving evidence for the role of wine in reducing the risk of four primary causes of death in Australia		
J.R. Bellon, A. Cordente	Novel yeast for new wine styles		

P.O. Williamson	Consumer insights in China		
D.L. Capone	Determining the role of polyfunctional thiols in Chardonnay wine aroma		
M.P. Day	The impact of air exposure during fruit processing		
S.A. Schmidt	Oxygen use during winemaking		
A. Cordente	Modulation of sulfur compound formation by yeast during fermentation		
T.E. Siebert	'Stone fruit' aroma and flavour in wine		
N. Scrimgeour	The impact of oxygen management on wine shelf-life		
P.J. Costello	High-throughput phenotyping of malolactic bacteria		
E.J. Bartowsky	The diversity of Oenococcus oeni – what it can mean for MLF		
J.R. Bellon	Breeding new life into the ancient art of winemaking		
P.J. Chambers	Microbial diversity at work in vineyards and wine	16th Australian Wine Industry Technical Conference, Main Program, Adelaide, SA	26 Jul 16
T.M. Parker	In-mouth flavour release from non-volatile grape-derived precursors	16th Australian Wine Industry Technical Conference, Fresh Science session, Adelaide, SA	
P.J. Costello	High-throughput phenotypic profiling of malolactic bacteria		
S.A. Schmidt	Genetic diversity in clones of Chardonnay	16th Australian Wine Industry Technical Conference, Main Program, Adelaide, SA	
P.R. Petrie	Practical options to manage vintage compression		
M.P. Day	Measuring up authentication: analytical tools to test wine provenance		27 Jul 16

S. Nordestgaard	Sources and properties of lees	16th Australian Wine Industry Technical Conference, Workshop Program, Adelaide, SA	
M.J. Herderich	Biological and environmental factors relating to rotundone formation		
T.E. Siebert	History of rotundone research		
M.P. Krstic	Using precision viticultural tools to better understand flavour variation in the vineyard		
C.S. Stockley	Wine labelling – the growing influence of the anti-alcohol lobby on health warning labels		
J.M. McRae	Hot news in heat tests!		
E.N. Wilkes	CMCs: more than just cold comfort		
P.W. Godden	Hot and cold stability in white wines		
E.N. Wilkes	Analytical testing – challenges for companies		
K.A. Bindon	Objective measures of grape quality	16th Australian Wine Industry Technical Conference, Fresh Science session, Adelaide, SA	28 Jul 16
N. Scrimgeour	Using cross-linked polymers to scavenge metals and extend shelf-life of wine		
D.L. Capone	Tropical flavours in Chardonnay wines	Workshop on varietal thiols in wine, AWRI, SA	1 Aug 16
M.P. Krstic	Effect of rootstock on Pinot Noir and Chardonnay performance	Post-vintage ‘work in progress’ tasting and rootstock seminar, Yarra Valley, Vic	11 Aug 16
S. Dillon, A. Rinaldo	Wine fermentation	A Big Glass of Wine Science, National Science Week event, North Adelaide, SA	16 Aug 16
J.L. Hixson, M.P. Day, M.Z. Bekker	Wine processes		
T.E. Siebert, P.O. Williamson, E. Kristianto	Wine flavours and aromas		

C.S. Stockley, V.F. Phillips	Wine and health		
J.M. McRae, E.N. Wilkes	Wine myth-busting		
G.A.P. Patacq	Do you really understand luxury in wine?	AWRI webinar	18 Aug 16
E.N. Wilkes	Copper - the good, the bad and the ugly	New Zealand Winegrowers Romeo Bragato Conference, Blenheim NZ	25 Aug 16
M.P. Day	Use of early oxygen exposure to modify style in white and red wines.		
P.A. Smith	Understanding how and why oxygen impacts on molecular drivers of taste and texture in wines		
A.D. Coulter	Stuck ferments, what can you do?	AWRI webinar	1 Sep 16
M.G. Holdstock	Introduction to the day	Sustainability and alternative energy options workshop, Padthaway, SA	5 Sep 16
M.L. Longbottom	Sustainable wine-grape production in Australia - drivers and measures		
	Natural capital management - impacts on the bottom line		
C.A. Simos, P.O. Williamson	How spice character makes Australian Shiraz unique?	Wine Australia, Australian Wine Grand Tasting 2016, Seoul, Republic of Korea	6 Sep 16
S. Nordestgaard	Innovations and options for grape sorting	Winery Engineering Association conference, McLaren Vale, SA	7 Sep 16
M.G. Holdstock	Shiraz and its regional differences		
S. Nordestgaard	Rapid extraction techniques for red wine production		8 Sep 16
T.J. Abbott	Real-time fermentation monitoring		
E.N. Wilkes	Closures - our changing understanding		
M.J. Herderich	New smoke taint R&D		

A.D. Coulter	Smoke taint	Accolade Wines viticulturists annual meeting, McLaren Vale, SA	
C.A. Simos, P.O. Williamson	How spice character makes Australian Shiraz unique?	Wine Australia, Australian Wine Grand Tasting 2016, Tokyo, Japan	
M.G. Holdstock	Wine flavours, faults and taints	Sommeliers Australia - Staff training, Sydney, NSW	12 Sep 16
G.D. Cowey		Sommeliers Australia - Staff training, Melbourne, Vic	
M.G. Holdstock	Simulated flavours, faults, taints and mouth-feel tasting	Sommeliers Australia, Sydney, NSW	19 Sep 16
P.O. Williamson	The right message: increasing choice of Australian wine in China	Creating consumer value workshop, Barossa Valley, SA	22 Sep 16
W.P. Pearson	Defining 'green' flavour in Cabernet Sauvignon wines using projective mapping and descriptive analysis	Cool Climate Oenology & Viticulture Institute, Brock University, St Catherines, Ontario, Canada	
T.M. Parker	Flavour precursors: contribution to wine flavour through in-mouth flavour release	University of South Australia, School of Pharmacy and Medical Science Postgraduate Symposium, Adelaide, SA	30 Sep 16
E.N. Wilkes	Performance of international laboratories for wine analysis, 2016 ring test results	APEC Wine Regulatory Forum, Ottawa, Canada	6 Oct 16
	Regulatory practices in wine - the example of methanol		7 Oct 16
M.P. Day	Measuring up authentication: analytical tools to test wine provenance	AWRI webinar	13 Oct 16
M.P. Krstic	Soil health – what is it and how can we manage it?	AWRI roadshow seminar, Geelong, Vic	
P.R. Petrie	Earlier, shorter, hotter? Is vintage compression really happening and what can you do about it?		
	How to improve fruit set in cool climates?		
E.N. Wilkes	Cold stability, hitting a moving target		

	Copper, the good, the bad and the ugly		
P.O. Williamson	Australian flavours	Tourism Australia staff conference, Sydney, NSW	19 Oct 16
G.D. Cowey	How spice character makes Australian Shiraz unique	Wine Australia China Awards & Best of Australia Showcase, China	24 Oct 16
P.W. Godden	Overview of the AWRI's RDEC capabilities	Wine Australia UK and EU media group visit, AWRI, Adelaide, SA	25 Oct 16
E.N. Wilkes	Impact of closures on wine		
P.O. Williamson	Linking the AWRI's wine flavour research with regionality		
P.J. Costello	Wine fermentation	Australian Institute of Food Science and Technology (AIFST) Microbiology Community of Interest Event, Professor Graham Fleet Memorial, Sydney, NSW	26 Oct 16
S. Nordestgaard	Hot extraction: a tool to manage compressed vintages?	AWRI webinar	27 Oct 16
C.A. Simos	Introduction to the Advanced Wine Assessment Course	Advanced Wine Assessment Course #40	31 Oct – 3 Nov 16
G.D. Cowey	Flavour, taints, faults and thresholds		
P.W. Godden	Introduction to wine show judging		
R. Gawel	Palate performance and statistical evaluation		
C.A. Simos, P.O. Williamson	Overview of the AWRI's RDEC capabilities	Wine Australia - Heads of Market group visit, AWRI, Adelaide, SA	1 Nov 16
V.T. O'Brien	Panel discussion on creating product premiums subject to country of origin perceptions of Australian wine	2016 Wine Australia exporter update, Adelaide, SA	2 Nov 16
P.W. Godden, C.A. Simos	Overview of the AWRI's RDEC capabilities	Wine Australia WSET group visit, AWRI, Adelaide, SA	7 Nov 16

CA. Simos	Introduction to the Advanced Wine Assessment Course	Advanced Wine Assessment Course #41	7 – 10 Nov 16
W.P. Pearson	Flavour, taints, faults and thresholds		
P.W. Godden	Introduction to wine show judging		
R. Gawel	Palate performance and statistical evaluation		
P.R. Petrie	Soil health – what is it and how can we manage it?	AWRI roadshow seminar, Mornington Peninsula, Vic	15 Nov 16
M. Essling	What are the positives and pitfalls of grazing sheep in your vineyard?		
	Organic vs conventional practices compared - what's stopping you from going organic?		
P.J. Costello	Can you influence your wine styles through MLF?		
I.L. Francis	Struck match and tropical fruit: the role of varietal thiols in Australian Chardonnay		
P.R. Petrie	How to improve fruit set in cool climates?	AWRI roadshow seminar, Gippsland, Vic	16 Nov 16
	Soil health – what is it and how can we manage it?		
I.L. Francis	Hidden flavour unlocked by saliva during tasting: a key to wine quality?		
	Struck match and tropical fruit: the role of varietal thiols in Australian Chardonnay		
P.J. Costello	Avoiding spoilage issues caused by wine bacteria: prevention is better than cure		
T.J. Abbott	Ferment simulator	AWRI webinar	17 Nov 16
P.R. Petrie	Earlier, shorter, hotter? Is vintage compression really happening and what can you do about it?	AWRI roadshow seminar, Yarra Valley, Vic	

	Soil health – what is it and how can we manage it?		
I.L. Francis	Struck match and tropical fruit: the role of varietal thiols in Australian Chardonnay		
	Complexity, texture and flavour … or green, hard and herbal? Incorporation of stems and leaves in cool climate Shiraz fermentation		
P.J. Costello	Can you influence your wine styles through MLF?		
G.D. Cowey	Your regional position - helpdesk, climate and wine composition trends	AWRI roadshow seminar, Clare Valley, SA	22 Nov 16
	Sugar and estimating potential alcohol		
	Filtration and impact on colour and wine quality		
M. Essling	Entwine Australia sustainability data - your regional position		
	Smart tools to manage vineyard variation		
P.R. Petrie	Canopy management		
A.D. Coulter	Saturation temperature cold stability test		
	Sulfides and copper treatment		
	Flotation to clarify juice		
M.G. Holdstock	Optimising MLF and preventing spoilage		
T.E. Siebert	Black pepper flavour in Shiraz: does the clone have any influence?	DPI NSW Shiraz clonal workshops, Majura, ACT and Hunter Valley, NSW	22 & 24 Nov 16
S. Nordestgaard	AWRI and the Australian wine sector	DLR Neustadt, Germany	25 Nov 16

	Wine movements and lees		
G.D. Cowey	Your regional position - helpdesk, climate and wine composition trends	AWRI roadshow workshop, McLaren Vale, SA	29 Nov 16
	Brett monitoring - aroma, chemical, micro and molecular tests		
	Brett - new treatment options		
M.L. Longbottom	Entwine Australia sustainability data - your regional position		
M. Essling	Benchmarking spray programs - how chemicals are used in your region versus other regions		
	Smart tools to manage vineyard variation		
V.T. O'Brien	Building product premiums through creating emotive appeal		
M.G. Holdstock	Update on manganese, water additions, nutrition labelling		
	pH and TA - getting it right		
P.R. Petrie	Soil health – what is it and how can we manage it?	AWRI roadshow seminar, Mount Barker, WA	
	Organic vs conventional practices compared - what's stopping you from going organic?		
K.A. Bindon	How can I predict wine tannin and colour in the vineyard?		
	How to maximise the phenolic potential of grapes through innovative winemaking		
P.R. Petrie	Soil health – what is it and how can we manage it?	AWRI roadshow seminar, Pemberton, WA	30 Nov 16

	What can I do to protect my vineyard from climate change?		
	What are the strategies to better manage the risk of *Botrytis* bunch rot?		
	Soil health – what is it and how can we manage it?	AWRI roadshow seminar, Margaret River, WA	1 Dec 16
K.A. Bindon	How can I predict wine tannin and colour in the vineyard?		
P.R. Petrie	Earlier, shorter, hotter? Is vintage compression really happening and what can you do about it?		
C.A. Simos	Causes and managements of slow and stuck fermentations		
I.L. Francis	Wine glycoconjugates as flavour precursors during consumption	Australasian Association for Chemosensory Science 17th Annual Meeting, Sydney, NSW	2 Dec 16
P.R. Petrie	Organic vs conventional practices compared - what's stopping you from going organic?	AWRI roadshow seminar, Swan Valley, WA	
C.A. Simos	What are the causes and management strategies of dealing with *Brettanomyces*?		
	Causes and management of slow and stuck fermentations		
K.A. Bindon	How to maximise the phenolic potential of grapes through innovative winemaking		
E.N. Wilkes	Can we get closure? Shining new light on the role of closures in wine faults	Wine Vision 2016, California, USA	5 Dec 16
C.A. Simos	Evaluation of winemaking treatments in Australian Pinot Noir	Pinot Noir winemaking trials tasting, Geelong, Vic	18 Jan 17
G.D. Cowey	Overview of the AWRI's RDE&C capabilities	Jim Gore, Principal of the WSET school in London and William Lowe (coordinated by Wine Australia) Adelaide, SA	20 Jan 17
P.O. Williamson	Linking the AWRI's wine flavour research with regionality		
C.A. Simos	Evaluation of winemaking treatments in Australian Pinot Noir	Pinot Noir winemaking trials tasting, Yarra Valley, Vic	

		Pinot Noir winemaking trials tasting, Mount Barker, WA	23 Jan 17
M.P. Krstic	Vineyard mechanisation: move to the 'no touch vineyard' - Australian perspective	Unified Symposium, California, USA	24 Jan 17
S.J. Nordestgaard	Grape sorting	Cabernet hang-time forum, Curtin University, Margaret River, WA	
C.A. Simos	Evaluation of winemaking treatments in Australian Pinot Noir	Pinot Noir winemaking trials tasting, Adelaide, SA	30 Jan 17
		Pinot Noir winemaking trials tasting, Canberra, ACT	2 Feb 17
		Pinot Noir winemaking trials tasting, Orange, NSW	3 Feb 17
P.R. Dry	Sourcing planting material, import requirements	Alternative varieties Research to Practice (RtP) workshop, Limestone Coast, SA	
P.R. Dry	Red varietal description		
M.L. Longbottom	Soil health – what is it and how can we manage it?	AWRI roadshow seminar, Launceston, Tas	7 Feb 17
	How to improve fruit set in cool climates		
K.A. Bindon	How to maximise the phenolic potential of grapes through innovative winemaking		
C.A. Simos	Evaluation of winemaking treatments in Australian Pinot Noir		
P.W. Godden	Overview of the AWRI's RDE&C capabilities	Wine Australia visit, Adelaide, SA	8 Feb 17
	How regionality and environmental factors drive wine style		
C.J. Day	AWRI business functions		
M.L. Longbottom	Soil health – what is it and how can we manage it?	AWRI roadshow seminar, Hobart, Tas	

	How to improve fruit set in cool climates		
K.A. Bindon	How to maximise the phenolic potential of grapes through innovative winemaking		
C.A. Simos	Evaluation of winemaking treatments in Australian Pinot Noir		
C.S. Stockley	Changing the upper limits of moderate alcohol consumption. The need for a worldwide politic on alcohol consumption.	WineHealth 2017, Logrono, Spain	17 Feb 17
	Round table innovation and future		
C.A. Simos	Evaluation of winemaking treatments in Australian Pinot Noir	Pinot Noir winemaking trials tasting, Adelaide, SA	21 Feb 17
A.D. Coulter	Industry development and support overview	A group of visitors from the University of Yamanashi, Japan, Adelaide, SA	28 Feb 17
M. Essling	Viticulture in Australia		
S.A. Schmidt	Overviews of AWRI bioscience research		
P.J. Costello	Malolactic fermentation		
P.A. Smith	Opportunities for creating value in wine production		
T.E. Siebert	What influences 'black pepper' flavour in Shiraz?		
C.S. Stockley	Dimethylpolysiloxane as a processing aid in winemaking	OIV expert group meetings, Paris, France	30 Mar 17
W.P. Pearson	Benchmarking Australian Shiraz terroir	Wine Australia 50 world sommeliers event, Australian Shiraz regional tasting	3 Apr 17
C.S. Stockley	Sub-Commission on Methods of Analysis question to Commission I on phthalates - Draft response - April 17	OIV Expert Group meeting, Paris, France	4 Apr 17

M.J. Herderich	Terroir effects on grape and wine aroma compounds	DLR Neustadt, Germany	7 Apr 17
E.N Wilkes	Closures – latest understanding of their impact	AWRI webinar	20 Apr 17
M.J. Herderich	Old friends in new bottles: aroma precursors in grapes and wine and the complexities of wine flavour	Denis Dubourdieu Symposium, Hochschule Geisenheim, Germany	26 Apr 17
C.A. Simos	*Brettanomyces* - causes and management strategies	AWRI Roadshow Seminar - Hunter Valley, NSW	3 May 17
P.R. Petrie	What can I do to protect my vineyard from climate change?		
E.N. Wilkes	Sulfur dioxide - what are we really measuring?		
	Copper - the good, the bad and the ugly		
M.G. Holdstock	Industry outcomes of emissions reduction and carbon sequestration for resilience and sustainability in the grape and wine sector	The Ag Climate Forum, Bunyah Mountains, QLD	10 May 17
	Wine faults and flavours		
C.A. Simos	Struck match and tropical fruit: the role of varietal thiols in Australian Chardonnay	AWRI Roadshow Winemaking Seminar - Griffith, NSW	
M.Z. Bekker	The beneficial style and performance effects of oxygen addition during fermentation		
	Copper - the good, the bad, the ugly		
S. Nordestgaard	Rapid extraction techniques for red wine production		

K.A. Bindon	How to maximise the phenolic potential of grapes through innovative winemaking		
P.R. Petrie	What are the strategies to better manage the risk of Botrytis bunch rot?	AWRI Roadshow Viticulture Seminar - Griffith, NSW	
	Scale and mealybug - what can I do to control these sap-sucking insects?		
K.A. Bindon	How can I predict wine tannin and colour in the vineyard?		
M.P. Krstic	Soil health – what is it and how can we manage it?	AWRI Roadshow Viticulture Seminar - Irymple, Vic	11 May 17
P.R. Petrie	Canopy management		
K.A. Bindon	How to maximise the phenolic potential of grapes through innovative winemaking	AWRI Roadshow Winemaking Seminar - Irymple, Vic	
S. Nordestgaard	Rapid extraction techniques for red wine production		
M.Z. Bekker	Copper - the good, the bad and the ugly		
E.N. Wilkes	Winemaking education, common wine faults and their impacts	7th APEC Wine Regulatory Forum, Hanoi, Vietnam	
	2016-17 ring test results and comparison to international proficiency test results		12 May 17
	Core elements of a strong laboratory quality management system		
	Presentation of the Methods of Analysis Compendium		
	Naturally occurring components in wine, including metals and regulatory limits that are imposed on such components		

M.P. Krstic	Best practice approaches to assessing harvest time - grape maturity and pest and disease levels	AWRI Roadshow Viticulture Seminar - Riverland, SA	
	Soil health – what is it and how can we manage it?		
P.R. Petrie	Organic vs conventional practices compared - what's stopping you from going organic?		

S. Nordestgaard	Rapid extraction techniques for red wine production	AWRI Roadshow Winemaking Seminar - Riverland, SA	12 May 17
K.A. Bindon	How to maximise the phenolic potential of grapes through innovative winemaking		
M. Z. Bekker	Sulfur dioxide - what are we really measuring?		
T.J. Abbott	How to predict the performance of your ferments: using the new AWRI Ferment Simulator		
V.T. O'Brien	How to build demand and premiums paid for Riverland wine		
E.N. Wilkes	APEC Wine Regulatory Forum Report- laboratory Capacity	6th APEC Food Safety Cooperation Forum, Hanoi, Vietnam	13 May 17
M. Essling	Organic vs conventional practices compared - what's stopping you from going organic?	AWRI Roadshow Seminar - Langhorne Creek and Adelaide Hills, SA	17 May 17
P.R. Petrie	Soil health – what is it and how can we manage it?		
C.A. Simos	Evaluation of winemaking treatments in Australian Pinot Noir	Pinot Noir Winemaking Trials Tasting #3 - Adelaide, SA	18 May 17
I.L. Francis	Struck match and tropical fruit: the role of varietal thiols in Australian Chardonnay	NSW DPI Chardonnay seminar, Orange, NSW	31 May 17

C.S. Stockley	National Health & Medical Research Council's alcohol drinking guidelines	Wine Victoria Board, Melbourne, Vic	6 Jun 17
	What is alcohol and its effects on the human body	Treasury Wine Estates, Melbourne, Vic	
T.E. Siebert	Determination of the potent flavour compound rotundone in grapes and wine using MDGC-MS and membrane-assisted solvent extraction	Gerstel Automated Sample Preparation & Innovation Throughout 50 years, celebration seminar day, Singapore	7 Jun 17
P.W. Godden	Tour of the Australian Wine Research Institute and overview of the RDE&C capabilities	Wine Australia Visit - George Gresty, Adelaide, SA	13 Jun 17
D.L. Capone	Thiols and tropical flavour in Chardonnay, rosé wine flavour and green flavour in red wine.	E. & J. Gallo Winery National Meeting, Lodi, California, USA	
P.R. Petrie	Smart tools to manage vineyard variation	AWRI Roadshow Workshop - Barossa Valley, SA	14 Jun 17
	Yield regulation - cost-benefit and the impact on quality		
G.D. Cowey	Your regional position - helpdesk, climate and wine composition trends		
	Brett monitoring - aroma, chemical, micro and molecular tests		
	Filtration and impact on colour and wine quality		
	Avoiding stuck fermentations		
M.G. Holdstock	Colour and tannin		
	Smoke taint analysis and interpretation		

	Carboxymethylcellulose (CMC) tartrate inhibitor		
A.D. Coulter	Avoiding stuck fermentations	AWRI Roadshow Workshop - Mornington Peninsula, Vic	20 Jun 17
	Carboxymethylcellulose (CMC) tartrate inhibitor		
	Sulfides and copper treatment		
K. DeGaris	Yield regulation - cost-benefit and the impact on quality		
M.G. Holdstock	Colour and tannin		
	New *Brettanomyces* treatment		
	Optimising MLF and preventing spoilage		
M.P. Krstic	Benchmarking spray programs - how chemicals are used in your region versus other regions	AWRI Roadshow Workshop - Mornington Peninsula, Vic	
E.N. Wilkes	Review of laboratory workshops and 17 ring test program	International Wine Technology Summit, Seattle, USA	
	Best practice in total SO_2 measurement		21 Jun 17
	Sugar free extract and the impact of sugar testing method		
M.G. Holdstock	Colour and tannin	AWRI Roadshow Workshop - Gippsland, Vic	
	pH and TA - getting it right		
	Oxygen use in winemaking		

K. DeGaris	Canopy management		
A.D. Coulter	Carboxymethylcellulose (CMC) tartrate inhibitor		
	Filtration and impact on colour and wine quality		
	Sulfides and copper treatment		
M.P. Krstic	Benchmarking spray programs - how chemicals are used in your region versus other regions		
A.D. Coulter	Filtration and impact on colour and wine quality	AWRI Roadshow Workshop - Yarra Valley, Vic	22 Jun 17
	Flotation to clarify juice		
	Sulfides and copper treatment		
M.G. Holdstock	pH and TA - getting it right		
	Optimising MLF and preventing spoilage		
	Oxygen use in winemaking		
M.P. Krstic	Stretching water further		
	Yield regulation - cost-benefit and the impact on quality		
S.A. Schmidt	The fight for dominance in grape juice and the genetics underpinning yeast strain performance	33rd International Specialised Symposium on Yeast, Cork, Ireland.	27 Jun 17

M.Z. Bekker	The effects of oxygen on “reductive aroma” compounds during fermentation, remediation, and post-bottling	New understandings in wine oxidation chemistry symposium, ASEV Annual Meeting in Bellevue, Washington, USA	
A.D. Coulter	Carboxymethylcellulose (CMC) tartrate inhibitor	AWRI Roadshow Workshop - Mt Barker, WA	
	Sulfides and copper treatment		
	Flotation to clarify juice		
G.D. Cowey	Your regional position - helpdesk, climate and wine composition trends		
	New *Brettanomyces* treatment		
	Oxygen use in winemaking		
G.D. Cowey	Sugar and estimating potential alcohol	AWRI Roadshow Workshop - Mt Barker, WA	
M. Essling	Benchmarking spray programs - how chemicals are used in your region versus other regions		
K. DeGaris	Yield regulation - cost-benefit and the impact on quality		
C.A. Varela,	Extensive genomic diversity and its effect on sulfite tolerance in the industrial yeast *Brettanomyces bruxellensis*	33rd International Specialised Symposium on Yeast, Cork, Ireland.	28 Jun 17
M.J. Herderich	Metabolomics tools and approaches for characterising flavours, phenolics and quality markers in grapes and wine	International Metabolomics Conference, Brisbane, QLD	
V. Hysenaj	Targeted metabolomics to provide insights into the role of trace sulfur compounds in wine aroma and quality		

K. DeGaris	Canopy management	AWRI Roadshow Workshop - Pemberton, WA	
M. Essling	Yield regulation - cost-benefit and the impact on quality		
A.D. Coulter	Smoke Taint		
	Carboxymethylcellulose (CMC) tartrate inhibitor		
	Removal/reduction of TCA/TBA, alcohol, VA, 4-EP and smoke taint compounds		
G.D. Cowey	Your regional position - helpdesk, climate and wine composition trends		
	Sugar and estimating potential alcohol		
	Update on manganese, water additions, nutrition labelling		
G.D. Cowey	Filtration and impact on colour and wine quality	AWRI Roadshow Workshop - Pemberton, WA	
M.P. Krstic	Seeing through the haze – insights into smoke taint management in wines	ASEV Annual meeting in Bellevue, Washington State, USA	
M.Z. Bekker	Myths and facts about the role of precursors in the formation of “reductive aromas” in wines post-bottling	Enology - Wine Aroma Session, ASEV Annual Meeting in Bellevue, Washington, USA	
M.P. Krstic	Outreach seminar – smoke taint	Smoke taint outreach workshop, ASEV Annual Meeting in Bellevue, Washington State, USA	29 Jun 17
P.R. Petrie	A thermal camera-based smart phone application to measure vine water status	ASEV Annual Meeting in Bellevue, Washington State, USA	
K. DeGaris	Yield regulation - cost-benefit and the impact on quality	AWRI Roadshow Workshop - Margaret River, WA	

M. Essling	Smart tools to manage vineyard variation		
A.D. Coulter	Analysis correlation with quality?		
	Colour and tannin		
	Removal/reduction of TCA/TBA, alcohol, VA, 4-EP and smoke taint compounds		
G.D. Cowey	Your regional position - helpdesk, climate and wine composition trends		
	Brett monitoring - aroma, chemical, micro and molecular tests		
	Oxygen use in winemaking		
A.D. Coulter	Bulk wine transport	AWRI Roadshow Workshop - Swan Valley, WA	30 Jun 17
	Oxygen use in winemaking		
G.D. Cowey	Your regional position - helpdesk, climate and wine composition trends		
	Brett monitoring - aroma, chemical, micro and molecular tests		
	Sugar and estimating potential alcohol		
G.D. Cowey	Update on manganese, water additions, nutrition labelling		
	Filtration and impact on colour and wine quality		
M. Essling	Benchmarking spray programs - how chemicals are used in your region versus other regions		

K. DeGaris	Yield regulation - cost-benefit and the impact on quality		

Appendix 2: AWRI publications recorded between 1 July 2013 and 30 June 2017

AWRI publication number	Reference
1542	Holt, H., Cozzolino, D., McCarthy, J., Abrahamse, C., Holt, S., Solomon, M., Smith, P., Chambers, P.J., Curtin, C. Influence of yeast strain on Shiraz wine quality indicators. *Int. J. Food Microbiol.*165 (2): 302-311; 2013.
1543	Stockley, C. Australia to host international wine and health conference in 2013. *Aust. N.Z. Grapegrower Winemaker* (594): p. 6; 2013.
1544	Cowey, G., Essling, M. Adapting to difficult vintages. *Aust. N.Z. Grapegrower Winemaker* (594): p. 26; 2013.
1545	Essling, M. Ask the AWRI: Vary strategies for successful weed management. *Aust. N.Z. Grapegrower Winemaker* (594): p. 36; 2013.
1546	Hoxey, L., Stockley, C., Wilkes, E., Johnson, D. What's in a label? *Wine Viti J.* 28 (4): 38-41; 2013.
1547	Pretorius, S. Beyond change. *WBM* (June): 30-31; 2010.
1548	Cordente, A.G., Curtin, C.D., Varela, C., Pretorius, I. S., Flavour-active wine yeasts. *Appl. Microbiol. Biotechnol.* 96 (3): 601-618; 2012.
1549	Ristic, R., Pinchbeck, K.A., Fudge, A.L., Hayasaka, Y., Wilkinson, K.L. Effect of leaf removal and grapevine smoke exposure on colour, chemical composition and sensory properties of Chardonnay wines. *Aust. J. Grape Wine Res.* 19 (2): 230-237; 2013.
1550	Curtin, C.D., Langhans, G., Henschke, P.A., Grbin, P.R. Impact of Australian Dekkera bruxellensis strains grown under oxygen-limited conditions on model wine composition and aroma. *Food Microbiol.*36 (2): 241-247; 2013.
1551	Dry, P., Longbottom, M., Essling, M. Is there a need for improved vineyard assessment for fruit grading? *Wines Vines* 94 (7): 52-55; 2013.
1552	Gawel, R., Van Sluyter, S.C., Smith, P.A., Waters, E.J. The effect of pH and alcohol on perception of phenolic character in white wine. *Am. J. Enol. Vitic.* 64 (4): 425-429: 2013.
1553	Dry, P. Marzemino. *Wine Vitic. J.* 28 (4): p. 63; 2013.
1554	Holdstock, M. Ask the AWRI: Snapshot of oak-related queries. *Aust. N.Z. Grapegrower Winemaker* (595): p. 68; 2013.
1555	Leske, P.A., Francis, I.L., Hunt, D. Sensory Evaluation. (eds) Bulleid, N., Jiranek, V. Australian Winemaking. Trivinum Press, Adelaideonline: www.trivinumpress.com.au/SEN: 35 p.; 2013.
1556	Bartowksy, E.J., Fleet, G.H. Malolactic Fermentation. (eds) Bulleid, N., Jiranek, V. Australian Winemaking. Trivinum Press, Adelaideonline: www.trivinumpress.com.au/MLF; xx p.; 2013.
1557	Fudge, A.L., Wilkinson, K.L., Ristic. R., Cozzolino, D. Synchronous two-dimensional MIR correlation spectroscopy (2D-COS) as a novel method for screening smoke tainted wine. *Food Chem.*139 (1-4): 115-119; 2013.

1558	Cozzolino, D., Cynkar, W.U., Dambergs, R.G., Shah, N., Smith, P. In situ measurement of soil chemical composition by near-Infrared spectroscopy: a tool toward sustainable vineyard management. *Comm. Soil Sci. Plant Anal.* 44 (10): 1610-1619; 2013.
1559	Muhlack, R. It's time to power up. *WBM* (August): 39-41; 2013.
1560	Dry, P. Ask the AWRI: Vines: Is an oldie necessarily a goodie? *Aust. N.Z. Grapegrower Winemaker* (596): p. 57; 2013.
1561	Varela, C., Chambers, P., Johnson, D. Trials turn up new strategies for softening the kick in wine. *Aust. N.Z. Grapegrower Winemaker* (596): 70-73; 2013.
1562	Carew, A.L., Smith, P., Close, D.C., Curtin, C., Dambergs, R.G. Yeast effects on Pinot noir wine phenolics, color, and tannin composition. *J. Agric. Food Chem.* 61 (41): 9892-9898; 2013.
1563	Van Sluyter, S.C., Warnock, N.I., Schmidt, S., Anderson, P., van Kan, J.A.L., Bacic, A., Waters, E.J. Aspartic acid protease from *Botrytis cinerea* removes haze-formation proteins during white winemaking. *J. Agric. Food Chem.* 61 (40): 9705-9711; 2013.
1564	Pojer, E., Mattivi, F., Johnson, D., Stockley, C.S. The case for anthocyanin consumption to promote human health: A review. *Comp. Rev. Food Sci. Food Safety* 12 (5): 483-508; 2013.
1565	Stockley, C. Key messages from WineHealth 2013 – International Wine and Health Conference. *Wine Vitic. J.* 28 (5): 16-18; 2013.
1566	Borneman, A., Herderich, M., Johnson, D. The DNA of innovation. *Wine Vitic. J.* 28 (5): 52-56; 2013.
1567	Dry, P. Tinto cao. *Wine Vitic. J.* 28 (5): p. 77; 2013.
1568	Kidman, C.M., Dry, P.R., McCarthy, M.G., Collins, C. Reproductive performance of Cabernet Sauvignon and Merlot (*Vitis Vinifera* L.) is affected when grafted to rootstocks. *Aust. J. Grape Wine Res.* 19 (3): 409-421; 2013.
1569	Mangoni, A.A., Stockley, C.S., Woodman, R.J. Effects of red wine on established markers of arterial structure and function in human studies: current knowledge and future research directions. *Expert Rev. Clin. Pharmacol.* 6 (6): 613-625; 2013.
1570	Coulter, A. Ask the AWRI: Understanding the ABCs of CMCs in stabilisation. *Aust. N.Z. Grapegrower Winemaker* (598): p. 72; 2013.
1571	McRae, J.M., Kassara, S., Kennedy, J.A., Water, E.J., Smith, P.A. Effect of wine pH and bottle closure on tannins. *J. Agric. Food Chem.* 61 (47): 11618−11627; 2013.
1572	Kidman, C.M., Mantilla, S.O., Dry, P.R., McCarthy, M.G., Collins, C. The effect of water stress on reproductive performance of Shiraz (*Vitis Vinifera* L.) grafted to rootstocks. *Am. J. Enol. Vitic.* 65 (1): 96-108; 2014.
1573	Jolly, N. P., Varela, C., Pretorius, I.S. Not your ordinary yeast: non- *Saccharomyces* yeasts in wine production uncovered. *FEMS Yeast Res.* 14 (2): 215-237; 2014.
1574	Longbottom, M. Greenhouse gas abatement in viticulture. *Aust. N.Z. Grapegrower Winemaker* (598): 35-38; 2013.
1575	Muhlack, R., Scrimgeour, N., Wilkes, E., Godden, P., Johnson, D. Optimising fermentation through simulation. *Wine Vitic. J.* 28 (6): 38, 40-43; 2013.
1576	Dry, P. Schönburger. *Wine Vitic. J.* 28 (6): p. 58; 2013.
1577	Viviers, M.Z., Smith, M.E., Wilkes, E., Smith, P. Effects of five metals on the evolution of Hydrogen sulfide, Methanethiol, and Dimethyl sulfide during anaerobic storage of Chardonnay and Shiraz wines. *J. Agric. Food Chem.* 61 (50): 12385–12396; 2013.

1578	Johnson, D. Fascinating story to tell. *Aust. N.Z. Grapegrower Winemaker* (599): p. 30; 2013.
1579	Dry, P. If the grape fits then you can grow it. *Aust. N.Z. Grapegrower Winemaker* (599): p. 57; 2013.
1580	Muhlack, R., Forsyth, K., Scrimgeour, N., Godden, P. There's gotta be a buck in those organic by-products. *Aust. N.Z. Grapegrower Winemaker* (599): 80-82; 2013.
1581	Johnson, D. 2013 AWRI Report: Planning for the future. *Aust. N.Z. Grapegrower Winemaker* (598): 81-84; 2013.
1582	Tran, T., Wilkes, E. How does CMC behave in NZ wines? *NZ Winegrower* (83): 67-69; 2013.
1583	Contreras, A., Hidalgo, C., Henschke, P.A., Chambers, P.J., Curtin, C., Varela, C. Evaluation of non-*Saccharomyces* yeast for the reduction of alcohol content in wine. *Appl. Environ. Microbiol.* 80 (5): 1670-1678; 2014.
1584	Viviers, M.Z., Smith, M.E., Wilkes, E., Smith, P.A. Effects of metals on the evolution of volatile sulfur compounds in wine during bottle storage. *Aust. N.Z. Grapegrower Winemaker* (600): 49-51; 2014.
1585	Wilkes, E. You've got to be a hot shot to hit the moving target of cold stability. *Aust. N.Z. Grapegrower Winemaker* (600): 43-46; 2014.
1586	Cowey, G. Ask the AWRI: Top tips for a successful yeast culture. *Aust. N.Z. Grapegrower Winemaker* (600): p. 42; 2014.
1587	Bindon, K.A., Madani, S.H., Pendleton, P., Smith, P.A., Kennedy, J.A. Factors affecting skin tannin extractability in ripening grapes. *J. Agric. Food Chem.* 62 (5): 1130-1141; 2014.
1588	Dry, P. Malvasia istriana. *Wine Vitic. J.* 29 (1): p. 53; 2014.
1589	Viviers, M., Smith, M., Wilkes, E., Smith, P., Johnson, D. The role of trace metals in wine 'reduction'. *Wine Vitic. J.* 29(1): 38-40; 2014.
1590	Francis, L. Des études multidisciplinaires à l'Australian Wine Research Institute. *Revue des Oenologues et des Techniques Vitivinicoles et Oenologiques* (149 Supp.): p. 9; 2013.
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1592	Bartowsky, E., Costello, P., Krieger-Weber, S., Silvano, A., Dumont, A., Francis, L., Travis, B. Au-delà de la fermentation malolactique. Évaluation et caracterisation de l'impact sensorial des bactéries oenologiques sur le caractère fruité des vins rouges. *Revue des Oenologues et des Techniques Vitivinicoles et Oenologiques* (149 Supp.): 61-65; 2013.
1593	Essling, M. Ask the AWRI: Canopy damage from herbicides. *Aust. N.Z. Grapegrower Winemaker* (601): p. 32; 2014.
1594	Dry, P. Terroir and the topic of wine typicity. *Aust. N.Z. Grapegrower Winemaker* (601): p. 40; 2014.
1595	McRae, J., Smith, P. Seal the deal on pH, red wine colour and tannin during bottle ageing. *Aust. N.Z. Grapegrower Winemaker* (601): 51-54; 2014.
1596	Gawel, R., Schulkin, A., Smith, P.A., Waters, E.J. Taste and textural characters of mixtures of caftaric acid and Grape Reaction Product in model wine. *Aust. J. Grape Wine Res.* 20 (1): 25-30; 2014.

1597	Mayr, C.M., Parker, M., Baldock, G.A., Black, C.A., Pardon, K.H., Williamson, P.O., Herderich, M.J., Francis, I.L. Determination of the importance of in-mouth release of volatile phenol glycoconjugates to the flavor of smoke-tainted wines. *J. Agric. Food Chem.* 62 (11): 2327-2336; 2014.
1598	Scrimgeour, N., Wilkes, E. Closure trials show volatile sulfur compound formation can still cause a stink. *Aust. N.Z. Grapegrower Winemaker* (602): 62, 64-67; 2014.
1599	Coulter, A. Ask the AWRI: Can 'Brett' affect white wines? *Aust. N.Z. Grapegrower Winemaker* (602): p.54; 2014.
1600	Dry, P. Souzao. *Wine Vitic. J.* 29 (2): p. 60; 2014.
1601	Krstic, M., Barlow, S. Vintage 2030 and beyond: Producing quality wines in warmer times. *Wine Vitic. J.* 29 (2): 52-57; 2014.
1602	Smith, P., Bindon, K., McRae, J., Kassara, S., Johnson, D. Tannin: impacts and opportunities along the value chain. *Wine Vitic. J.* 29 (2): 38-41; 2014.
1603	Cordente, A.G., Cordero-Bueso, G., Pretorius, I.S., Curtin, C.D. Novel wine yeast with mutations in YAP1 that produce less acetic acid during fermentation. *Fems Yeast Research* 13 (1): 62-73; 2014.
1604	Uberti, F., Danzi, R., Stockley, C., Peñas, E. Ballabio, C., Di Lorenzo, C., Tarantino, C., Restani, P. Immunochemical investigation of allergenic residues in experimental and commercially-available wines fined with egg white proteins. *Food Chem.* 159: 343-352; 2014.
1605	Capone, D.L., Herderich, M.J., Pardon, K.H., Hayasaka, Y., Cordente, A.G., Grant-Preece, P.A., Sefton, M.A., Elsey, G.M., Jeffery, D.W. Formation of varietal thiol aroma compounds in wine: Synthetic and analytical studies of grape and wine thiol conjugates. In Hofmann, T., Meyerhof, W., Schieberle, P. (eds) *Advances and Challenges in Flavor Chemistry and Biology: Proceedings of 9th Wartburg Symposium on Flavour Chemistry and Biology*, *Eisnach, Germany* Deutsche Forschungsanstalt für Lebensmittelchemie: Freising, Germany 242-248; 2011.
1606	Mateo, E., Torija, M.J., Mas, A., Bartowsky, E.J. Acetic acid bacteria isolated from grapes of South Australian vineyards. *Int. J. Food Microbiol.* 178: 98-106; 2014.
1607	Song, J., Smart, R.E., Dambergs, R.G., Sparrow, A.M., Wells, R.B., Wang, H., Qian, M.C. Pinot Noir wine composition from different vine vigour zones classified by remote imaging technology. *Food Chem.* 153: 52-59; 2014.
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1609	Borneman, A.R., Zeppel, R., Chambers, P.J., Curtin, C.D. Insights into the *Dekkera bruxellensis* genomic landscape: Comparative genomics reveals variations in ploidy and nutrient utilisation potential amongst wine isolates. *PLoS Genetics* 10 (2): e1004161; 2014.
1610	Scarlett, N.J., Bramley, R.G.V., Siebert, T.E. Within-vineyard variation in the 'pepper' compound rotundone is spatially structured and related to variation in the land underlying the vineyard. *Aust. J. Grape Wine Res.*20 (2): 214-222; 2014.
1611	Mayr, C., Geue, J., Holt, H., Pearson, W., Francis, I.L. Characterization of the key aroma compounds in Shiraz wine by quantitation, aroma reconstitution, and omission studies. *J. Agric. Food Chem.* 62 (20): 4528-4536.; 2014.

1612	Dry, P. Ask the AWRI: Bunches with ripe and unripe berries. *Aust. N.Z. Grapegrower Winemaker* (603): p.49; 2014.
1613	Essling, M. The role of potassium in grapevine function. *Aust. N.Z. Grapegrower Winemaker* (603): p.74; 2014.
1614	Contreras, A., Henschke, P., Chambers, P., Curtin, C., Varela, C. New yeast approach is aiming to produce a lower alcohol wine. *Aust. N.Z. Grapegrower Winemaker* (603): 82-83; 2014.
1615	Kutyna, D.R., Cordente, A.G., Varela, C. Genetic engineering of industrial *Saccharomyces cerevisiae* strains using a selection/counter-selection approach. Mapelli, V. (ed.) Yeast metabolic engineering: methods and protocols. Totowa, N.J.: Humana Press. Chapter 9: 157-168; 2014.
1616	Bindon, K.A., Kassara, S., Cynkar, W.U., Robinson, E.M.C., Scrimgeour, N., Smith, P.A. Comparison of extraction protocols to determine differences in wine-extractable tannin and anthocyanin in *Vitis Vinifera* L. Cv. Shiraz and Cabernet Sauvignon grapes. *J. Agric. Food Chem.* 62 (20): 4558-4576; 2014.
1617	Dry, P. Pruning systems: a short history of a long practice. *Aust. N.Z. Grapegrower Winemaker* (604): 54-55; 2014.
1618	Holdstock, M. Ask the AWRI: Here's the hot topics of vintage 2014. *Aust. N.Z. Grapegrower Winemaker* (604): p. 79; 2014.
1619	Viviers, M., Smith, M., Wilkes, E., Smith, P., Johnson, D. Role of trace metals in wine 'reduction'. *Wines Vines* 95 (5): 86-88; 2014.
1620	Carew, A.L., Lloyd, N.D.R., Close, D.C., Dambergs, R.G. Wine phenolic and aroma outcomes from the application of Controlled Phenolic Release to Pinot Noir must. Beames, K.S., Robinson, E.M.C., Godden P.W., Johnson, D.L. (eds.) Proceedings of the 15th Australian Wine Industry Technical Conference: Sydney, New South Wales 13-18 July 2013. Urrbrae, South Australia; The Australian Wine Industry Technical Conference Inc.: 80–84; 2014.
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1624	Viviers, M.A., Smith, M.E., Wilkes, E., Smith, P.A. Effect of metals on the evolution of volatile sulfur compounds during wine maturation. Beames, K.S., Robinson, E.M.C., Godden P.W., Johnson, D.L. (eds.) Proceedings of the 15th Australian Wine Industry

	Technical Conference: Sydney, New South Wales 13-18 July 2013. Urrbrae, South Australia; The Australian Wine Industry Technical Conference Inc.: 97–101; 2014.
1625	Muhlack, R.A., Forsyth, K.K., Scrimgeour, N., Godden, P.W. Creating value from by-products – and industry review and insights into practical case studies. Beames, K.S., Robinson, E.M.C., Godden P.W., Johnson, D.L. (eds.) Proceedings of the 15th Australian Wine Industry Technical Conference: Sydney, New South Wales 13-18 July 2013. Urrbrae, South Australia; The Australian Wine Industry Technical Conference Inc.: 117–119; 2014.
1626	Hayman, P.T., Thomas, D.S., Longbottom, M.L., McCarthy, M.G. Predicting and preparing for heatwaves. Beames, K.S., Robinson, E.M.C., Godden P.W., Johnson, D.L. (eds.) Proceedings of the 15th Australian Wine Industry Technical Conference: Sydney, New South Wales 13-18 July 2013. Urrbrae, South Australia; The Australian Wine Industry Technical Conference Inc.: 133–137; 2014.
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1628	Curtin, C.D., Borneman, A.R., Zeppel, R., Cordente, A.G., Kievet, R., Chambers, P.J. Harnessing genomics to ensure a 'Brett'-free future for Australian wine. Beames, K.S., Robinson, E.M.C., Godden P.W., Johnson, D.L. (eds.) Proceedings of the 15th Australian Wine Industry Technical Conference: Sydney, New South Wales 13-18 July 2013. Urrbrae, South Australia; The Australian Wine Industry Technical Conference Inc.: 158–160; 2014.
1629	Dry, P. Gamay. *Wine Vitic. J.* 29 (3): p.59; 2014.
1630	Gawel, R., Godden, P., Williamson, P., Francis, L., Smith, P., Waters, L., Herderich, M., Johnson, D. Influence of phenolics on white wine quality and style. *Wine Vitic. J*. 29 (3): 34-36; 2014.
1631	Wilkes, E., Warner, L. Accurate mid-infrared analysis in wine production – fact or fable? *Wine Vitic. J*. 29 (3): 64-67; 2014.
1632	Williamson, P.O., Loose, S.M., Lockshin, L., Francis, L. Thinking outside the bottle: information about Australia can increase the choice of Australian wines by Chinese consumers. *Wine Vitic. J*. 29 (3): 71-74; 2014.
1633	Longbottom, M. Grape and wine sustainability program to launch on 1 July. *Aust. N.Z. Grapegrower Winemaker* (605): p.85; 2014.
1634	Dry, P. Ask the AWRI: Can you achieve yield control in the vineyard without using bunch thinning? *Aust. N.Z. Grapegrower Winemaker* (605): p.40; 2014.
1635	Bindon, K., Holt, H., Williamson, P.O., Varela, C., Herderich, M., Francis, I.L. Relationships between harvest time and wine composition in *Vitis vinifera* L. cv. Cabernet Sauvignon 2. Wine sensory properties and consumer preference. *Food Chem.* 154: 90-101; 2014.
1636	McRae, J.M., Kirby, N., Mertens, H.D.T., Kassara, S., Smith, P.A. Measuring the molecular dimensions of wine tannins: comparison of small-angle x-ray scattering, gel-permeation chromatography and mean degree of polymerization. *J. Agric. Food Chem.* 62 (29): 7216-7224; 2014.
1637	Cowey, G. Ask the AWRI: the ABCs of filtration and what works for you. *Aust. N.Z. Grapegrower Winemaker* (606): p.60; 2014.

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1639	Coulter, A., Cowey, G., Dry, P., Essling, M., Holdstock, M., Stockley, C., Simos, C., Johnson, D. Vintage 2014 – trends from the AWRI helpdesk. *Wine Vitic. J*. 29 (4): 34-36; 2014.
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1642	Longbottom, M. Adaptation, mitigation and innovation in a changing climate. *Aust. N.Z. Grapegrower Winemaker* (607): p. 16; 2014.
1643	Longbottom, M. Ask the AWRI: Viticulture and greenhouse gas emissions. *Aust. N.Z. Grapegrower Winemaker* (607): p. 54; 2014.
1644	Ruiz-Garcia, Y., Smith, P.A., Bindon, K.A. Selective extraction of polysaccharide affects the adsorption of proanthocyanidin by grape cell walls. *Carbohyd. Polym.* 114: 102-114.; 2014.
1645	Stockley, C.S. Chair of WineHealth 2013 scientific advisory committee. *Nutr. Aging* 2(2-3): 77-79; 2014.
1646	Scholey, A., Benson, S., Stough, C., Stockley, C. Effects of resveratrol and acohol on mood and cognitive function in older individuals. *Nutr. Aging* 2(2-3): 133-138; 2014.
1647	Jolly, N.P., Varela, C., Pretorius, I.S. Role of non-*Saccharomyces* yeasts in wine production. *Wines Vines* 95 (7): 52, 54, 56; 2014.
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1651	Marty, P., Dry, P. Sparkling wine production in the southeast of England. *Wine Vitic. J.* 29 (4): p. 60; 2014.
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Australian Government

Australian Grape and Wine Authority

The Australian Wine Research Institute

Defining the nutritional drivers of yeast performance and matching yeast to must

FINAL REPORT to
AUSTRALIAN GRAPE AND WINE AUTHORITY

Project Number: **AWR 1302**

Principal Investigator: **Simon Schmidt**

Research Organisation:
The Australian Wine Research Institute

Date: **22 September 2017**

Project title: Defining the nutritional drivers of yeast performance and matching yeast to must
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Table of Contents

1. Abstract:

The complex interactions between yeasts and their environment are brought sharply into focus when wine fermentations fail to complete. Retrospective analyses of such failures are difficult or impossible. The relationship between yeast strain genetics and its interaction with the grape juice environment is the subject of this work. Two hundred commercially available wine yeasts, or yeasts isolated from wine, were sequenced to evaluate wine yeast genomic diversity. A representative subset of those sequenced yeasts was used to evaluate the fitness impacts of grape juice variables commonly associated with poor fermentation performance and the genetic determinants of some traits identified.

2. Executive summary:

The complex interactions between yeasts and their environment are brought sharply into focus when wine fermentations fail to complete. Retrospective analyses of such failures are difficult or impossible because of the many combinations of factors that may lead to this undesirable outcome. These factors include choice of yeast strain, of which there are many, grape juice composition and winemaking interventions.

Comparative genomics of yeast

The relationship between yeast strain genetics and a yeast's interaction with the grape juice environment is the subject of this work. Two hundred commercially available wine yeasts, or yeasts isolated from wine, were sequenced to evaluate wine yeast genomic diversity. Whole genome sequence data is now available for 200 wine yeast via the National Center for Biotechnology Information short read archive under the accession number SRP066835. This data archive contains genomic sequence information on wine, ale and cider yeasts of commercial and environmental origin, including species of *Saccharomyces cerevisiae*, *Saccharomyces uvarum* and hybrids of *S. cerevisiae* with non-*cerevisiae Saccharomyces* yeast including *S. eubayanus*, *S. paradoxus* and *S. kudriazevii*. The comparative analysis of this data has been published in the journal *Genes|Genomes|Genetics* (Borneman et al. 2016).

Yeast response to environment - comparative analysis of molecularly barcoded yeast

A fundamental property of any commercial yeast is the degree to which it can compete for resources in their working environment. Yeast competitiveness was assessed directly in this project using a representative collection of barcoded wine yeasts. Strain selection for this representative subset of 94 yeasts was informed by the results of comparative genomics. Each strain was tagged with a unique DNA barcode introduced into a phenotypically neutral location. The tags were required to identify strains in mixed cultures, thus enabling the parallel determination of fitness profiles in a range of industrially relevant media formulations. This was achieved through competition experiments in which differential fitness was assessed in response to environmental challenges.

Environmental variables commonly associated with poor fermentation performance, such as sugar concentration and temperature, were not discriminating factors of yeast strain fitness. Copper concentration and nitrogen availability were, however, powerful contributors to fitness variations between wine yeast strains. Fitness-based predictions of performance were validated using single inoculum fermentations. These experiments showed a high concordance between pooled culture fitness and individual strain performance profiles.

Following initial explorations of yeast and environment interactions that were assessed in defined medium, later stages of the project progressed to fitness profiling experiments using freshly prepared unfiltered juice. This enabled a comparison with defined medium results (fitness validation) using an environment complete with the rich microbial ecology that comprises a standard grape juice. Fitness of strains was evaluated in grape juice with different additives (modified

nitrogen, SO_2, increased sugar concentration etc.) and against a panel of juices. This work showed that juice preferences exist for some strains and lends weight to the idea that strain/must matching may offer tangible benefits when selecting strains for fermentation.

Uncovering the genetic basis of performance

To uncover the genetic basis of performance in specific environmental conditions, classical genetics (mating and screening of progeny) and modern genomics (whole genome sequencing of progeny) have been combined to identify regions of the genome that positively or negatively contribute to the performance of different strains. The primary focus has been on sulfite and copper tolerance, both of which are highly discriminatory for wine yeasts. The genetic basis of resistance to both of these stresses is well researched and much is already known about the evolutionary strategies that yeast use to tolerate these powerful selective agents. However, this work is uncovering an unlikely interdependence between these strategies, the exact nature of which is still being explored.

The project benefitted substantially from the involvement of several industry partners including Yalumba, Treasury Wine Estates through their Wolf Blass winery, and Pernod Ricard through their Orlando winery at Rowland Flat. The project also benefitted through access to sequencing resources at the Ramaciotti Centre for Genomics (UNSW Sydney, Australia).

3. Background:

There are many yeast strains available to the wine industry where there is limited detail available about how they perform in different contexts, both in terms of fermentation kinetics and flavour outcomes. The aim of this project was to build a knowledge base on how different yeast strains perform in different environments (i.e. grape juices of widely varying composition, representing a range of wine styles) and how winemakers can optimise fermentation efficiency, and best minimise the risk of suboptimal (stuck and sluggish) fermentations while delivering wine styles to winemaker specifications through collaborations with AWR 1301 ('Fit-for-purpose yeast').

To address the perennial problem of suboptimal (i.e. slow, sluggish and stuck) fermentations, a survey of Chardonnay grape juice composition was undertaken by the AWRI in 2009 to better understand juice parameters that contribute to fermentation performance. This survey built on previous of work at the AWRI and elsewhere that identified nitrogen and oxygen availability as being critical factors in fermentation efficiency and reliability. It led to the identification of potassium concentration and pH (and particularly their interaction) as key parameters influencing performance outcomes, and different yeast strains were found to differ in their sensitivity to these parameters (Schmidt 2011). However, serious limitations associated with characterising yeast strains one at a time became apparent during this work.

There have been numerous studies on the influences of specific environmental conditions on individual yeast strains. Some studies have simultaneously investigated two or three environmental conditions, for example lipids and oxygen, nitrogen and biotin, nitrogen and pantothenate, and pH and potassium. Due to the limited scope of work performed to date there is a lack of information about the broader context of nutrient interactions. There is also limited information on the wider range of nutrient interactions beyond pair-wise interactions. This is due to the number of experiments required to experimentally implement a multi-factorial design using conventional experimental approaches to fermentation where one of the design variables is yeast strain.

Technological developments in DNA sequencing and statistical approaches to analysis of sequence data have now enabled the simultaneous evaluation of large numbers of strains in many combinations of environments to assess how individual yeast strains behave in differing environmental conditions. The establishment of baseline performance maps allowed the evaluation of different winemaking practices on strain-specific performance profiles. This approach highlighted strain-specific limitations and essential requirements so that suboptimal fermentation conditions can be objectively minimised. Prior to this project, no such detailed information was available for a broad spectrum of strains.

This project contributed to objectives in Wine Australia's Programs 3.1, 3.2 and 3.3 by augmenting winemaking practices that will deliver improved fermentation efficiency with reduced risk of faults though informed use of available wine yeast (germplasm).

4. Project aims and performance targets:

a. Build a genomic database of wine yeast to facilitate strain selection and delineating available diversity
b. Define yeast strain-specific performance parameters for at least 100 commercially available wine strains, enabling the matching of strain to must
c. Evaluate the yeast strain specific efficacy of fermentation additives in modulating the performance of yeast in wine fermentations
d. Contribute to studies in AWRI Project 3.1.1, undertaking the identification of compounds that lead to undesirable (attenuated) sensory characters in wine arising from suboptimal fermentations
e. Identify robust wine yeast strains to be exploited in AWRI Project 3.2.2
f. Provide winemakers with knowledge that will enable them to deliver efficient and reliable fermentations and produce quality wine to market specification.

5. Method:

a. A genetically diverse set of commercially available wine yeast was selected through sequencing of genomic DNA extracted from 200 yeast strains. Sequencing was performed using Illumina HiSeq platforms running off Nextera prepared libraries. Relationships between strains were assessed through whole genome based phylogenetic cluster analysis (Borneman et al. 2016). All sequencing work was performed at the Ramaciotti Centre for Genomics (UNSW Sydney, Australia).

b. A barcoded commercial yeast strain collection was created and a pooled inoculum with equal representation of all barcoded strains generated. Choice of strains for this collection was informed by whole genome based phylogenetic analysis.

c. Pooled inocula were used to perform fermentations in a defined medium and in panels of different grape juices. Iterative rounds of experiments were performed with different combinations of key grape juice variables using factorial experimental designs. Grape juice variables chosen included those commonly measured in wineries and controllable by the winemaker.

d. Representative samples of all barcodes from individual, pooled inoculum fermentation replicates, treatments and time points were obtained by polymerase chain reaction (PCR). The PCR products were molecularly tagged using Illumina compatible indexing primers to allow concurrent analysis. Up to 96 samples could be analysed in a single sequencing run.

e. Relative frequencies of strains at the completion of the exponential growth phase in different environmental conditions were determined through enumeration of barcodes within molecularly tagged amplicon pools. Statistical evaluation of enumerated barcodes was performed via fitting of a Quasi-likelihood Generalised Linear Model (GLMs) using an empirical Bayes approach and then computing an Anova style F-test for each individual comparison that was made (Robinson et al. 2010). Data were filtered to remove strains with little or no informative data. After filtering, 87 strains generally remained in the data set. Prior to model fitting the data was normalised using the relative log expression (RLE) method described by Anders and Huber (2010). In addition,

the common and tagwise dispersions were also calculated. The different variances and the sources of variation were used in the modelling step to estimate significance.

f. Findings from pooled experiments were validated by evaluating individual strains in defined environmental conditions using 100 mL ferments fitted with non-return valves to limit exposure to atmospheric gas. The fermentation medium for validation experimental work was generally a minimal defined medium that models the composition of a Chardonnay juice as described in Schmidt et al. (2011). Fermentation vessels were stirred at 150 rpm and all fermentations were carried out at 17°C except where stated otherwise. Inoculation densities of 5 x10^6 were used for pooled inoculum work and 1 x 10^6 for single strain validation experiments.

6. Results and discussion:

a. Comparative genomics of wine yeast

Genome sequences are now available for 200 wine yeast, comprising *Saccharomyces cerevisiae*, *Saccharomyces uvarum* and hybrids of *S. cerevisiae* with non-*cerevisiae Saccharomyces* yeast including *S. eubayanus*, *S. paradoxus* and *S. kudriazevii*. The strains sequenced included commercial and non-commercial yeast. Commercial yeast strains were purchased as Active Dry Yeast from industry suppliers. Non-commercial strains were accessed from the AWRI wine microorganism culture collection and represent isolates collected from numerous wineries, both in Australia and overseas since the 1950s; many of these have not been previously characterised. Older wine yeast isolates provide some historical context to the relationships between the various wine yeasts in this study and offer a potential untapped source of diversity for strain phenotypic assessment.

Initial inter-genomic comparisons of the 200 sequenced wine yeast strains were made via alignment of each genome with that of the laboratory strain *S. cerevisiae* S288c. The shared differences of the various wine yeast, compared to S288c, form the primary comparative dataset used to construct a dendrogram (tree) of relationships between strains (Figure 1). Additional genetic elements not found within the genome of s288c as well as alignments to other *Saccharomyces* yeasts were made in parallel.

The overall structure of the dendrogram is flat; it has a shallow root structure. This indicates a high level of genetic similarity between strains, consistent with a recent point of divergence. The relationships between the different clades (higher order structures of the tree branches) are poorly defined, with all being equally related to each other. A more robust order begins to appear higher up in the branches of the tree within the small and tightly defined clades. Here the longer branches (and stronger statistical support for tree structure) speak to the influence of domestication through strain selection and breeding on the generation of increased levels of genetic divergence. Surprisingly, there are fewer directly identical strains than were expected prior to commencing this work.

Of the 200 strains sequenced, 54 (27%) form a clade (group) with a large degree of similarity to strain EC1118. EC1118 is a robust 'workhorse' yeast isolated many years ago in France from secondary fermentation of champagne. Subgroups within the larger EC1118 clade show particularly high levels of genetic divergence and evidence of breeding and selection. For example, seven strains are interspecific hybrids arising from crosses of non-*cerevisiae* strains with an EC1118-like parent (Borneman et al. 2016).

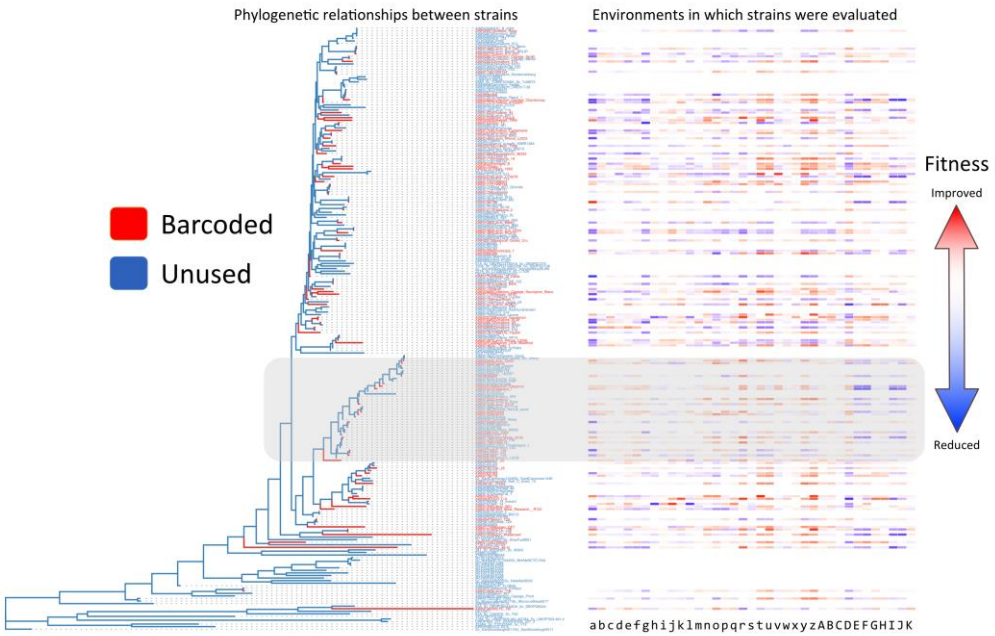

Figure 1. Heat map of yeast fitness profiles mapped against wine yeast phylogeny. In the heat map, red indicates improved fitness and blue indicates reduced fitness. On the dendrogram, strains marked in red indicate those that were barcoded. The subgroup shaded in grey indicates the predominant group of highly related yeasts closely associated with sparkling wine manufacture.

b. Construction of wine yeast barcode collection

The strategy for comparing relative fitness levels of many wine yeast strains in different environments required development of a methodology that enabled strains to be discriminated when harvested from mixed populations. This required the construction of a molecularly tagged (barcoded) wine yeast collection. The strains to be barcoded were chosen based on prior knowledge of their traits and genetic relatedness as determined from genome sequence data.

Barcodes were introduced into 94 strains. This was achieved in two stages. In the first a selectable marker (KanMX), flanked by LoxP sites and a pair barcodes (Caporaso et al. 2012) was integrated via homologous recombination into the HO locus of each selected strain. A second transformation with a non-integrative plasmid containing Cre recombinase stimulated the removal of the selectable marker between the LoxP sites (Carter and Delneri 2010), creating a 150 base pair barcode comprised of a LoxP scar flanked by a molecular tag at each end. Each strain was then cured of the non-integrative plasmid by growing overnight in a non-selective medium and screening for the loss of the antibiotic resistance encoded by the plasmid. The integrity of the barcode within each of these strains was verified by individually sequencing across the region in which the barcode was introduced. In the process of building the barcoded collection some strains were identified that had unanticipated inherent resistance to the selectable marker (G418). The barcoded strains are shown in red on the dendogram in Figure 1.

Inoculation ready pools were created by growing each barcoded strain individually in 50 mL liquid cultures to a defined optical density, mixing the cultures, changing the medium to

fresh YPD + Glycerol, rapidly freezing them in liquid nitrogen and storing at -80°C. This provided a ready stock for use directly in fitness profiling experiments. The creation of the barcoded wine yeast collection provides a resource for the investigation of yeast strain performance and genetic analysis of wine yeast for years to come. The direct effect of disruption of the HO locus in these wine yeasts is that spores dissected from strains in the collection can no longer mate with themselves. Therefore, strains from this collection can be used directly in genetic dissection experiments such a bulk segregant and quantitative trait analysis, an approach that will be discussed in greater detail later in this report.

c. *Defining optimised methods for the conduct of pooled inoculum fitness profiling experiments.*

At the outset of this project a demonstration was required that detecting a change in the abundance of a strain was possible within a mixed population. This was achieved by creating two pools of strains, one in which all strains were in equal proportions and one in which a subset of strains was mixed into a pool at a reduced concentration compared to other strains. These two pools were sequenced and analysed as described above. As shown in Figure 2, a subset of strains is clearly detected at a reduced frequency in the population, demonstrating the capability of the pooled sequencing approach for the detection of altered representation of individuals in mixed populations.

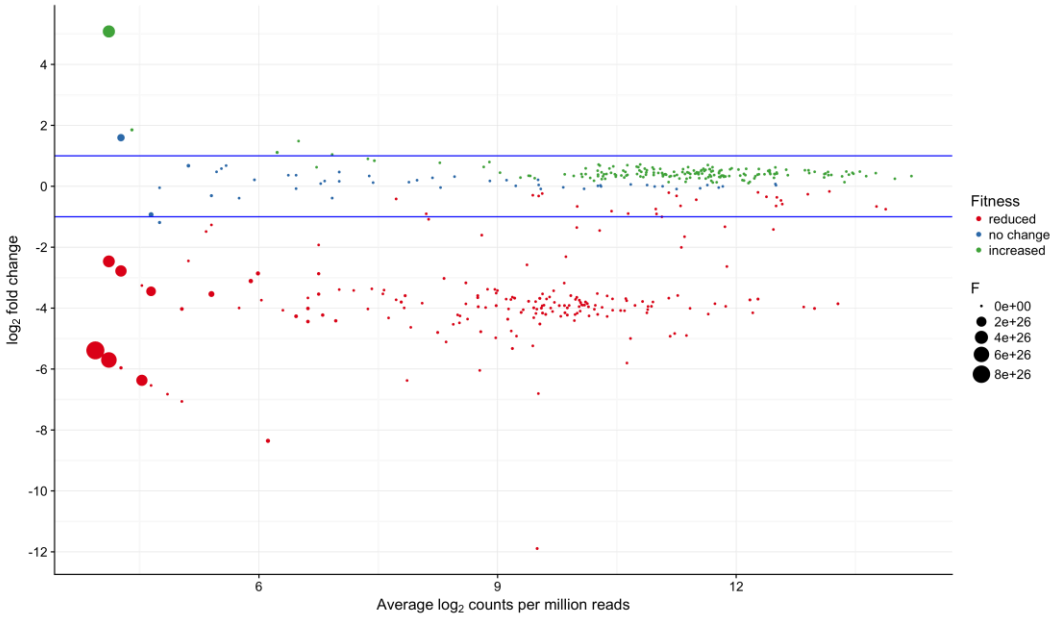

Figure 2. Detection of strain frequency changes in mixed populations by barcode sequencing. Each point represents an individual strain.

Two possible approaches to the conduct of fitness experiments is possible. One involves initiating a continuous culture where a treatment regime is applied to a culture in steady state and changes in population dynamics are compared before and after the change. The other approach is to use a serial batch regime where strains are used to initiate a standard fermentation. A sample from the first culture is then used to inoculate a second culture (serial batch). In this work, the relative merits of continuous culture and serial batch culture for the profiling of yeast strain fitness were determined. The comparison was made using a pooled inoculum comprised of 96 wine yeast gene deletion library

(WYGDL) mutants. Strains in the WYGDL collection have predictable phenotypes and therefore were the most suitable for such an evaluation. Ninety-six mutants were used to reflect the scope and scale of anticipated wine yeast barcode experiments.

Both continuous culture and serial batch culture successfully delivered expected fitness profiles for deletion mutants that had known phenotypes under the conditions tested. However, the resolution provided by serial batch cultures was clearly superior. Strains with a strong phenotype (e.g. a strain with deletion of the CUP2 gene indicating copper sensitivity) could be detected after only one passage through a treatment condition. Increased discrimination of strains with marginal fitness differences could be obtained through additional rounds of serial inoculation.

These results clearly identified serial batch inoculation as the method of choice for assessing the fitness of barcoded wine yeast strains. In addition, while chemostat culture is historically the method of choice for fitness profiling, the technical difficulties associated with its conduct make it difficult to implement in the context of experiments where many different conditions are examined simultaneously.

Finally, standard methods for the extraction of DNA from laboratory yeast had to be adapted to permit the uniform extraction from wine yeast with different morphologies. This was achieved through a modification to the Qiagen yeast genomic DNA extraction kit method involving a threefold increase in the concentration of lyticase used prior to cell lysis and a doubling of the lyticase incubation time.

d. Yeast response to environment - comparative analysis of molecularly barcoded yeast

A general approach adopted for comparing yeast fitness was devised in which eight environmental variables were tested in triplicate at four time points, each sample corresponding to the end of exponential growth phase in a serial inoculation regime. The overall approach was modelled on that taken by (Robinson et al. 2014). This configuration results in 96 samples, which is the limit imposed by the number of index sequences available in an Illumina dual indexed sequencing run on a HiSeq 2500 sequencer. Experiments were performed either in defined medium as described by (Schmidt et al. 2011) when testing the impacts of variations to specific compositional variables, or grape juice that had either been stored or freshly pressed. Grape juices were all derived from white grape varieties, either Chardonnay, Riesling, Pinot Gris or Sauvignon Blanc.

Some strains do not appear at all in the dataset or only occasionally. These strains are not even recoverable from the pooled inoculum. The missing strains are: 739, 793, 1430, 1501, 1787 and 2865. As a result, data for only 87 strains are reported. In addition, some strains deviate in their representation substantially from the inoculating pool once grown up in controlled defined medium. Some examples are 1427, 1716 and 1722, 1077 and 767. These strains are either detrimentally affected by the freeze-thaw process used to prepare the pools and inoculate the ferments or they may grow substantially less well in defined medium compared to YPD.

Table 1 summarises the compositional variables by experimental batch that were evaluated over the course of the project either using defined medium as a matrix for the exploration of effects on strain specific fitness or juice to explore gross matrix effects on the strain performance.

Table 1. Compositional variables assessed during this project

	Defined medium					Grape juice	
	Exp 1	**Exp 2**	**Exp 3**	**Exp 4**	**Exp 5**	**Exp 6**	**Exp 7**
1	High sugar	High potassium	DM_12°C	No vitamins	High copper	High YAN	YPD
2	High copper	High copper	High sugar_12°C	No biotin	Low biotin	High sugar	Defined medium
3	Low pH	High copper/ high potassium	DM_18°C	Biotin only	Low pH	High SO_2	Chardonnay RF
4	Low YAN	Low pH	High sugar_18°C	Biotin 0.1µg/L	High copper/ Low biotin	High sugar/ High YAN	Chardonnay BR
5	High potassium	Low pH/ High potassium	DM_28°C	No thiamine	Low pH/ Low biotin	High sugar/ High SO_2	Riesling EV
6	Low pH/ High potassium	Low pH/ High copper	High sugar_28°C	Thiamine 10 µg/L	Low pH/ High copper	High SO_2/ High YAN	Riesling WB
7	High sugar/Low YAN	Low pH/ High copper/ High potassium		No Pantothenate	Low pH/ High copper/ Low biotin	High sugar/ High SO_2/ High YAN	Sauvignon Blanc WB
8							Pinot Gris Y

The importance of matrix on the fitness response of strains is shown in Figure 3. In this experiment eight different medium types were compared: yeast extract peptone (YPD), defined medium and six different white grape juices. Most of the variation occurs with time as would be expected. With each re-inoculation there is more opportunity for strains to grow and therefore change the relative proportion of strains in the community. The changes in the make-up and relative representations of strains within the community are most obvious between time points 1 and 2. By time point 3 the structure of the community has stabilised and does not change between time points 3 and 4.

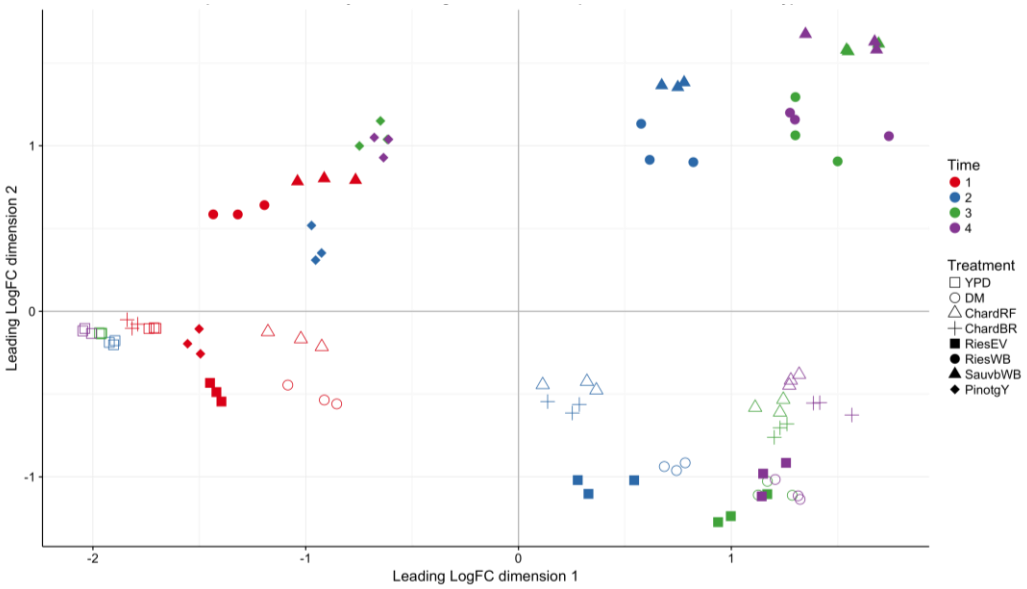

Figure 3. Multi-dimensional scaling plot showing general matrix effects on strain profiles over time. Different point shapes indicate different medium or juice matrices and the colour of the points indicates different time points.

There are some surprising features of this dataset. Firstly, the defined medium formulation is most similar in its effect on yeast strain representation to a Riesling from Eden Valley, despite having a substantially higher pH (3.5 vs 3.1). The other Riesling in the dataset has an impact similar to a Sauvignon Blanc. While the impact of the two Chardonnays is similar to each other despite substantial differences in the available nitrogen of the two juices (338 mg N/L and 170 mg N/L), the Pinot Gris does not have as great an impact over time as any of the other matrices, with the exception of YPD.

Figure 4 shows a more detailed picture of the impacts that the different juices have on specific strains. Compared to RF Chardonnay, there are there are only a few strains that show marginal juice preference for either the BR Chardonnay or EV Riesling. Specifically, strain 2078 does relatively better in the low YAN BR Chardonnay and strains 1502 and 2863 do marginally better in the low pH EV Riesling. Overall strains do not appear to perform differently in any of these juices. However, strain preferences are immediately evident when comparing RF Chardonnay with either WB Riesling or WB Sauvignon Blanc. In these cases, strains 1504, 228, 2280 and 934 all perform much worse in the Riesling and Sauvignon Blanc than they do in the RF Chardonnay. The compositional feature that these two juices have in common is their low pH; both are below 3.0.

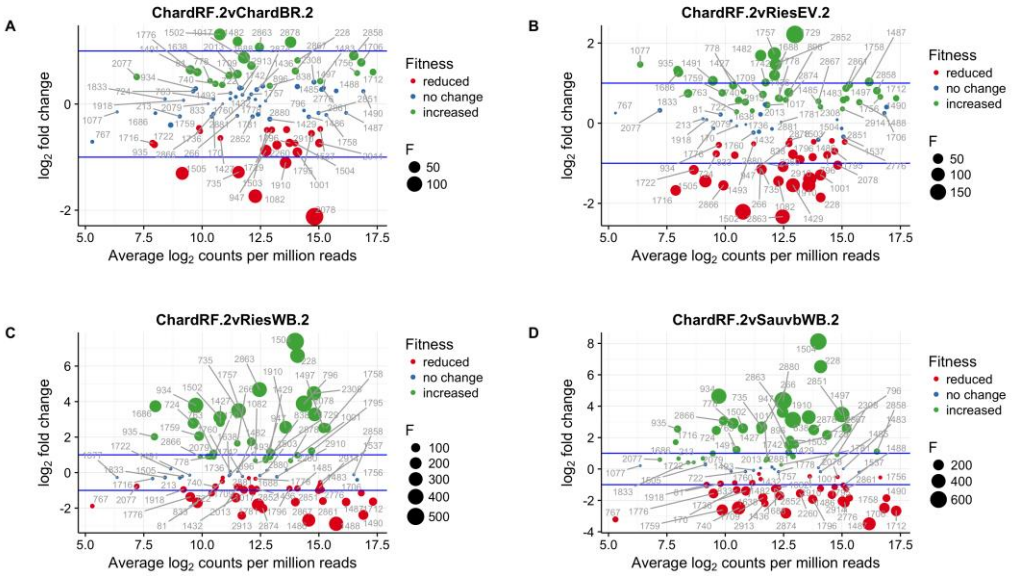

Figure 4 Strain specific performance profiles in RF Chardonnay compared to four other juices; A) RF Chardonnay – BR Chardonnay, B) RF Chardonnay – EV Riesling, C) RF Chardonnay – WB Riesling, D) RF Chardonnay – WB Sauvignon Blanc.

Of the many compositional variables assessed during this project only a handful were demonstrated to substantially alter the competitive fitness of yeasts in a strain specific manner. These variables were copper, sulfite, nitrogen and vitamin concentration. Low temperature, low YAN and high sugar had similar impacts across all strains and combinations of harsh conditions such as low pH, high copper and low biotin or high sugar, low YAN and high SO_2 put such a downward pressure on growth that no individual strain was able to thrive.

There are, however, some notable examples in particularly harsh conditions. In the complete absence of vitamins only one strain dominated. AWRI 1505, a hybrid of *S. cerevisiae* and *S. bayanus,* was able to grow and substantially dominate the culture where all other strains could not. This advantage was completely eroded by the addition of biotin alone, supporting previous work showing that biotin is one of the few vitamins critical for healthy fermentations. In subsequent experimental work it was shown that exceedingly small concentrations of this vitamin (0.1 µg/L) were sufficient to sustain normal growth in the presence of other vitamins, suggesting that in standard winemaking scenarios low concentrations of biotin would be unlikely to be growth limiting for wine yeast.

A second example of an individual yeast being particularly strong in a specific condition is the performance of another hybrid, this time between *S. cerevisiae* and *S. kudriavzevii* (AWRI 1503). This was one of a handful of strains whose competitive fitness was improved at 12°C compared to 28°C. Other strains were more competitive in the warmer temperature, specifically 796 and 1482. This type of temperature-based stratification in performance could be a basis for strain choice for red and white wine fermentations.

Two traits were studied in more detail: resistance to copper and sulfite. Both copper and sulfite can be present in high and unpredictable concentrations in juice because of intensive spaying in the vineyard or protective additions to the bins or crushers. Both copper and sulfite are anti-microbial agents and therefore their effects are inhibitory rather than stimulatory. They are notable because of their capability to discriminate amongst wine yeasts.

Panel A of Figure 5 shows the relative fitness of strains under elevated copper conditions. Although strongly inhibitory to growth for some strains, copper (at least at moderate concentrations) allows some strains to thrive, presumably because of a relief from nutrient constraints due to the suppressive effect on growth of competitor strains. Strains that not only survived but thrived under these conditions were 2913, 1432 and 796. On the other hand, there were a substantial number of strains on which elevated copper placed a particularly heavy burden. Strains 1487, 1537 and 2856 were representatives of that group. For these strains, copper limited their ability to form biomass; so, not only did they grow more slowly but the maximum number of cells they could form became limited. This can lead to ferments inoculated with them failing to complete in a timely manner when copper concentrations are substantially higher than normal.

Panel B in Figure 5 shows the relative fitness of strains under elevated sulfite conditions. In contrast the to the fitness profiles under high copper conditions, there were no major winners when concentrations of sulfite were high. There were many strains that survived, but did not necessarily thrive. However, the inhibitory effect was equally severe. While the impact on strains 228 and 1504 seemed to be particularly critical, there were many other strains for which inoculation into a high SO_2 must would result in extended lag times before obvious signs of fermentation became evident or possibly other strains would come to dominate the ferment before the inoculated strain was able to grow sufficiently. Another feature of the two conditions was that strains that performed well under high copper conditions tended not to not perform well under high sulfite conditions. Different sets of strains appeared to be better suited to either one condition or the other.

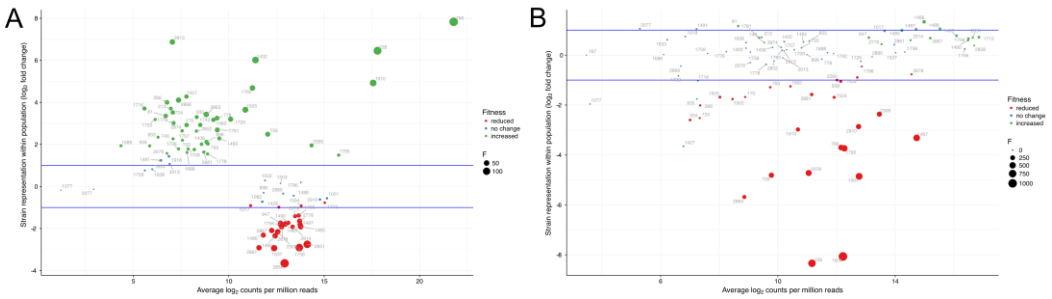

Figure 5. Strain specific performance profiles in media containing A) 10 mg/L copper, B) 18 mg/L free SO_2, compared to a medium with minimal or no copper or SO_2 respectively. Each point represents the fold change representation of a strain under the stated condition. Red indicates significant reduction in representation of a strain, green represents a significant improvement in the representation of a strain.

In summary, this work revealed many aspects of the yeast/environment interaction. A case can be made for the idea that at least one component of yeast strain choice for inoculated fermentations should be consideration of the conditions under which the ferment is to be carried out. A strain that performs well at low temperature may be at a competitive disadvantage at elevated temperature and strains that are competitive in high SO_2 conditions may be at a substantial disadvantage in high copper conditions. In this report a limited number of specific examples have been given but observations can be extended to a larger number of conditions including available nitrogen and pH. The relationship between copper and sulfite will be explored in more detail in the following section.

e. Genetic basis of tolerance to defined environmental challenges

The genetic aspects of copper and sulfite tolerance are rigorously studied fields with long histories. Yeast have evolved different approaches to dealing with these two inhibitory factors. Despite being inhibitory, both are required for the healthy growth of yeast. Copper is a cofactor required for protection against reactive oxygen species (Nevitt et al. 2012) and sulfite is a metabolic intermediate in the sulfate assimilation pathway required for the *de novo* synthesis of key amino acids (Yoshida et al. 2011). As a consequence, the cellular concentrations of both are tightly regulated in yeast.

The regulation of intracellular copper is complex. One major solution to the difficulty of managing free copper ions within the cell is to keep copper bound up. Yeast use a protein known as CUP1 as this sequestering agent (Fogel and Welch 1982). The number of copies of CUP1 in different yeasts has been related to a strain's ability to tolerate high concentrations of copper (Fogel et al. 1983). Copper concentrations in grape juice have been reported to vary from below detection thresholds to 7 mg/L (Schmidt et al. 2011). With wine strains varying greatly in their resistance to copper (Figure 5), a survey of CUP1 copy number was undertaken in the 87 strains for which copper tolerance information was available. These strains had between 2 and 60 copies of CUP1. However, the correlation between CUP1 copy number and copper tolerance was not strong. There were many examples of strains with a high CUP1 copy number but low fitness in a high copper medium. There are clearly other factors impinging on copper tolerance.

A classical genetics approach was undertaken to understand why a strain with high CUP1 copy number can be sensitive to copper. A copper resistant strain (796) was crossed with two copper sensitive strains (1537 and 1487) in two separate matings. The diploid progeny were sporulated and 100 spores of each assessed for their copper tolerance in 100 mL ferments. The spores were assigned to copper sensitive and tolerant pools and the genotypes of the pools assessed in a bulked segregant analysis (BSA), mapping single nucleotide polymorphism segregation in the bulks to identify alleles associated with copper tolerance or sensitivity (Magwene et al. 2011).

The cross between 796 and 1537 identified the CUP1 locus as the major contributor to differences in tolerance between those two strains. Quantitative PCR showed that 1537 has 18 fewer copies of CUP1 than 796. These findings are consistent with previous work that understands copper tolerance to be a function of CUP1 copy number. The cross between 796 and 1487 was between two strains that both have a large number of CUP1 copies. In this cross, BSA identified an allele tightly linked to SSU1. This gene encodes a sulfite efflux pump whose activity is associated with sulfite tolerance. The two parents used in this cross (796 and 1487) have low and high sulfite tolerance respectively which is contrary to their copper tolerance profiles. SSU1 has not previously been associated with copper sensitivity.

The relationship of SSU1 to copper sensitivity was established by deleting SSU1 in the haploid parents that were used to make the crosses used in the BSA analysis. It was shown that deletion of SSU1 in the 1487 haploid relieved it from copper sensitivity.

It is not yet clear why having improved sulfite tolerance may compromise a strain's copper tolerance. Answering this question is the basis of ongoing work. The finding suggests that yeast strains can be either copper tolerant or sulfite tolerant, but not both. This has significant implications for the isolation of broadly stress-tolerant yeasts that work in a range of commercial applications. In summary, CUP1 copy number and SSU1 activity status have been mapped for all 87 strains in the barcoded collection. This information can now be used to predict copper or sulfite tolerance in newly isolated strains, with potential for use in the wine industry or as markers in ongoing breeding efforts to generate strains with more robust performance characteristics.

7. Outcome/conclusion:

a. Performance against planned outputs

A summary of project performance against specific objectives is given below. Overall the project achieved the objectives set out in the original application.

i. A genomic database of wine yeasts was created. Information about the relationships between wine yeasts and the underlying sequence data relating to each strain are publicly available and can be accessed by other wine researchers.

ii. Yeast strain-specific performance parameters for 87 commercially available wine strains was generated, which is short of the 100 originally planned. While attempts were made to barcode 100 strains, only 94 were ultimately possible due to incompatibilities of some strains with the barcoding technique employed. As explained in the results, only 87 of these were routinely detected in sufficient quantity from a mixed pool to permit robust statistical evaluation of their fitness.

iii. Yeast strain-specific efficacy of fermentation additives in modulating the performance of yeast in wine fermentations was evaluated in both defined medium and Chardonnay juice. Yeasts that benefit from complex nitrogen addition were identified, as were strains whose performance was unchanged in response to more generous nutrient conditions.

iv. Identification of compounds contributing to attenuated characters was not pursued beyond working with project AWR 1301 to identify aromatic profiles of stuck ferments from a screen of wine yeast strains in red wine. In that work, clustering of strains based on aromatic profile did not identify a unique aromatic signature in ferments that failed to finish. As a result, this was not pursued further.

v. Six yeasts have been identified for use in future strain development work. These have undergone a rigorous screening process to evaluate their ability to complete fermentation of Chardonnay juice in a timely manner and to understand their baseline aromatic profiles.

vi. Knowledge derived from this project has been and continues to be delivered to stakeholders through trade journals and Wine Australia-funded extension mechanisms. Planned integration of data with the online ferment simulator will provide access to information in a usable form for all levy payers.

b. Could changing the methodology/technology have improved the outcome?

Some aspects did not progress to the extent that was originally envisaged, at least in part due to technical limitations resulting from evolution in sequencing technology. Specifically, the project was planned around use of a HiSeq 2000. Dual indexing, which was a requirement built into the design of the wine yeast barcode library, only became available on MiSeq and HiSeq 2500 sequencing platforms. The yield of sequence data from MiSeq platforms was insufficient to permit a rigorous statistical treatment of the experiments and sequencing on a HiSeq 2500 was more expensive than originally budgeted. Therefore, fewer experiments exploring yeast-environment interaction could be performed within the project budget than were originally envisaged. With sequencing being a continually evolving technology, it was important to adapt to the conditions and therefore all strain profiling work was performed on a HiSeq 2500. Had the design of the wine yeast barcode collection been approached differently, using a single barcode instead of two to define each yeast strain, there may have been more flexibility in the sequencing platforms that could be used in experimental work. This could have been

achieved through increasing the budget for the construction of the library and this may have saved subsequent costs in the analysis of experiments.

c. Practical implications

The work outlined in this report has three main practical implications

i. By understanding the relationships between a broad range of commercial yeasts and environmental isolates it is immediately possible to choose representative strain sets for research work. This capability will reduce redundancy in experimental designs and facilitate coordination across projects. With an increased focus on fewer strains, a more comprehensive picture can emerge about which strains to use and when.

ii. Through revealing strain-specific limitations in fermentation it will be possible to inform winemakers about potential problems before they arise and diagnose fermentation problems after the fact. Through understanding the nutrient requirements of individual yeast it is possible to advise which yeasts will benefit from nutrient additions and when an addition might have limited impact. Resources have also been developed that will continue to expedite research on fermentation performance issues and methodologies have been established that enable the efficient use of those resources.

iii. By uncovering the genetic basis of various phenotypic traits it becomes possible to categorise the performance attributes of strains without having to engage in expensive and time-consuming phenotyping trials for each new strain that is encountered. This will be of use in bioprospecting projects that aim to isolate many thousands of novel strains from natural and winery environments. These molecular markers can also be put to use in strain development programs where antibiotic-selectable markers cannot be used if those strains are to be deployed in the food and beverages industry.

d. Benefits to the industry

The AWRI's extensive experience in consulting and advising winemakers on rescue of suboptimal fermentations during vintage has made it abundantly clear that prediction of suboptimal fermentations is extremely poor beyond knowledge of sugar and nitrogen impacts. A detailed understanding of the performance interaction of yeast strain and environment has highlighted known, and identified previously unknown, predictive factors thereby improving the tools available to winemakers to better manage fermentation through objective matching of strain to must.

8. Recommendations:

Fitness profiling using a pooled inoculum was an effective and efficient tool to explore the impacts of environmental conditions on strain performance. Supported with appropriate statistical tools and assuming access to appropriate sequencing resources this is an approach that should find multiple applications in future research. Its main limitation is that it effectively assesses a strain's ability to grow, and fermentation performance issues are not always due to an impedance to growth, but may result from higher order nutritional deficiencies that have an impact on general resilience.

Future work will look to increase the complexity of the environment under investigation. Much of the work reported here was done in defined medium. While this is an important stepping stone, the limitations that stem from its basis in our limited understanding of the full complexity of the grape juice medium mean that this methodology must ultimately move to natural juice environments. This comes with its own cost to reproducibility; however, it promises to reveal much about both the phenotypic variability of yeast and also the as yet unseen components of juice that give rise to variable fermentation outcomes. One small window into this is evident in the work presented here

showing yeast strain fitness profiles between a Sauvignon Blanc and a Riesling from the same winery to be more similar than between two Rieslings. It may turn out to be the case that yeasts and their preferences can tell us as much about juice and its composition as experiments manipulating their environment can tell us about the yeasts themselves.

There is still substantial scope to investigate the genetic basis of physiological yeast traits. Gaining a better grasp of this aspect of yeast biology is a long-term thread that underpins much of the work reported here. The tools that are now available to address these questions have never been more powerful and accessible. The projects that have just been completed in the last four years have laid the foundations for the exploitation of this emergent and evolving technology. Staying on top of these developments and what they offer demands a continuous effort in their application because the lead times involved in re-establishing that knowledge is long.

Finally, it is anticipated the yeast/bacterial interactions is an area that requires closer attention. With the increase in popularity of simultaneous alcoholic/malolactic fermentations, warmer climates that bring new disease pressures and changes in the way winemakers approach the use of SO_2 both in the vineyard and the winery there is more opportunity for yeast and bacteria to interact in ways that the current generation of industry practitioners may not have experienced.

9. Appendix 1: Communication:

a. Communication of outcomes

Outcomes and knowledge generated during the progress of this project have been communicated to peers through peer-reviewed publications and conference presentations, and annual meetings with researchers at the University of Adelaide. Representatives of Wine Australia attend these meetings and therefore have had the opportunity to hear updates on project progress outside of the six- monthly reports that were provided for the duration of the project.

Information has been extended to industry stakeholders through articles in trade journals, presentations in the AWRI roadshow program and webinars.

In addition, results generated during this project will also be built into the AWRI Ferment Simulator. This application, which is available to levy payers through the AWRI-hosted WineCloud, enables users to specify compositional variables of their grape juice matrix. When yeast strains are selected that are not suitable in the conditions described the user can be given a warning that will alert them to the risk associated with the choice of that strain.

b. Journal articles written during project

Borneman, A. R., Forgan, A. H., Kolouchova, R., Fraser, J. A., Schmidt, S. A. 2016a. Whole genome comparison reveals high levels of inbreeding and strain redundancy across the spectrum of commercial wine strains of *Saccharomyces cerevisiae*. *G3: Genes|Genomes|Genetics 6*(4): 957–971.

Borneman, A. R., Schmidt, S. A., Pretorius, I. S. 2013. At the cutting-edge of grape and wine biotechnology. *Trends Genetics29*(4): 263–271.

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Schmidt, S. A., Henschke, P. A. 2015. Production, reactivation and nutrient requirements of active dried yeast in winemaking: theory and practice. *Aust. J. Grape Wine Res. 21*(S1): 651–662.

Schmidt, S. A., Borneman, A. R., Kolouchova, R., McCarthy, J. M., Bellon, J., Herderich, M., Johnson, D. 2017. Spoilt for choice: Picking the right yeast in a vibrant market. *Wine Vitic. J.,* in press.

c. Presentations

Webinar - Simon Schmidt (2014) What you need to know about rehydration nutrients and nutrient additives in the management of alcoholic fermentation. Available from: https://www.youtube.com/watch?v=EQBfvw9CjNs&feature=youtu.be

Roadshow presentation - Managing stuck fermentations – Simon Schmidt (2015)

Schmidt, S. The fight for dominance in grape juice and the genetics underpinning yeast strain performance. 33rd International Specialised Symposium on Yeast, Cork, Ireland. 26-29 June 2017

Schmidt, S. Yeast competitive fitness in wine-like fermentation environments. 6th Yeast Products and Discovery, Adelaide, Australia. 2-4 December 2015.

10. Appendix 2: Intellectual Property:

This project has generated knowledge, know-how and data on the the nutritional drivers of yeast performance and matching yeast to must, which will be published and communicated as part of the project outcomes.

A barcoded strain collection of 87 strains has been created and CUP1 copy number and SSU1 activity status have been mapped for these strains. This information can be used to predict copper or sulfite tolerance in newly isolated strains with potential for use in the wine industry or as markers in ongoing breeding efforts to generate strains with more robust performance characteristics.

Six yeast strains have been identified for use in future strain development work, based on a rigorous screening process to evaluate their ability to complete fermentation of Chardonnay juice in a timely manner and their baseline aromatic profiles (AWRI 947, AWRI 1719, AWRI 1736, AWRI 1742, AWRI 1787 and AWRI 1939).

11. Appendix 3: References

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Yoshida, S., Imoto, J., Minato, T., Oouchi, R., Kamada, Y., Tomita, M., Soga, T., Yoshimoto, H. 2011. A novel mechanism regulates H_2S and SO_2 production in *Saccharomyces cerevisiae*. *Yeast* 28(2): 109–121.

12. Appendix 4: Staff

Simon Schmidt, Anthony Borneman, Radka Kolouchova, Jane McCarthy, Angus Forgan, Paul Chambers

13. Appendix 5:

Nothing additional to report

14. Appendix 6: Budget reconciliation

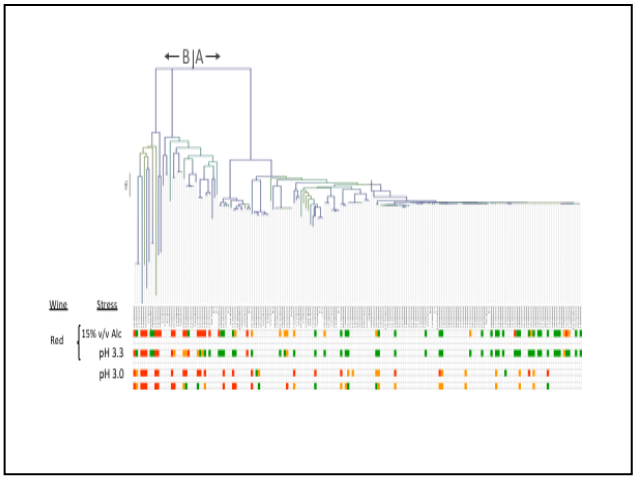
The project's budget reconciliation statement will be submitted separately.

Australian Government

Australian Grape and Wine Authority

The Australian Wine Research Institute

Efficient and reliable malolactic fermentation to achieve specification wine style



FINAL REPORT to

AUSTRALIAN GRAPE AND WINE AUTHORITY

Project Number: **AWR1303**

Principal Investigators: **Eveline Bartowsky until July 2016; Simon Schmidt from August 2016**

Research Organisation:

The Australian Wine Research Institute

Date: **22 September 2017**

Project title: Efficient and reliable malolactic fermentation to achieve specification wine style

Project No.: AWR 1303

Author: Dr Simon Schmidt

Date: 22 September 2017

Table of Contents

1. Abstract:

Malolactic fermentation (MLF) is a fundamental aspect of winemaking that can be difficult and lengthy. This project characterised the diversity of bacterial fitness that remained undiscovered in the rich resource of the AWRI wine microorganism culture collection (AWMCC). Strains were identified that lend themselves well to challenging environmental conditions. Nutrient additions and other treatment regimens were explored that might facilitate robust and reliable malolactic fermentations. Finally, the characterisation of genetic factors responsible for bacterial fitness and wine quality attributes through gene expression analysis was attempted. Candidate genes that contribute to diacetyl production were identified.

2. Executive summary:

The conversion of malic acid to lactic acid by lactic acid bacteria (known as malolactic fermentation or MLF) is a fundamental aspect of winemaking. MLF is important because it helps provide microbial stability to the finished product through the removal of a potential carbon source for spoilage organisms (malic acid), it reduces acidity and can impart various sensory characteristics, some subtle, others less so.

This project's primary aim was to improve the reliability of MLF. This goal was approached from multiple angles. The first was to better understand and characterise the bacterial resources available to winemakers. By defining the limits of what was possible using available resources, areas for development were identified. Specifically, the conduct of MLF in low pH or higher ethanol wines was identified as a high priority for achieving tangible improvements in winemaking, given the limited fundamental knowledge and practical resources available to draw upon.

A program of genetic and physiological screening was undertaken to identify strains of *O. oeni* that could perform better in such conditions, particularly low pH. Pilot and industry trials with a range of bacterial isolates led to information about winemakers' MLF requirements and enabled the identification of specific bacterial isolates that can conduct efficient MLF at industrial scale.

The second approach to improvement of MLF reliability was through an investigation of process options that had the potential to substantially reduce the overall time taken to get a wine to a point where is can be stabilised and protected through SO_2 addition and barrel topping. While MLF is traditionally conducted following completion of alcoholic fermentation, it is also possible for MLF to be conducted concurrently. This is an option increasingly being used by winemakers, but its successful deployment requires a complete understanding of when and where it is most effective such that risks can be managed. Work conducted in this project identified substantial time savings that can be achieved in the production of red wine. Co-inoculation was a reliable and efficient strategy regardless of bacterial strain used. However, the same cannot be said for white and sparkling wine production and some work is still required to better understand how to get co-inoculation to work effectively in these environments.

Other process-driven interventions were also explored, specifically, the use of nutrient additives to support MLF and the impact of oxygen. Nutrient addition has the potential to be stimulatory in nutrient-limiting conditions. Several industry trials over two vintages failed to identify any improvement resulting from nutrient addition. Oxygen or air addition to ferments can be used to stimulate alcoholic fermentation but has the potential to have a negative impact on MLF. Experiments conducted to determine if aeration of alcoholic fermentation could affect subsequent MLF found no inhibitory or stimulatory effects. Work on the potential impact of oxygen use on co-inoculated ferments is ongoing. This area is especially important to winemakers wanting to use oxygen additions in their ferments without adversely affecting MLF, especially when MLF is performed after completion of primary fermentation.

The third approach was to identify genetic markers that might help in more efficiently identifying robust bacterial strains from microbial surveys carried out by others. Both gene expression studies and genome wide association approaches were used. Candidate genes that could be used as markers for diacetyl production and others that indicated potential variation in strain-specific sugar utilisation in genomically highly related strains were identified. Furthermore, variations in metabolic pathways were found that indicated varied capacity of bacterial strains to utilise citric acid.

The key benefit to industry of this work is the provision of evidence-based advice on how to conduct and manage malolactic fermentation under challenging conditions. In this project, different approaches to the conduct of MLF have been investigated, some substantially outside of standard industry practice, with the aim of pushing the boundaries of what is achievable in MLF. Such risks can be undertaken in a research environment so that commercial risks taken by Australian winemakers can be objectively minimised. This approach contributes to the maintenance of a sustainable and profitable Australian wine industry.

The project benefitted substantially from the involvement of several industry partners including Yalumba, Treasury Wine Estates through their Wolf Blass winery, Hardy's Wines through their Tintara winery and Pernod Ricard through their Orlando winery at Rowland Flat.

3. Background:

Malolactic fermentation (MLF) is conducted in all red wines and in many white and sparkling base wines. Even though pH, alcohol concentration, SO_2 and temperature of wine are useful guides to predicting the success of an MLF, this winemaking step remains unreliable, and can add extra costs to the winery through suboptimal performance of MLF bacteria, microbial spoilage and quality downgrades.

The AWRI has helped the Australian industry improve MLF efficiency in red wines by developing co-inoculation strategies (yeast and bacteria inoculated together) that are more efficient than standard sequential inoculations. However, difficulties remain for MLFs in both red and white winemaking. Currently there are no MLF starter strains available that are specifically suited to Australian winemaking conditions; commercially available strains are largely targeted to overseas markets.

Improving MLF efficacy in winemaking is a significant opportunity for the Australian wine industry as highlighted through numerous articles in industry journals and reviews. AWRI studies on co-inoculation in red wine have assisted in addressing this problem (Abrahamse and Bartowsky 2012), but there are limited studies in white wine. Several studies detailing the impact of *O. oeni* and MLF on wine aroma and wine style have been conducted (Swiegers et al. 2005), yet there is limited research undertaken on specific flavour compounds, the pathways involved and their modulation through winemaking. Recent studies by the AWRI have identified the role of MLF in modulating fermentation-derived volatile compounds that impact on 'fruity' and 'berry' aromas in red wine (Costello et al. 2012, 2013). Research by the AWRI and others on glycosidases have demonstrated that MLF bacteria possess these enzymes which are typically absent in yeast (D'Incecco et al. 2004, Grimaldi et al. 2005). However, the knowledge of winemaking conditions required for the expression of such enzymes involved in liberation of the latent aroma compounds is missing. Genome sequencing of 12 *O. oeni* strains by AWRI has revealed the large genetic diversity amongst strains (Borneman et al. 2012) and the link between genome and phenotype is only beginning to be unravelled (Bartowsky 2017).

MLF is used in production of all red and some white wines. A large amount of time, energy and profit is lost through stuck or sluggish MLF. Slow MLF increases wine spoilage risks (such as *Brettanomyces* spoilage) and leads to increased costs (e.g. due to extended periods of heating tanks/barrel halls). Ensuring timely MLF completion is therefore paramount to reliable, cost-effective, quality wine

production. In addition, MLF has been shown to affect wine style but research is required to determine how this can be reliably harnessed by winemakers to tailor their wines.

4. Project aims and performance targets:

This project was designed to take promising candidate O. oeni strains (identified in Project AWR 1305), including Australian isolates, and evaluate their performance under difficult winemaking conditions. Evaluations were to be performed in red, white and sparkling base wines. Additionally, genomic data generated in AWR 1305, was to be used to identify enzymatic pathways involved in the formation of desirable aroma compounds, leading to the development of genetic markers for the isolation of individual strains with flavour-enhancing properties that provide winemakers with the opportunity to shape wine style. Together, the findings were to be captured in a comprehensive knowledge base of the impact of MLF strain, inoculation regime and compatibility with a broad range of yeast strains on MLF efficiency and flavour development.

The specific project aims were to:

a. Identify *O. oeni* strains that tolerate MLF-limiting wine parameters, grow rapidly and efficiently metabolise malic acid

b. Validate performance of target strains in a wide range of conditions typically found in red, white and sparkling winemaking

c. Identify additional factors that ensure an efficient MLF and augment aroma and flavour development, such as MLF inoculation regime (in white wines), impact of winemaking additives (e.g. nutrients), and compatibility with current and 'in development' yeast strains

d. Identify genes for metabolic processes that impact on wine aroma and flavour

e. Determine winemaking practices and/or conditions required to modulate aroma and flavour compounds and their impact on wine style

f. Develop a comprehensive knowledge base of potential metabolic pathways in *O. oeni* that can be used to shape wine style

g. Provide the wine industry with knowledge generated from the above, particularly with regard to strategies for improving MLF outcomes

h. Develop commercial MLF bacterial strains that are suited to Australian winemaking conditions.

5. Method:

5.1. *O. oeni* strain selection

Using data from Project FPA 390, *O. oeni* strains from the AWRI germplasm collection recognised as having potential for use in MLFs were assessed for tolerance to extreme MLF-limiting wine parameters found in red, white and sparkling wine production. Candidate strains were trialled at lab-, pilot- and winery-scale for MLF efficiency in red and white wines under Australian winemaking conditions. Pilot- and winery-scale wines underwent sensory analyses to ensure sound wines were produced with the candidate strains.

5.2. Chemical analysis of wine

Wine ethanol, pH, TA and glucose plus fructose were determined by Fourier-transform spectroscopy (mid-IR) using a Winescan FT2 (Foss) apparatus calibrated for table wine. Free and total sulfur dioxide concentration were determined by flow injection analysis (FIA, Lachat). In some cases, ethanol was determined by near infrared (NIR) spectroscopy

(Alcolyser, MEP). The initial L-malic acid concentration of test wines was determined enzymatically (Randox) using a Daytona automated spectrophotometric analyser (Randox).

5.3. RNA extraction and transcriptomic analysis

Transcriptome analysis was conducted as reported in Sternes et al. (2017).

5.4. Phenotypic screening

Prior to inoculation into test wine, bacterial strains were adapted to wine conditions by pre-culture through two successive stages. In the first, strains were cultured in a modified MRS (mod.MRS) medium prepared by supplementing MRS with D-fructose (10 g/L), DL-malic acid (6 g/L), ethanol (5 % v/v), and cysteine (1.6 g/L or 0.5 g/L for experiments conducted in either Cabernet Sauvignon or Shiraz wines, respectively), with final pH 4.5. Strains were cultured aerobically at 27˚C for 7-10 days, after which time they were subcultured (1-2 % v/v) into a second pre-culture stage (mod.MRS/wine) comprising mod.MRS medium (50% v/v) and wine used for screening (Cabernet Sauvignon or Shiraz) (50% v/v). Strains in this second stage were cultured anaerobically (anaerobic chamber; Coy, USA) at 22˚C for 10-12 days, and cell biomass determined by absorbance (600 nm, 1 cm path length). Using a pre-determined standard curve correlating absorbance (600 nm, 1 cm) values and cell counts (determined microscopically using a bacterial counting chamber, 1000x magnification) of representative strains (stationary phase cultures in mod.MRS/wine), the biomass of each culture was standardised to achieve $1x10^8$ cells/mL by appropriate dilution with mod.MRS/wine medium.

5.5. Tolerance screening of malolactic bacteria

Screening for tolerance to ethanol or low pH stress was conducted in two red wines:

(i) Cabernet Sauvignon: ethanol 13.9 % v/v; pH 3.49; total acidity (TA), 6.9 g/L; total SO2 <4 mg/L, glucose plus fructose, 1.1 g/L; L-malic acid, 1.2 g/L

(ii) Shiraz: ethanol, 13.2 % v/v; pH 3.41; TA, 9.2 g/L; total SO2, <4 mg/L; glucose plus fructose, 0.4 g/L; L-malic acid, 3.4 g/L).

Cabernet Sauvignon was obtained as commercially vinified wine (Barossa Valley, South Australia) from the 2013 vintage; Shiraz wine was prepared from grapes (Robe, South Australia) from the 2015 vintage and vinified at the Hickinbotham Roseworthy Wine Science Laboratory. Wines were obtained after completion of alcoholic fermentation and prior to MLF, and stored at 0-2˚C prior to use. Prior to screening, the L-malic acid content of the Cabernet Sauvignon wine was adjusted to 2.0 g/L. Three sub-lots of respective test wines were prepared from each wine by adjustment to the following parameters: (i) 12 % v/v ethanol, pH 3.5 (reference wine); (ii) 15 % v/v ethanol, pH 3.5 (ethanol stress wine), and (iii) pH 3.3, 12 % v/v ethanol (low pH stress wine). Media and test wines were sterilised by filtration using a sterile membrane filter (0.22 µm pore size).

5.6. Evaluating the effect of nutrient additives

The influence of winemaking additives, such as nutrients, and compatibility with yeast strains was investigated to improve MLF performance.

6. Results and discussion:

6.1. Understanding the genetic basis of malolactic bacterial contribution of wine flavour

Comparative genomic studies of hundreds of *O. oeni* isolates have demonstrated substantial genomic variation between strains of *O. oeni*, with up to 10% variation in protein coding genes observed between any two strains, including those predicted to be involved in sugar utilisation and transport, exopolysaccharide biosynthesis, amino-acid biosynthesis and natural

competence (Bartowsky and Borneman 2011, Borneman et al. 2012). However, little is understood regarding the *O. oeni* transcriptome. To characterise how the *O. oeni* genome interacts with its environment, RNAseq analysis of this important industrial microbe, including the global mapping of operon architecture, was undertaken.

MLFs were initiated separately for three *O. oeni* strains, AWRIB551, AWRIB552 and AWRIB419. Samples were taken at three stages of MLF for RNAseq and metabolite analyses, MLF-start (~ 2.1 g/L L-malic acid), mid-MLF (~ 1.6 g/L L-malic acid) and late-MLF (~0.5 g/L L-malic acid). The three *O. oeni* strains completed MLF at different rates: finishing after 5, 8 and 20 days respectively.

This study has progressed the understanding of genetic variability for *O. oeni* by conducting the first whole transcriptome analysis of this bacterium. There were 47 such novel RNAs identified, of which two were highly abundant. These two most abundant novel RNAs were also the longest in length, comprising 880 nt and 1086 nt (average novel RNA length of 118 nt). In general, these 47 novel RNAs did not appear to encode functional proteins; for example, the 880 nt novel RNA contained an open reading frame (ORF) that encoded part of galactose mutarotase and the 1083 nt novel RNA contained no substantive ORFs.

Given the potential variation that is present between strains of *O. oeni*, *de novo* transcript assembly was employed to investigate genes that may be lacking from the reference strain PSU-1, but present in the three strains used in this study. Analysis of the *de novo* transcripts revealed the presence of a single operon in AWRIB419 that was absent from the PSU-1 genome and which consisted of ORFs encoding a putative NADH-flavin reductase, a transposase and a threonine dehydrogenase followed by a downstream inverted repeat of the first two genes.

In AWRIB551 and AWRIB552 this transposon appears to have inserted into an alternate genomic locus, residing within a large, multi-genic operon, disrupting an ORF that is predicted to encode an arginine deiminase. Insertion of the fGI173 transposon into this alternate genomic locus in AWRIB551 and AWRI552 has resulted in the gene expression of threonine dehydrogenase involved in vitamin B6 metabolism, reducing its abundance between 1.7- to 3.2-fold relative to AWRIB419 across all sampling points.

Pair-wise comparisons between sampling points within individual strains failed to reveal any significant difference in gene expression when comparing T1 and T2, however the comparison of T1 and T3 yielded 10, 24 and 7 differentially expressed genes in AWRIB551, AWRIB552 and AWRIB419, respectively. This shows that individual *O. oeni* strains do not respond dramatically during progression of MLF.

Despite the relative temporal stability of the transcriptional profile for a strain over time, substantial differences between strains were observed. Most notably, the IIA subunit of a non-specific phosphotransferase system was highly upregulated in AWRI419. The bacterial phosphotransferase system is a major mechanism for the uptake of various sugars including extracellular D-glucose, arbutin and salicin. In the same strain fifteen amino-acyl transfer RNAs (tRNAs) were observed to be significantly downregulated. tRNAs play a variety of roles in cell biology. Whether the substantially reduced expression of them in AWRIB419 is a cause or a consequence of the slow progression of malolactic fermentation in this strain is worthy of further exploration.

Dramatic differences were observed between AWRIB419 and the other two strains in the expression of genes related to diacetyl metabolism. This result is somewhat expected since the strains were chosen, at least in part, because of their different profiles of diacetyl production, with AWRIB419 being the higher producer. The most significant difference was in an open reading frame encoding L-lactate dehydrogenase, a gene that is lacking in the other two strains. This gene allows the production of pyruvate from L-lactate which is a potential

substrate in the production of diacetyl. This would explain the different in diacetyl production between AWRIB419 and the other two strains.

Identification of differentially expressed genes in *O. oeni* lays the foundation for further transcriptome profiling of a larger cohort of strains. In conjunction with publicly available comparative genomic analysis, further transcriptomic profiling of *O. oeni* will aid the identification of fit-for-purpose strains which exhibit desirable combinations of genetic characteristics. The final results of this work were published in Sternes et al (2017).

6.2. Enhancing malolactic bacterial performance through process management

Evaluating inoculation regimes as a tool in the management of malolactic fermentation duration

Different winemakers and wineries have different needs from malolactic fermentation. Depending on the situation, faster is not always better. A slow yet steady malolactic fermentation provides the time and space to take care of other matters during the hectic vintage months, giving some assurance that wines have a level of protection resulting from the slow release of CO_2 while they are in barrel. Yet for others rapid completion of malolactic fermentation is a primary scheduling issue that enables efficient use of limited winery resources. Different inoculation strategies can result in substantially different completion times for wines, as demonstrated by Abrahamse and Bartowsky (2012). With co-inoculation, completion of malolactic fermentation can occur concurrently with alcoholic fermentation. At the other end of the spectrum sequential inoculation can result in malolactic fermentation occurring over a period of months. However, these two extremes are not always predictable and can depend on several factors including yeast strain used for alcoholic fermentation and matrix conditions such as pH, SO_2 and temperature of fermentation.

The effect of inoculation regime has been progressively explored across the duration of this project. This has occurred primarily through the vehicle of pilot- and winery-scale trials with winery-scale trials being conducted in collaboration with industry partners.

Trials over three vintages in 2015, 2016 and 2017 assessed the impact of co-inoculation and sequential inoculation at pilot- and industry-scale. The aim was to extend the observations that were made at laboratory scale by Abrahamse and Bartowsky (2012). These trials looked at three different systems: Shiraz, Chardonnay and sparkling base. Over the three vintages different *O. oeni* strains were trialled to take account of varying conditions.

Evaluation of inoculation regimes - 2015 vintage trials

In 2015 three strains – one commercially available and two non-commercially available – were trialled in in Shiraz and Chardonnay (AWRIB551, AWRIB710 and AWRIB714). The duration of malolactic fermentation in Shiraz ferments (12 days) were similar regardless of whether they were initiated as part of a co-inoculation or sequential inoculation regimes (Figure 1A). The overall process time was therefore reduced in the co-inoculated ferments by 20 days from 42 to 22 days. All three strains performed similarly, with regional isolates demonstrating a performance profile equivalent to that of an industry 'workhorse' strain.

In contrast, bacterial strain differences were evident in malolactic fermentation of Chardonnay (Figure 1B). While all strains performed similarly in sequentially inoculated ferments, with a total process time of 36-40 days from the beginning of alcoholic fermentation, only one strain efficiently completed co-inoculated fermentation with a total process time of 18 days (B710). The other two strains (B706 and B551), one of which is the commercial benchmark in this work, took longer to complete MLF in the co-inoculation regime than in the sequential inoculation regime (74 days). The cause of MLF delay in the co-inoculation regime remains undetermined.

Concentrations of diacetyl, a key compound contributing to the perception of butteriness in wines, did not appear to be related to either co-inoculation or sequential inoculation regimes, but rather appeared to increase in concentration when the duration of MLF became extended, especially in Chardonnay. However, even at their highest concentrations the Chardonnay concentration of diacetyl was 10-fold lower than that which was observed in Shiraz (Figure 2A). Despite this overall lower concentration of diacetyl, the relative perception of butteriness was higher in the Chardonnay for those Chardonnay wines that had the highest concentration of diacetyl (Figure 2B).

In summary, chemical and sensory analysis of the 2015 pilot and industry trials showed that, except in one case, novel *O. oeni* strains produced sound wines with no obvious faults. One strain produced wine with a slight reduced character (data not shown). Red wines exhibited higher diacetyl concentrations than white wines. Strains apparently differed in their suitability for co-inoculation work.

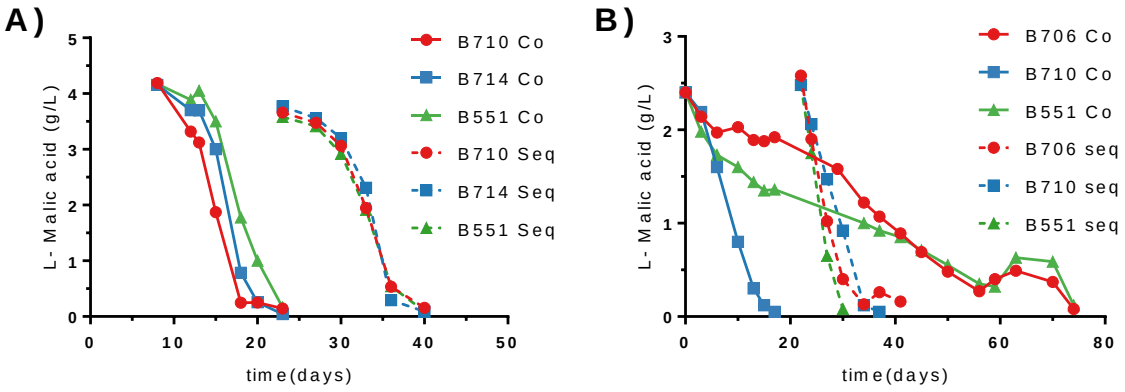


Figure 1. Progress of MLF in Shiraz (A) and Chardonnay (B) using co-inoculation and sequencing inoculation regimes with three different bacterial strains**.**

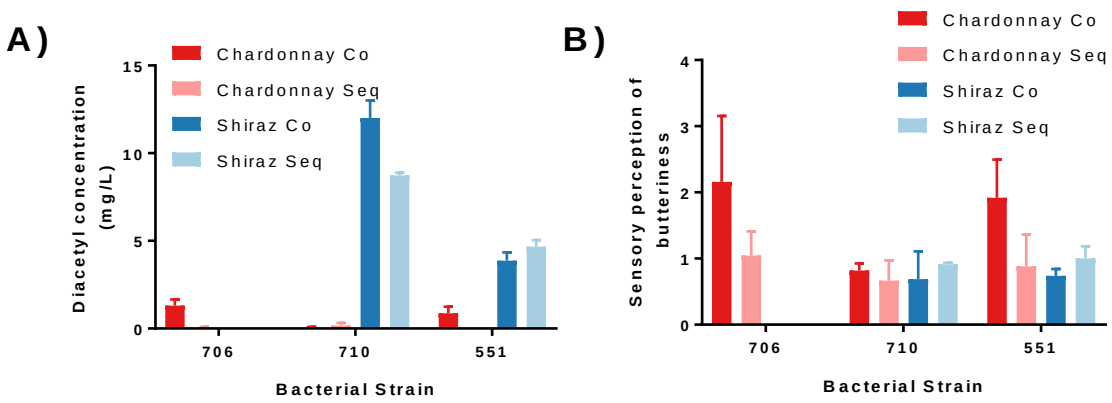


Figure 2. Effect of inoculation regime and wine type on diacetyl concentration and perception of butteriness.

Evaluation of inoculation regimes - 2016 vintage trials

Three trials were undertaken in the vintage of 2016. These trials again examined MLF inoculation regimes at pilot- and winery-scale, focusing on two novel *O. oeni* strains that were chosen based on data from AWR 1305 and trials undertaken in the previous year (data not shown). Strain AWRIB447 was identified in laboratory-scale trials as a high ethanol tolerant strain and was therefore chosen as a candidate for red wine MLF trials. AWRIB706 showed tolerance to low pH in the same laboratory trials and good performance in sequential inoculation pilot-scale vintage trials in 2015 and was therefore selected as a promising candidate for industry trials in Chardonnay table wine and sparkling base wine production.

Shiraz fermentation was undertaken in open tank fermenters inoculated with yeast AWRI796. Co-inoculation MLF treatments were inoculated with AWRIB447 48 hours post-initiation of alcoholic fermentation. At 0° baumé wines were pressed to barrel and left to complete MLF. Sequential treatments were inoculated post-pressing in barrel at 0° baumé at standard bacterial addition rates. Co-inoculation of AWRIB447 was extremely beneficial (Figure 3A). MLFs were complete almost concurrently with alcoholic fermentation. This contrasts with sequential inoculation which, for this strain, required 80 days before any indication of malic acid utilisation was apparent. The co-inoculated fermentation eventually finished after 120 days.

MLF of Chardonnay was carried out in triplicate in 20 L stainless steel kegs. Co-inoculation with AWRIB706 was initiated 48 hours post-initiation of alcoholic fermentation by addition of Qa23. Fermentations were carried out at 17°C. Alcoholic fermentation was completed, at which point sequential inoculation was initiated in a separate replicate set of 20 L wines. The pH values of these wines were between 3.3 and 3.4. In a pattern that reflected the 2015 trial with this bacterial strain (Figure 1B), both sequentially inoculated MLFs finished 6 days prior to co-inoculated wines (24 and 31 days respectively). This data indicates that in relatively soft conditions AWRIB706 is not well suited to co-inoculation.

Finally, trials with AWRIB706 in the production of sparkling base wine showed the inverse of previous observations of this strain. Trials were performed in duplicate, 300 L stainless steel barrels. As with previous trials co-inoculated MLFs were initiated 48 hours post-yeast inoculation whereas sequential MLFs were initiated after the ferments had become ‘sugar dry’. Although co-inoculated sparkling base MLFs showed no sign of malic acid utilisation for almost 60 days (compared to 25 days for the sequential ferments) these MLFs ultimately finished substantially faster (82 days) than sequentially inoculated MLFs, which failed to complete malic acid utilisation (0.8 g/L after 125 days).

In summary, it is difficult to discern a consistent pattern of performance of individual bacterial strains. Co-inoculation of red fermentation was generally reliable with a variety of bacterial strains and substantial reductions in the overall duration of the combined alcoholic and malic acid fermentations were observed. This was not always the case for white wine MLFs where the duration of co-inoculated ferments often exceeded that of sequentially inoculated MLFs. These observations were consistent over several bacterial strain/juice matrix/vintage combinations. The one bacterial strain that demonstrated a consistent pattern of performance in co-inoculated and sequentially inoculated red and white fermentations was strain AWRIB710. However, this strain suffered from sensory defects that precluded it from further study but may warrant closer attention in future work.

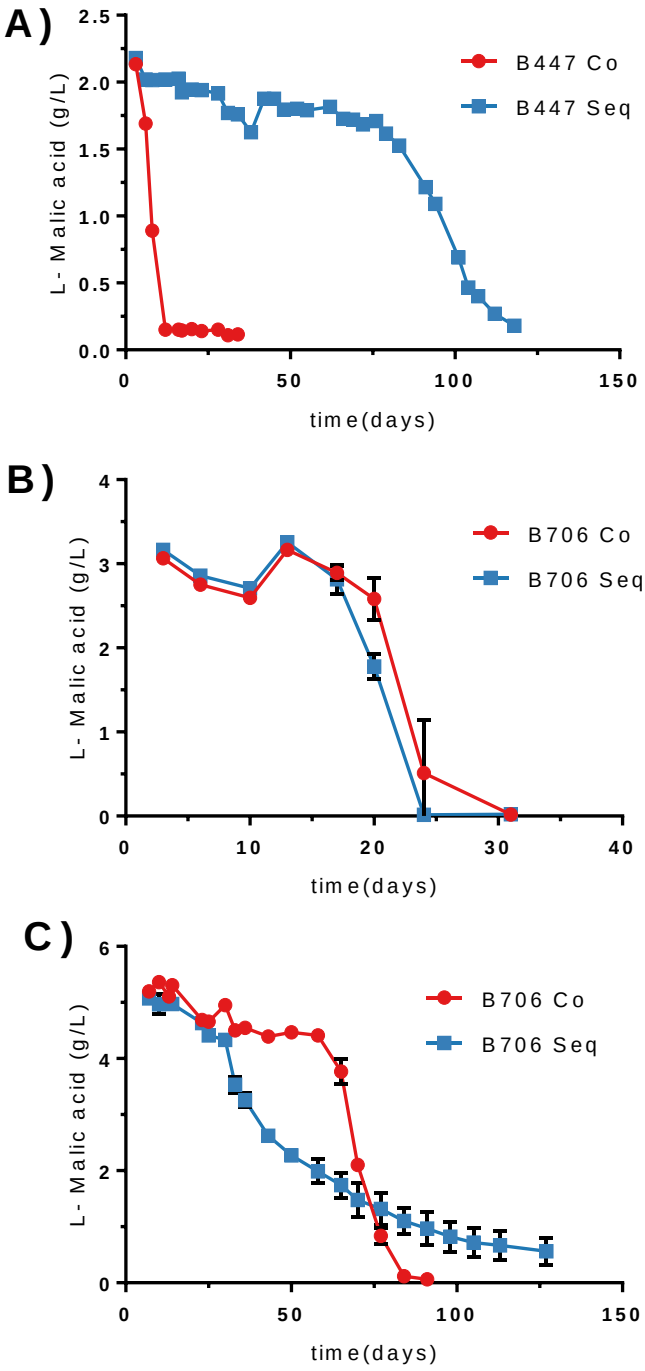


Figure 3. 2016 vintage co-inoculation and sequential inoculation MLF trials in Shiraz (A), Chardonnay (B) and sparkling base (C). These trials were conducted in 10 tonne, 20 L and 300 L volumes respectively.

Nutrient addition as an aid to malolactic fermentation

Just as with yeast nutrient additives, additives claiming to support reliable malolactic fermentation are increasingly becoming available via a number of commercial nutrient suppliers. Technical information provided by inactive dry yeast suppliers generally indicates that these products provide a source of bacterial nutrients such as amino acids and parietal polysaccharides. Some brands may also include other specific components such as yeast ghosts which, evidence suggests, facilitate absorption of toxic medium chain fatty acids produced by yeast. Proprietary recommendations for use of IDY bacterial nutrients include cases where, for example, nutrient status of the wine may be limiting, conditions are difficult for MLF induction, and where MLF is slow or stuck.

At the initiation of this work it was unclear under what circumstances nutrient addition would be helpful in malolactic fermentations and whether individual strains might respond differently to nutrient supplementation.

In the development of a small volume bacterial screening method used for comparison of bacterial strains in section 6.3, several different MLF nutrient additives were evaluated against eight different bacterial strains. In this work, which evaluated strain performance in a range of different matrices and with nutrients from a number of different suppliers, almost all strains benefitted from the addition of nutrients. Without nutrient addition, most bacterial strains were only capable of partial MLF in most of the conditions tested. An example of this is given in Figure 4A. Nutrient addition to small-scale MLFs facilitated completion of MLF by all but two strains under these conditions (Figure 4B). While the magnitude of the effect of some additives was greater than others, the general trend was consistent, that nutrient addition helped. As a result, MLF nutrients have become a standard additive in the AWRI's small-scale bacterial assessment protocol.

Based on the results observed in small-scale trials, industry trials were undertaken over two successive vintages. Two nutrient additives with two *O. oeni* strains (one that responded well to nutrient addition in previous trials and an industry reference) were trialled in a Shiraz wine with low free alpha-amino nitrogen (considered as stressful for *O. oeni*). Neither nutrient addition increased the rate of MLF for either of the two strains. This suggests that the stress of low alpha-amino nitrogen in this wine was insufficient to compromise MLF. In the second vintage, a commercial nutrient was used to treat Shiraz wine and sparkling wine that were undergoing MLF in two independent trials. Both of these wines were challenging and MLF was slow in both cases. Shiraz took 55-65 days to complete and sparkling base between 37 and 63 days. In neither case did nutrient addition reduce the duration of MLF.

In summary, nutrient additions were observed to be beneficial in small-scale MLF fermentations where wines were highly clarified and the dissolved oxygen concentration of the wines can be presumed to be high. However, in barrel or tank MLF of red or white wine no evidence for enhancement of MLF was observed over two vintages.

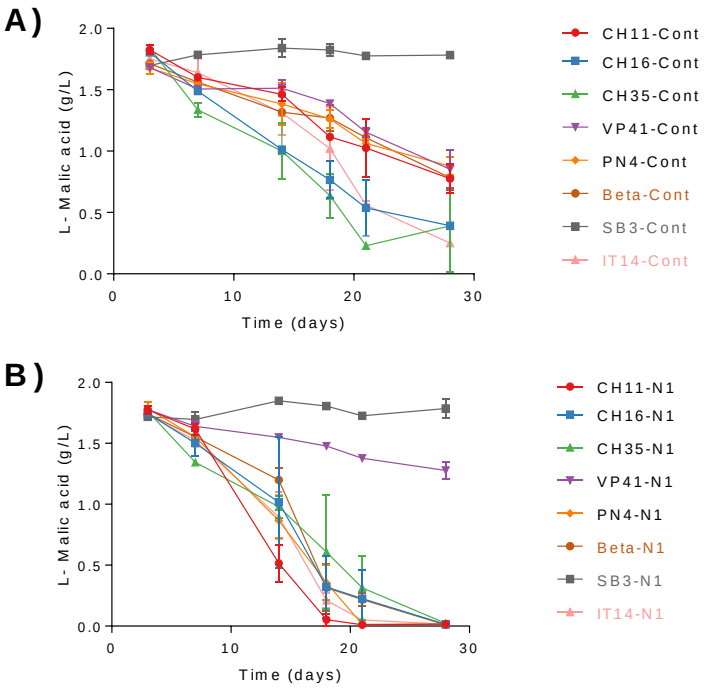


Figure 4. Effect of nutrient addition on progress of malolactic fermentation in small scale fermentations of Chardonnay wine by eight different strains of *O. oeni*. Control (A) and nutrient treatment (B).

Oxygen and malolactic fermentation

Data derived from trials in the application of oxygen during fermentation of red wines in rotary fermenters suggested that the oxygen exposure history of a ferment could influence the performance of subsequent malolactic fermentations (AWRI Project 3.3.2). While malolactic fermentation was a feature of that trial, the trial was not designed to detect changes to malolactic fermentation as a consequence of fermentation treatments. To explore the impact of fermentation oxygen treatments on malolactic fermentation more rigorously, a set of experiments was conducted in which potential confounding influences could be better controlled.

Four Chardonnay wines were made with two different oxygen addition regimes during fermentation with two different exposure levels with each of the two regimes. Ferments experienced a high level of exposure using a regime of either 2 hours or 72 hours, or a low level of exposure using a regime of 2 hours or 24 hours. Alcoholic fermentation completed after nine days for the high-level exposure regime and three days later for the low-level exposure regime. In each case, wines were inoculated with *O. oeni* strain VP41 immediately upon completion of alcoholic fermentation. The completed alcoholic ferments were transferred to a separate vessel under anaerobic conditions. Malolactic fermentation was carried out on yeast lees. Progress of malolactic fermentation was monitored through enzymatic assay. The results of this work are presented in Figure 5. There was no consistent relationship between the type of oxygen treatment applied and the progress of malolactic fermentation. In all cases, complete consumption of malic acid occurred extremely quickly, less than 0.1 g/L L-malic acid was reached in 8 – 11 days. Similar results were obtained in replications and variations of this experiment.

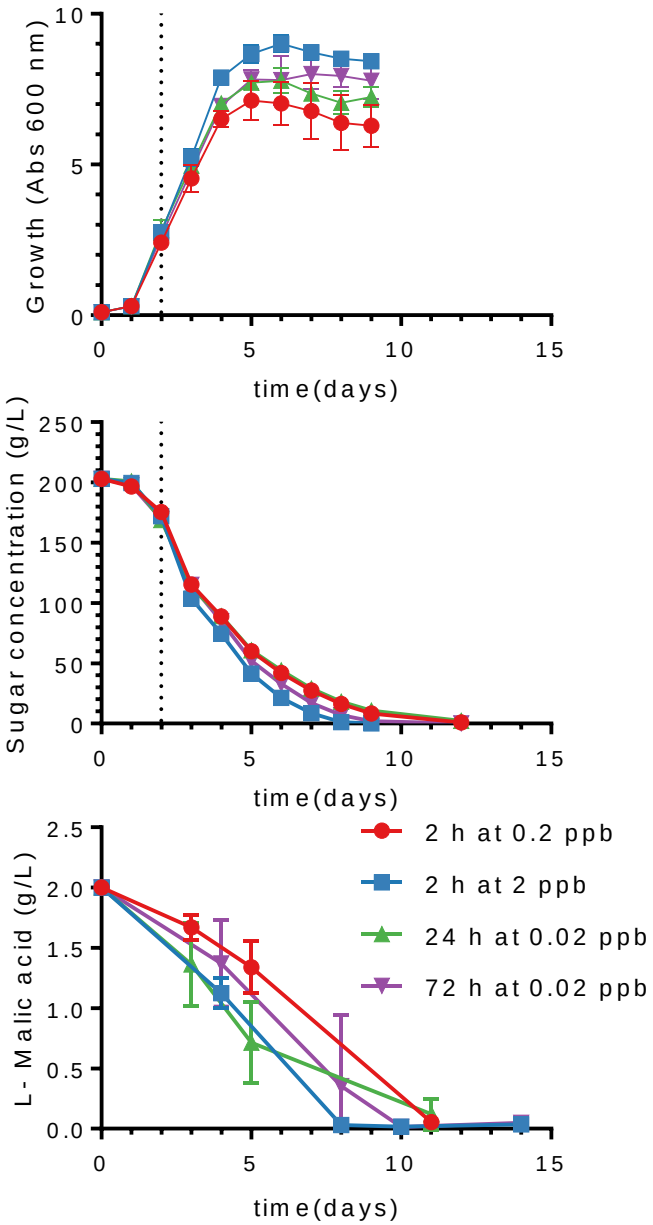


Figure 5. Effects of oxygen addition on alcoholic and malolactic fermentation in Chardonnay. Two regimes were used to deliver high level exposure: 2 hours at 2.0 mg/L (red) and 72 hours at 0.02 mg/L (purple), and another two regimes were used to deliver a low level exposure: 2 hours at 200 mg/L (blue) and 24 hours at 0.02 mg/L (green).

6.3. Isolation and characterisation of robust malolactic bacterial strains from Australian wine regions

Characterisation of *O. oeni* genetic diversity through whole genome sequencing and comparative genomics in project AWR1305 revealed substantial inter-strain genetic variation with large clusters of highly related strains (Borneman et al. 2012, Sternes and Borneman 2016). In general *O. oeni* can be split into two groups (shown as A and B in Figure 6) with group A being the least diverse and comprised largely of strains isolated in Australia. The knowledge gained through these comparative genomics efforts was employed to evaluate the breadth of oenologically useful traits. To this end, an *O. oeni* subset was created to reflect the broader genetic diversity of this species. The source of many of these strains was the AWRI wine microorganism culture collection (AWMCC), which is a repository for strains isolated over many decades. The representative subset was characterised using a high throughput phenotyping approach (Costello et al. 2017) and their ability to complete malolactic fermentation under a range of challenging, wine relevant conditions recorded. An overview of this work is presented in Figure 6.

The most alcohol-tolerant strains were found amongst group A and this physiological trait appeared to be the most common among all the characterised strains. Tolerance to low pH was neither as common or as well clustered, with a broader cross section demonstrating tolerance to low pH. Nevertheless, group B strains also tended to be almost uniformly sensitive to low pH.

Individual strains that demonstrated robustness to a range of conditions were selected for more detailed analysis through small-scale laboratory work and pilot-scale trials. The small-scale trials approximated moderately tough conditions of pH and SO_2. Three strains outperformed all others including a commercially available strain used for comparison (Figure 7).

One of these strains (AWRIB1062) was evaluated in industry trials in the 2017 vintage. This strain was compared with a commercially available reference strain and two previously trialled regional isolates in MLF of sparkling base in barrel. The strain was evaluated in co-inoculation and sequential inoculation regimes (Figure 8A and B). This strain performed competitively with the commercial reference strain when used in a co-inoculation and outperformed all other strains in sequential inoculation. This indicates a robust tolerance of AWRIB1062 toward low pH and cooler temperatures, which is consistent with earlier small-scale laboratory trials.

In summary, large-scale small volume screening followed by larger-scale laboratory trials have identified several candidate bacterial strains that demonstrated robust performance characteristics in industry trials. Strains varied in their capacity to perform in both co-inoculation and sequential inoculation regimes and it may be that different strains will need to be used depending on the inoculation regime employed. At least one strain was identified that outperformed an industry benchmark strain in low pH/low temperature MLF of sparkling base wine, a style of winemaking that for many requires short process times.

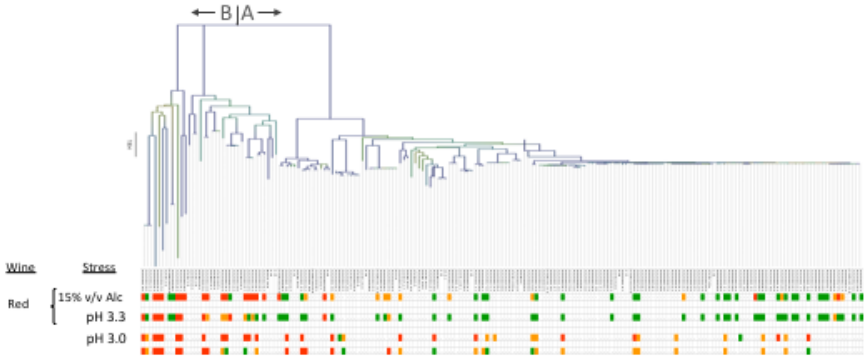


Figure 6. Bacterial strain MLF performance in wine relative to a baseline condition. Green - top 25% of performers; orange - intermediate performers, red - did not complete MLF. Performance profiles in high alcohol (15%), pH 3.3 and pH 3.0 is aligned against a phylogenetic tree that illustrates the relationship between *O. oeni* strains.

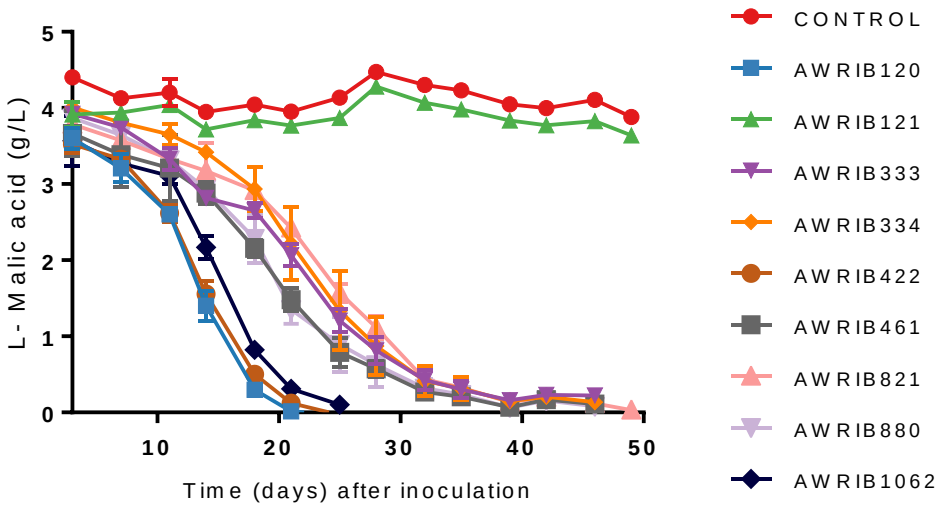


Figure 7. Comparison of *O. oeni* performance in 2 L Chardonnay wine. Sterile filtered Chardonnay wine (12.5% ethanol, 28 mg/L total SO_2, pH 3.18) was inoculated with acclimatised starter cultures at ~1 x 10^7 cells/mL and ferments were carried out at 22°C. Progress of MLF was monitored enzymatically. A commercially available strain was included for comparison (control).

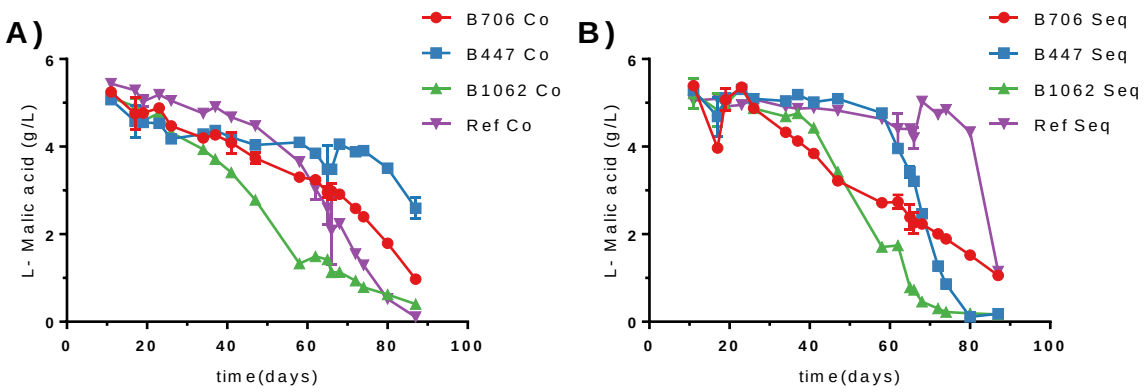


Figure 8. Comparison of novel isolate performance with a commercially available reference strain in MLF of sparkling base wine in barrel. MLFs were performed in duplicate.

7. Outcome/conclusion:

7.1. Performance against planned outputs

Identify O. oeni *strains that tolerate MLF-limiting wine parameters, grow rapidly and efficiently metabolise malic acid; Validate performance of target strains in a wide range of conditions typically found in red, white and sparkling winemaking*

Through an extensive evaluation of bacterial traits using novel screening methodologies developed during this project, numerous strains were identified that are tolerant of specific conditions generally considered to be inhibitory to MLF. Some of these strains are available through commercial suppliers, others were isolated from industrial fermentations over several decades. Target conditions in which strains needed to perform well were primarily high ethanol concentration (>15%), low pH (<3.1) and high SO_2. While many strains capable of sustaining effective MLF in higher ethanol wines were identified, far fewer were able to tolerate low pH. Few wines with a low pH are required to undergo MLF (primarily sparkling wines) and because this represents a niche application it is likely that selection for strains that can perform well in such conditions has not been a priority for commercial suppliers.

Performance of strains identified as robust in various conditions was validated through larger-scale laboratory fermentations (2 – 20 L). At this scale characterisation also involved sensory assessment using the AWRI's quality panel. A prerequisite for the progress of strains to industry trials is that they should not only efficiently consume L-malate but that the resultant wines should be free of faults or taints. A small number of strains were noted as imparting sulfidic aroma profiles to wines. The basis and degree of volatile sulfur compound production in wine by *O. oeni* and the strain-specific nature of that contribution is poorly understood. As such, this is an area worthy of further research. Strains that consistently performed well in these conditions and were fault-free were subsequently assessed in industry trials varying in scale from replicated barrel fermentations to singular 10-tonne fermentations. Making the transition from 20 or 50 L fermentations to >5000 L remains a challenge in evaluation work. Preparation of sufficient quantities of acclimatised starter culture places a burden on project resources and this naturally limits the number of such trials that can be undertaken in any given vintage. Future work will look to minimise the number of such trials through increased focus on specific experimental questions and optimise production of bacteria at scales that facilitate interaction with industry partners.

Determine winemaking practices and/or conditions required to modulate aroma and flavour compounds and their impact on wine style; Identify additional factors that ensure an efficient MLF and augment aroma and flavour development, such as MLF inoculation regime (in white wines), impact of winemaking additives (e.g. nutrients), and compatibility with current and 'in development' yeast strains

Several winemaking practices were evaluated from both performance and wine style perspectives. The MLF regime employed can have a substantial impact on both MLF efficiency and sensory qualities. The specifics are highly wine matrix dependent.

In red wines, co-inoculation regimes generally proceeded reliably with a substantial reduction in total process time. Many of the bacterial strains evaluated in red wine performed equivalently and while the production of a key metabolite, diacetyl, did vary substantially in a strain-dependent manner, this variation did not have large impacts on the sensory quality of the finished wine.

This was not the case in white wines. In white wines, there could be large differences in total process time between co-inoculated and sequentially inoculated ferments but not in a way that was predictable. While some co-inoculated strains were able to complete MLF in a time comparable with sequentially inoculated strains, therefore reducing the overall process time, other strains took substantially longer, thereby extending total process time beyond that which was required for sequentially inoculated strains. MLFs that became sluggish in this way also tended to have higher concentrations of diacetyl. While the concentrations of this metabolite were much lower than what was observed in red wines, the sensory impact appeared to be much higher, with perception of buttery characters doubling over that of wines that proceeded efficiently. These results indicate that co-inoculation of white and sparkling base wines is still a risker operation than it is for red wines.

Other treatments investigated included nutrient addition and oxygen treatment. Neither treatment had a measurable effect on MLF performance, either positive or negative. Nutrient addition carries the risk that if the nutrients are not consumed by *O. oeni*, residual nutrient in the finished wine could act as a source of sustenance for spoilage organisms. Oxygen use is more commonly associated with stimulation of alcoholic fermentation and that was at least in part the inspiration for this series of experiments. This work demonstrated that oxygen could be used during alcoholic fermentation without negative consequences for subsequent MLF. This work is being continued as part of ongoing projects investigating the use of oxygen during winemaking.

Identify genes for metabolic processes that impact on wine aroma and flavour; Develop a comprehensive knowledge base of potential metabolic pathways in O. oeni that can be used to shape wine style

The contribution to wine aroma and flavour by *O. oeni* and the genetic factors that underpin that contribution have been approached through the extensive sequencing of more than 200 *O. oeni* strains and the characterisation of physiological traits in a subset of those strains. Gene expression profiling of three strains throughout malolactic fermentation identified likely candidate genes that could be used as markers for diacetyl production and others that indicated potential variation in strain-specific sugar utilisation in genomically highly related strains. Furthermore, variations in metabolic pathways indicating varied capacity to utilise citric acid were identified. Extending these findings to a broader range of bacterial strains requires further work as does the testing of predictions made on the basis of a single gene expression study. However, the experiments undertaken in the course of this project have provided a framework for a deeper understanding of MLF and have laid the foundation of future work in this area.

Provide the wine industry with knowledge generated from the above, particularly with regard to strategies for improving MLF outcomes

The primary vehicle for industry engagement and extension activities has been the AWRI roadshow program. Several talks are routinely given that aim to inform industry stakeholders about specific project activities, progress of industry trials and also more broadly about how learnings can be put into practice to minimise risk of problem MLFs. These roadshow activities are supported through the provision of fact sheets that were either created or updated upon completion of the project and are available through a number of channels including the AWRI helpdesk, the AWRI library and AWRI website.

Develop commercial MLF bacterial strains that are suited to Australian winemaking conditions

Through an extensive evaluation of bacterial traits, several promising bacterial strains were identified that exhibited strong performance in either higher ethanol concentrations or low pH conditions. Some of these strains have been trialled extensively at pilot and industry scale and represent promising candidates for commercialisation.

7.2. Could changing the methodology/technology have improved the outcome?

The approaches to specific aspects of the work undertaken in this project have, naturally, evolved over the duration of the projects as different elements were found to work or not. For example, preparation of starter cultures for industry trials began by simply growing bacterial strains in larger and larger volumes of commercially sourced juice until sufficient biomass was generated to inoculate a large volume of wine. However, wine conditions are substantially different to those of the growth media used to generate biomass and experiments initiated in this way generated unreliable outcomes. Starter culture preparation then evolved to include increasingly extensive acclimatisation procedures to get the strains ready for the wine they were to encounter in the trial. Acclimatisation of starters came at the cost of staff time and limited the capacity to be responsive to rapidly changing vintage realities. Long lead times were required as well as samples of equivalent wine prior to the wine being ready. The development of methods to prepare cultures in the inter-vintage period and store them as frozen stocks dramatically shortens the lead time involved in starter culture preparation during vintage periods, improves the ability to respond to evolving vintage needs and reduces commitment of staff time during an already busy period. While these changes are now in place at the end of the project, it is likely that some of the vintage trials that were undertaken during the project would have benefitted substantially had they been in place earlier.

Much of the screening work was performed in wine. When work such as this takes place over many vintages the wine matrix can change, or if a particular source is exhausted and a new one sought, it will be completely different. This occurred during this project making a continuous data set difficult to compile. This would occur in almost any context if, after completion of a project, additional work was performed to extend the findings. It would have been better had the initial phenotyping work been undertaken in a defined wine matrix rather than an actual wine made from grape juice. The results can always be validated in real wine matrices after initial findings have been made but it is difficult or impossible to extend a set of data from one real wine to another to obtain a consistent foundational data set that spans multiple matrices.

7.3. Practical implications

The molecular markers identified in this project will facilitate more efficient screening of novel bacterial isolates. Potential sources of novel isolates include ongoing Wine Australia projects such as AWR1501. Given the difficulty and time involved in performance screening of *O. oeni* isolates, methods that help to streamline bacterial strain characterisation will be particularly beneficial.

All of the work on co-inoculation, nutrient addition and the effects of oxygen has generated a dataset that will form the basis for advice on how best to apply different winemaking practices and the risks and rewards associated with them. This information is being disseminated through multiple channels and will become a resource for researchers and industry practitioners alike.

7.4. Benefits to the industry

The key benefit to industry is the provision of evidence-based advice on how to conduct and manage malolactic fermentation under challenging conditions. In this project, different approaches to the conduct of MLF have been investigated, some substantially outside of standard industry practice, with the aim of pushing the boundaries of what is achievable in MLF. Such risks can be undertaken in a research environment so that commercial risks taken by Australian winemakers can be objectively minimised. This approach contributes to the maintenance of a sustainable and profitable Australian wine industry.

7.5. Recommendations

One area of malolactic performance that was only partially addressed in this project was the constraining effect of SO_2 concentration in juice and wine on MLF. This will become a more important consideration in light of increased adoption of co-inoculated MLFs. If it is considered at all, analysis of juice will focus on free SO_2 because of the potentially inhibitory effect on primary fermentation. However, *O. oeni* and other lactic acid bacteria can strip components from bound SO_2, thus liberating free SO_2 from the total. The degree to which this is a fundamental consideration for co-inoculated fermentation regimes and the mechanisms by which *O. oeni* resists SO_2 are natural progressions of the current work.

8. Appendix 1: Communication:

Outcomes and knowledge generated during the progress of this project have been communicated to peers through peer-reviewed publications, conference presentations, and annual meetings with researchers at the University of Adelaide. These are itemised in sections 9.1, 9.2 and 9.3. Representatives of Wine Australia attended these meetings and therefore have had the opportunity to hear updates on project progress outside of the six- monthly reports that were provided for the duration of the project.

Information has been extended to industry stakeholders through articles in trade journals, presentations in the AWRI roadshow program, webinars and workshops at both the 2013 and 2016 Australian Wine Industry Technical Conferences. Updated fact sheets have been prepared that provide the latest information to winemakers and are available upon request from the AWRI helpdesk or as a download from the AWRI website.

8.1. Journal publications

Bartowsky, E.J. 2017. *Oenococcus oeni* and the genomic era. *FEMS Microbiol. Rev.* 41: S84–S94.

Bartowsky, E.J., Costello, P.J., Chambers, P.J. 2015. Emerging trends in the application of malolactic fermentation. *Aust. J. Grape Wine Res.* 21: 663–669.

Borneman, A., Bartowsky, E., Costello, P., Sternes, P., Chambers, P., Herderich, M., Johnson, D. 2015. Unravelling the capricious nature of *Oenococcus oeni*. *Wine Vitic. J.* 30(3): 34, 36-37.

Cappello, M.S., Zapparoli, G., Logrieco, A., Bartowsky, E.J. 2017. Linking wine lactic acid bacteria diversity with wine aroma and flavour. *Int. J. Food Microbiol.* 243: 16–27.

Costello, P.J., Siebert, T.E., Solomon, M.R., Bartowsky, E.J. 2013. Synthesis of fruity ethyl esters by acyl coenzyme A: alcohol acyltransferase and reverse esterase activities in *Oenococcus oeni* and *Lactobacillus plantarum*. *J. Appl. Microbiol.* 114: 797–806.

Schmidt, S.A., Costello, P.J. 2014. Nutrients to support primary and secondary fermentations: what are they and do they work? *AWRI Tech. Rev.* 211: 6-11.

Sternes, P.R., Borneman, A.R. 2016. Consensus pan-genome assembly of the specialised wine bacterium *Oenococcus oeni*. *BMC Genom*. 17(308): 1-15.

Sternes, P.R., Costello, P.J., Chambers, P.J., Bartowsky, E.J., Borneman, A.R. 2017. Whole transcriptome RNAseq analysis of *Oenococcus oeni* reveals distinct intra-specific expression patterns during malolactic fermentation, including genes involved in diacetyl metabolism. *Int. J. Food Microbiol.* 257: 216–224.

8.2. Conference Presentations

Genomic analysis of 80 *Oenococcus oeni* strains and connecting the genome with winemaking properties. Presenter: E. Bartowsky; 11th Lactic Acid Bacteria Symposium, Egmond aan Zee, The Netherlands, 31 Aug-3 September 2014.

Genomic analysis of 80 *Oenococcus oeni* strains and connecting the genome with winemaking properties. Presenter: E. Bartowsky; Crush 2014 - Grape and Wine Science Symposium, Adelaide, 25-26 Sepembert 2014.

High-throughput phenotyping of malolactic bacteria. Presenter: P. Costello; 16th Australian Wine Industry Technical Conference, Adelaide, 24-28 July 2016.

High-throughput phenotyping of malolactic bacteria. Presenter: P. Costello; 16th Australian Wine

Industry Technical Conference, Adelaide, 24-28 July 2016 (MLF Workshop).

Wine Fermentation – Research and innovation with an age-old process. Presenter: P. Costello; AIFST Seminar – Sydney, October 2015.

8.3. Extension presentations

Titles of AWRI roadshow presentations

- Can I improve MLF performance and reliability?
- Avoiding spoilage issues caused by wine bacteria: prevention is better than cure
- Novel *O. oeni* strains with appropriate winemaking properties
- Can you influence wine styles through MLF?

Fact sheets – available from the AWRI website

Updates for the AWRI fact sheet 'Achieving successful MLF' have been prepared for upload to the AWRI website.

A new protocol 'Preparation of a liquid malolactic fermentation (MLF) starter from an agar slant culture of *Oenococcus oeni*', will also be available for wineries wishing to culture 'in-house' or other malolactic strains from the AWRI wine microorganism culture collection.

A new AWRI fact sheet 'MLF in white and sparkling base wines' has been prepared for upload to the AWRI website.

9. Appendix 2: Intellectual Property

This project has generated knowledge, know-how and data on the subject of malolactic fermentation and wine quality which will be published and communicated as part of the project outcomes.

10. Appendix 3: References

Abrahamse, C.E., Bartowsky, E.J. 2012. Timing of malolactic fermentation inoculation in Shiraz grape must and wine: influence on chemical composition. *World J. Microbiol. Biotechnol.* 28: 255–265.

Bartowsky, E.J. 2017. *Oenococcus oeni* and the genomic era. *FEMS Microbiol. Rev.* 41: S84–S94.

Bartowsky, E.J., Borneman, A.R. 2011. Genomic variations of *Oenococcus oeni* strains and the potential to impact on malolactic fermentation and aroma compounds in wine. *Appl. Microbiol. Biotechnol.* 92: 441–447.

Borneman, A.R., McCarthy, J.M., Chambers, P.J., Bartowsky, E.J. 2012. Comparative analysis of the *Oenococcus oeni* pan genome reveals genetic diversity in industrially-relevant pathways. *BMC Genom.* 13: 1-13.

Costello, P.J., Chambers, P.J., Bartowsky, E.J. High-throughput phenotyping of malolactic bacteria. Beames, K.S, Robinson, E.M.C., Dry, P.R., Johnson, D.L. (eds.) *Proceedings of the 16th Australian Wine Industry Technical Conference: Adelaide, South Australia, 24-28 July*. Glen Osmond, S.A.; The Australian Wine Industry Technical Conference Inc: 115-117; 2017.

Costello, P.J., Francis, I.L., Bartowsky, E.J. 2012. Variations in the effect of malolactic fermentation on the chemical and sensory properties of Cabernet Sauvignon wine: interactive influences of *Oenococcus oeni* strain and wine matrix composition. *Aust. J. Grape Wine Res.* 18: 287–301.

Costello, P.J., Siebert, T.E., Solomon, M.R., Bartowsky, E.J. 2013. Synthesis of fruity ethyl esters by acyl coenzyme A: alcohol acyltransferase and reverse esterase activities in *Oenococcus oeni* and *Lactobacillus plantarum*. *J. Appl. Microbiol*. 114: 797–806.

D'Incecco, N., Bartowsky, E., Kassara, S., Lante, A., Spettoli, P., Henschke, P. 2004. Release of glycosidically bound flavour compounds of Chardonnay by *Oenococcus oeni* during malolactic fermentation. *Food Microbiol.* 21: 257–265.

Grimaldi, A., Bartowsky, E., Jiranek, V. 2005. A survey of glycosidase activities of commercial wine strains of *Oenococcus oeni*. *Int. J. Food Microbiol.* 105: 233–244.

Sternes, P.R., Borneman, A.R. 2016. Consensus pan-genome assembly of the specialised wine bacterium *Oenococcus oeni*. *BMC Genom*. 17(308): 1-15.

Sternes, P.R., Costello, P.J., Chambers, P.J., Bartowsky, E.J. Borneman, A.R. 2017. Whole transcriptome RNAseq analysis of *Oenococcus oeni* reveals distinct intra-specific expression patterns during malolactic fermentation, including genes involved in diacetyl metabolism. *Int. J. Food Microbiol.* 257: 216–224.

Swiegers, J.H., Bartowsky, E.J., Henschke, P.A., Pretorius, I.S. 2005. Yeast and bacterial modulation of wine aroma and flavour. *Aust. J. Grape Wine Res.* 11: 139–173.

11. Appendix 4: Staff

Peter Costello, Eveline Bartowsky, Anthony Borneman, Paul Chambers

12. Appendix 5:

Nothing additional to report

13. Appendix 6: Budget reconciliation.

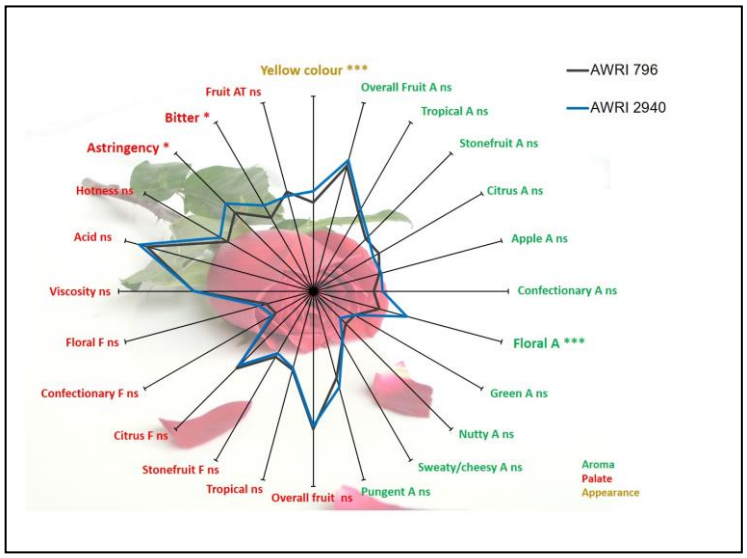
The project's budget reconciliation statement will be submitted separately.

Australian Government

Australian Grape and Wine Authority

The Australian Wine Research Institute

Enhanced winemaking outcomes and wine style diversification through provision of fit for purpose yeast starter cultures



FINAL REPORT to

AUSTRALIAN GRAPE AND WINE AUTHORITY

Project Number: **AWR 1301**

Principal Investigator: **Christopher Curtin (to August 2016), Simon Schmidt (from August 2016)**

Research Organisation:

The Australian Wine Research Institute

Date: **22 September 2017**

Australian Government

Australian Grape and Wine Authority

Project title: Enhanced winemaking outcomes and wine style diversification through provision of fit for purpose yeast starter cultures

Project No.: AWR 1301

Author: Dr Chris Curtin and Dr Simon Schmidt

Date: 22 September 2017

Disclaimer/Copyright Statement:

Contents

1. Abstract:

While it is common practice for winemakers to choose yeast starter cultures based on their perceived impact on wine style, as much as on their capacity to reliably complete fermentation, this choice has generally been guided by anecdotal evidence rather than objective data. In this project, replicated small-scale fermentations and pilot-scale winemaking revealed the extent to which existing yeast strains can influence composition and sensory properties of red wine. Through correlation to genome sequences, genetic markers were identified for key flavour compounds. Finally, novel *Saccharomyces* interspecies hybrids were generated along with variants of AWRI796 that impart 'rose' aromas in wine.

2. Executive summary:

Once grapes have been harvested and crushed, Australian winemakers are confronted by the need to choose from more than one hundred commercially available yeast strains to start their fermentations. For some winemakers the choice is straightforward, they base their decision solely upon reliability of fermentation completion and may in fact use the same yeast strain for all wine styles. Others make use of yeast germplasm resources to accentuate desired flavours and minimise the production of off-flavours, choosing strains that are 'fit-for-purpose' considering the wine style they are seeking to achieve. To date there has been a lack of objective data winemakers could draw upon to make this choice, typically limited to technical information for a single supplier's strain portfolio, or scientific studies involving a relatively small number of strains. This project was designed to generate knowledge concerning the potential flavour impacts various commercially-available strains may have on wine style, in parallel with work to better understand their fermentation performance and reliability (Project AWR1302). In other words, the aim was to better define what purpose available strains are fit for. The data sets generated were then used to guide development of novel yeast strains that offer Australian winemakers unique opportunities to craft new wine styles and achieve their stylistic goals.

Outcome 1: Knowledge of potential value that can be derived from available yeast genetic diversity to shape wine flavour profile and texture.

In previous work at the AWRI, the potential for yeast strain to shape white wine style had been demonstrated for a small number of strains chosen based upon anecdotal evidence. To provide a more systematic dataset informing winemakers on choice of yeast, for this study strains were selected based on genome sequencing performed in Project AWR1302. This ensured that available genetic diversity was represented across an even split of commercially available and Australian winery isolates.

Small-scale replicated laboratory fermentations were performed using 94 yeast strains in a standardised model grape juice and a Shiraz juice. The latter was modified first-run juice to which commercially available grape phenolic extracts were added to mimic the phenolic composition of a complete Shiraz must. This enabled comparison of flavour compound production in real juice to that observed in a model system, and also exploration of the impact of yeast strain on phenolic composition of red wine.

The results show that model grape juice is suitable for evaluating production of many key yeast-derived volatile aroma compounds, though for a subset of analytes it was evident that yeast metabolism was substantially altered in real grape juice relative to defined medium. This is consistent with other research that has examined the influence of grape composition on yeast-derived flavour compound formation. Nevertheless, when considered together, the experiments in model grape juice and real grape juice provide insight into the extent of variation between existing yeast strains with regards to their potential flavour impacts. Strains could be grouped according to their overall aromatic profiles by applying clustering algorithms. Winemakers interested in harnessing yeast that boost production of desired aroma compounds can use these datasets to make an informed choice from

amongst several strains, taking into consideration their performance-related properties and any preference for a particular supplier.

A major focus in this study was to determine the extent of yeast influence on red wine style, extending prior research that suggested Shiraz quality parameters were modulated according to yeast strain. In addition to laboratory-scale fermentations exploring yeast-phenolic interactions in the absence of grape solids, winemaking trials were performed in collaboration with AWRI Project 3.1.4 (texture). A follow-up winemaking trial was performed incorporating yeast strains studied throughout this project, shown at laboratory scale to yield the greatest differences in wine composition. Data on wine volatiles confirm that profiles observed at laboratory scale were replicated in pilot-scale fermentations. Together, these datasets emphasise that choice of yeast has a marked influence on red-wine style, and amongst available yeast strains there are several groups that have distinct properties that can be harnessed by winemakers.

Outcome 2: Knowledge of the potential to control yeast flocculation late in fermentation to improve processing efficiency

Cell-to-cell binding can result in the formation of large clusters of cells that settle to the bottom of the fermentation vessel, a process known as flocculation. This process is particularly helpful at the end of fermentation, making wine clarification and filtration easier. As a proof-of-concept study, gene technologies were used to construct a wine strain where the onset of flocculation could be triggered at any point during fermentation by modifying an external signal.

To gain insight into natural variation amongst wine yeast regarding the timing and strength of flocculation, two complementary approaches were taken. First, a novel high-throughput sedimentation-rate assay was devised and applied to end of fermentation samples of yeast from model grape juice fermentations described under outcome 1. The samples were transferred into model wine solutions where known triggers of flocculation were varied, namely pH and ethanol concentration, and some samples were transferred to lower temperatures. Second, a collection of 94 barcoded wine yeast strains developed in project AWR1302 were pooled and used to perform fermentations under varied conditions (pH, ethanol, temperature). The barcodes were used to detect which strains remained in suspension throughout fermentation and which strains flocculated at different stages of fermentation. Together these datasets revealed some strains that flocculate throughout fermentation and are unaffected by fermentation conditions, and other strains that flocculate strongly at the end of fermentation.

Fermentation and processing difficulties can also be associated with strains that are overly flocculent or flocculate inappropriately. The methods developed as components of this project were ultimately used not only to characterise flocculation behaviour but to alter it. Work on low alcohol yeasts in AWRI Project 3.3.1 identified strains capable of producing wine with reduced alcohol that were excessively flocculent and had only limited application in winemaking. Non-flocculating versions of those yeast have been isolated by applying the methods originally designed to characterise yeast flocculation behaviour.

Outcome 3: Knowledge of the impact that genome composition of wine yeast interspecies hybrids has on their potential to modulate wine style

Genome composition of hybrids generated in this project was assessed using custom-designed molecular assays that detect the presence or absence of representative chromosomal regions from each parent species, and by whole-genome sequencing. Genome stability was assessed by comparison of genome structure shortly after hybrid generation with structure following fermentation completion or in some cases after passaging cultures for hundreds of generations. Due to their method of creation *cerevisiae*/non-*cerevisiae* hybrids are generally triploid as determined by flow cytometry. The hybrids generated at the AWRI have demonstrated a surprising degree of genetic stability during mitotic growth. Examples of partial chromosome loss were encountered but these were often the

consequence of the hybridisation event itself rather than propagation. Once a lineage was established its genetic makeup and phenotypic profile remained unchanged and thus represent suitable source material for commercial-scale propagation.

Outcome 4: Development of novel wine yeast strains

While results generated in this project demonstrate the extent of natural variation that exists amongst wine yeasts, and their potential to impact wine style, there is no single 'perfect' yeast. Each exhibits a combination of desirable and undesirable attributes. Furthermore, among commonly used wine yeast strains some properties are not available. Genome sequences (AWR1302) and wine compositional data from fermentations performed in this project were drawn upon to explore the potential use of genome-wide-association (GWAS) to identify genetic markers for strain development. Several potential markers were identified for production of volatile aroma compounds, such as those responsible for 'rose' aroma in wine, and work was initiated to confirm their relevance.

In parallel to the generation of these foundational datasets and platforms for future strain development, in this project classical strain development approaches were used to generate new interspecies hybrids and variants of an industry 'workhorse' wine strain (AWRI796) that improved one of its known defects and introduced a novel property.

The portfolio of interspecies hybrids generated by the AWRI was expanded to include hybrids with recently discovered *Saccharomyces* species: *S. arboricolus* and *S. eubayanus*. The winemaking potential of these two species was unknown prior to initiation of this work, though the industrial relevance of *S. eubayanus* is well established through its role as one parent of lager yeast. The capacity of lager yeast to ferment wort at low temperature prompted investigations of the low-temperature fermentation potential of wine strain x *S. eubayanus* hybrids alongside hybrids with another known cold-tolerant species, *S. kudriavzevii*. Screening of wine yeast interspecific hybrids generated between these species identified a number of hybrid yeast strains that complete fermentation at 12°C, but with a protracted timeline (15-18 days). However, two hybrid strains showed robust fermentation properties at 12°C and completed fermentation in a timely manner (9-12 days).

Newly generated hybrids, along with others generated in earlier projects, were also evaluated for use in sparkling wine production. Some of this work was performed in collaboration with Plumpton College in the UK and this has led to considerable interest in using interspecific wine yeasts for sparkling wine production.

AWRI796 was chosen as a starting point for strain improvement activities due to its relatively simple genomic organisation (its chromosomal copies are almost identical to one another) and widespread use in the wine industry. One of the known flaws of this strain is its tendency to produce high levels of succinic acid, which can lead to an imbalance in acidity and 'salty' flavour in wine (Coulter et al. 2004). Mutants of AWRI796 were successfully isolated that produced 40% less succinic acid than AWRI796 in white wines in laboratory trials.

Drawing upon knowledge of genes involved in production of volatile compounds responsible for 'rose' aroma in wine, 2-phenylethanol and 2-phenylethyl acetate, a selection strategy was implemented to isolate variants of AWRI796 with enhanced production of these compounds. This strategy succeeded without extensive accumulation of other mutations, meaning that isolated variants were essentially identical to AWRI796 aside from a very small number of changes to their genomes (< 10 nucleotides). These changes translated to production of between 5- and 50-fold increases in 'rose' aroma compounds relative to AWRI796, dependent upon grape variety fermented. Pilot- and industry-scale trials of these novel strains demonstrated a profound impact of these strains on wine sensory properties. Generally, favourable profiles were seen in white varieties; however, descriptors associated with these strains in red varieties were more varied and not always positive.

3. Background:

Previous AWRI projects (AWRI 1.3.1 and AWRI 1.3.3) were successful in developing new yeast starter cultures for Australian wine producers that minimise hydrogen sulfide formation, enhance 'fruity' volatile thiol formation, and create opportunities for wine style diversification. With the exception of low H_2S strains, which drew upon detailed understanding of genes involved in the sulfate reduction pathway, these products were developed empirically. Despite the important 'imprint' yeast can leave on a wine's flavour profile, there is an incredibly shallow understanding of the genetics of yeast-derived flavour. Even for the Ehrlich pathway (which produces higher alcohols and volatile fatty acids), many genes involved are known, but it is not clear why one yeast has a tendency to produce more of a particular higher alcohol than another. The AWRI is well placed, with a reservoir of Australian yeast germplasm diversity to draw upon (AWRI Project 3.2.5), to develop understanding of flavour profile diversity and underlying genetics.

The review published on flavour-active wine yeasts (Cordente et al. 2012) discussed the yeast 'flavour phenotypes' currently available to winemakers, while highlighting a lack of progress towards genomics-assisted yeast breeding. A lack of comprehensive yeast flavour profiling datasets has limited systematic studies examining the genetic basis of flavour compound formation to a few targets – specifically, production of acetic acid (Marullo et al. 2007) and the volatile thiol 4MMP (Roncoroni et al. 2011). Attempts have been made to correlate production of esters and higher alcohols to transcriptomic data (Rossouw et al. 2008). Genome wide association studies are much more likely to facilitate targeted yeast breeding, particularly where performing interspecies hybridisation. The concept that particular alleles (copies) of flavour genes may determine overall flavour profile has received relatively little attention to date (Bisson and Karpel 2010).

Inoculation with selected active dry wine yeast represents one of the lowest cost inputs in wine production, yet has a significant impact on winemaking outcomes. Even though some strains are favoured over others for particular applications, often due to their perceived flavour effects, there is a lack of systematic data on wine yeast flavour profile diversity. Improved understanding of diversity already available amongst commercial starter cultures will ensure winemakers can choose fermentation yeast most congruent with their stylistic intentions, and will underpin development of fit for purpose starter cultures tailored for Australian winemaking requirements.

4. Project aims and performance targets

The objectives of this project were to:

1. Understand the potential value that can be derived from yeast genetic diversity to shape wine flavour profile and mouth-feel, predominantly for *Saccharomyces* species (complementary objectives in AWRI Projects 3.2.3 and 3.5.3) but also selected non-*Saccharomyces* yeasts.
2. Understand the potential for more efficient processing of wine post-alcoholic fermentation through controlled timing and strength of yeast flocculation.
3. Develop knowledge of relative contributions of divergent genomes in interspecies hybrids to wine flavour profiles and winemaking outcomes, to support effective breeding and hybridisation methodology.
4. Use genomics-assisted breeding and interspecies hybridisation to develop novel yeast strains targeting traits identified as important for Australian winemakers by Wine Australia and through industry consultation, with initial priority targets of enhanced red wine flavour, production of 'rose' aromas, avoidance of faults (H_2S, VA, succinic acid), optimal flocculation behaviour, and stability in performance. Yeast strains identified in AWRI Project 3.2.3 as having desirable traits will be incorporated in strain development.

5. Methods:

5.1. Conduct and analysis of small-scale fermentations for aromatic profiling

A large number of commercial, indigenous, and genetically diverse yeasts were used to separately ferment chemically defined and real grape juices. The choice of yeasts was guided by genomic data generated in AWR1302. Metabolomic analyses of fermentation volatiles were performed, enabling the relative proportions of >50 compounds to be determined, and multivariate yeast strain 'flavour phenotypes' were defined. Fermentations were performed in triplicate. Hydrogen sulfide production during fermentation was monitored using mercuric chloride detector tubes as described by Ugliano and Henschke (2010). Volatile metabolites were measured as previously described (Siebert et al. 2005, 2010). Concentrations of glycerol, acetate, ethanol and organic acids were determined as described by Schmidt et al. (2011).

5.2. Flocculation characteristics of these yeasts were assessed using complementary approaches:

5.2.1.High-throughput sedimentation rate assay of individual strains

Multifactorial screening experiments exploring yeast strain x environmental interactions on flocculation behaviour were conducted by taking end of fermentation yeast samples from 5.1 and resuspending them in media with varied composition (pH, ethanol), and incubating them for 24 hours at different temperatures. A high-throughput sedimentation rate assay, developed in this project, was then used to determine how quickly the yeast samples dropped out of suspension. Briefly, in deep 96-well plates the samples were agitated, and subsampled into a standard 96-well plate for measurement of optical density. After a defined period, the deep well plates were resampled for measurement of optical density. Difference between the two measures was indicative of yeast in the deep-well plates falling out of suspension due to enhanced flocculation behaviour under the experiment conditions.

5.2.2. Competitive sedimentation rate assay

A sedimentation rate assay using the barcoded yeast collection developed in AWR1302 was developed to independently compare the flocculation behaviour of yeast. In this assay a mixed population of yeast were grown under varied conditions (sugar concentration, pH, temperature) in fermentation vessels, then subsampled into a tall cylindrical flask with a sample port. After a defined period, subsamples were taken from the top and bottom of the cylinder, for comparison with the original sample. Custom-amplicon sequencing as described in AWR1302 was used to generate quantitative data on the strain composition of the samples, enabling detection of strains that were over-represented in 'bottom samples' due to their flocculation behaviour. The 'cylinder-sedimentation' approach was used to inform design of a selection strategy for generation of non-GM wine yeasts with altered flocculation properties.

5.3. Determining genomic factors contributing to variations in aromatic profiles

Building on the knowledge of variation in strain aromatic profiles and genomic datasets (AWR1302), bioinformatic approaches were used to correlate genomic features with desirable strain traits. Genomic correlations were used to develop genetic markers for strain development, with the goal of optimising more than one trait relevant to red winemaking. The intention was to perform quantitative-trait-loci (QTL) analysis to validate these and generate additional markers, and the foundations were laid for this work through selection of stable haploid variants (through sporulation) of wine yeast displaying traits of interest. During this project, new methods for yeast modification were developed and published (Ryan et al. 2016), based upon the bacterial CRISPR-cas9 system. These methods enable precise and efficient manipulation of industrial yeast strain genomes, with one common application being the verification of genetic markers. In this project, CRISPR protocols were implemented and used to verify genetic markers for production of 'rose' aroma compounds, by 'swapping' gene alleles (copies) between strains.

5.4. Methods for the development of new strains

5.4.1.Mutagenesis and selection

Two traits were targeted for introduction to the industry workhorse yeast strain AWRI796 – enhanced production of 'rose' aroma compounds, and reduction of this strain's tendency to overproduce succinic acid, which can lead to perceived mouth-feel and taste faults. AWRI 796 was mutagenised with 6% ethyl methane sulfonate for a duration that resulted in a survival rate of ~ 60%. Cultures of AWRI 796 that had either been mutagenised or not were plated onto YPD agar containing toxic analogues of phenylalanine (p-Fluoro-DL-phenylalanine [PFP] or o-Fluoro-DL-phenylalanine). Isolates able to grow on the toxic analogues were subsequently screened for alterations to their aroma profiles.

5.4.2. Rare mating for interspecies hybridisation

Saccharomyces cerevisiae wine strains previously shown to hybridise efficiently with other *Saccharomyces* species were used to evaluate the potential for new hybrids to be constructed with recently described species *S. eubayanus* (one of the parental species of lager yeast) and *S. arboricolus*. The methods for their creation are described in Bellon et al (2011). Winemaking potential of novel hybrids generated by rare-mating was investigated through laboratory-scale fermentation experiments.

5.5. Conduct of pilot-scale winemaking experiments

In parallel to small-scale laboratory fermentations, pilot-scale benchmarking trials were performed across three vintages, with commercial or newly generated yeast strains in each trial, to evaluate sensory impact on Shiraz, Cabernet Sauvignon and/or Grenache. Wines produced in year 1 of the project were evaluated over two years to determine the effects of storage on red wines with different yeast-generated flavour profiles.

6. Results/discussion:

6.1. Aroma profiling of representative wine yeast

6.1.1.Establishing baseline profiles in defined medium

A small-scale fermentation protocol was established and validated using three commercial yeast strains known for their divergent aromatic profiles (AWRI796, AWRI838, AWRI1688 – a monosporic isolate of wine strain Zymaflore VL3). These strains were used to ferment a defined medium representative of Chardonnay juice (formulation described in Schmidt et al. 2011) in 100 mL fermentation vessels fitted with non-return valves to minimise any impact from access to oxygen. An early issue was batch variation in reported concentrations of aroma compounds due to the technical approach employed for aroma compound quantification. Various strategies to address this were explored including C^{13} labelled internal standards and various data normalisation approaches. Geometric-mean normalisation (across three control strains) proved to be an effective method of standardising the data so that comparisons across multiple batches of fermentations could be made. This necessitated the inclusion of all three control strains in every fermentation batch, reducing the number of strains included in the study.

Aromatic profiling of 94 strains (listed in Appendix 5, Table 1) was undertaken. Volatile metabolite production profiles and VSC formation datasets were compiled for fermentations conducted in chemically defined grape juice (CDGJ) in year 1 of the project, and heat maps depicting the relative concentration of each analyte for all strains were constructed (see Figure 1 for example). Two-way clustering groups yeasts together based upon their overall volatile aroma compound profiles, and shows which analytes are correlated. Some examples are evident where genetically related strains are also grouped by their aroma compound profiles, for example AWRI838 and others from the 'PDM' clade described in Project AWR1302 – in this

case AWRI2260, AWRI1001, AWRI2910 (Qa23, Petaluma 1 and Elegance). Interestingly, interspecies hybrids that have AWRI838 as a parent also displayed a similar profile for key compounds, namely volatile fatty acids and their ethyl esters. Also evident was a cluster of strains that could release significantly higher concentrations of tropical volatile thiols (3MH, 3MHA, 4MMP), including AWRI1487, AWRI1537, AWRI2858 and AWRI2861 (L2056, Vin13, VL1 and X5 respectively).

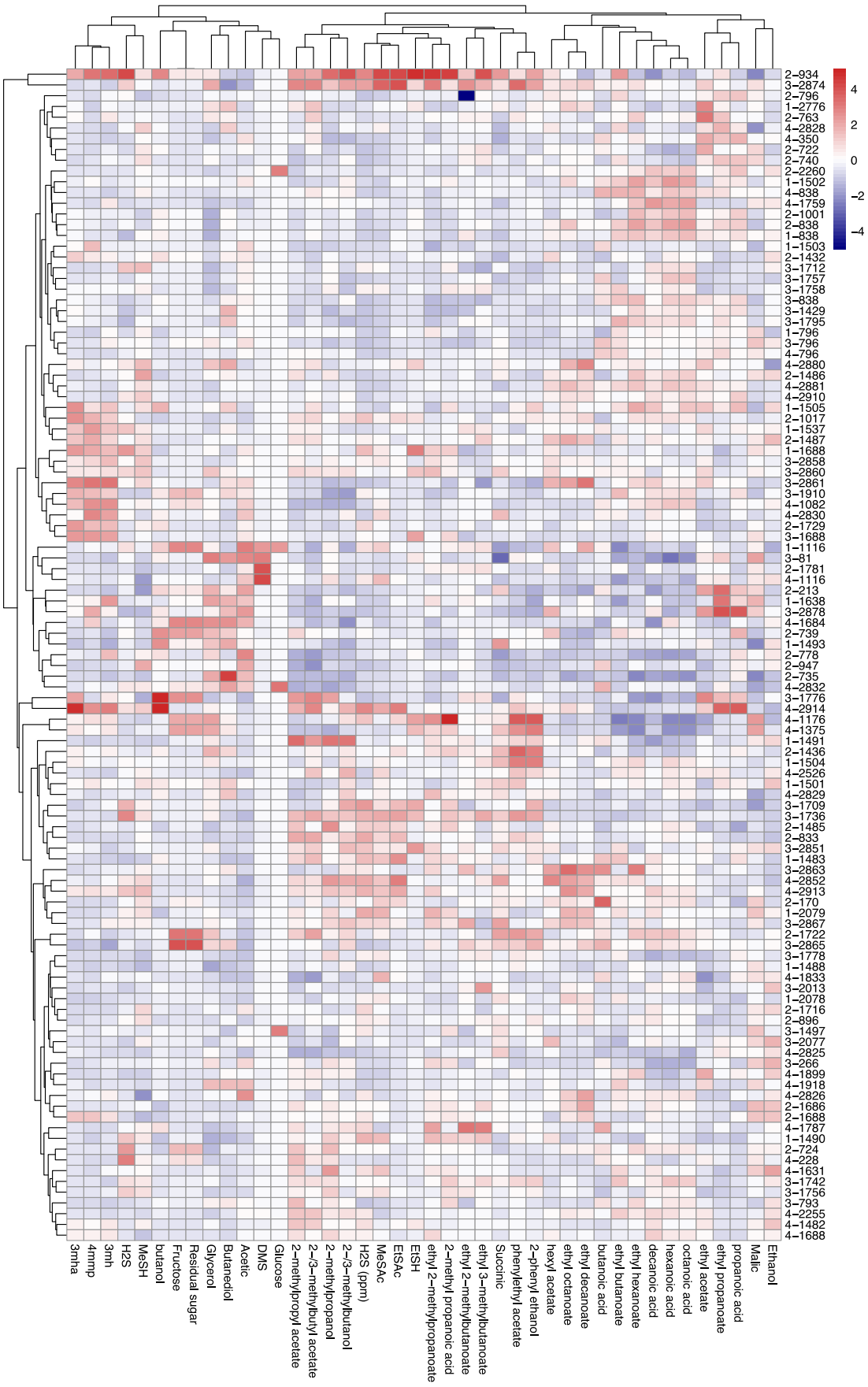


Figure 1. Clustered heat map showing how yeast-derived fermentation products and wine yeast strains vary in their aromatic profiles. Yeast strains are shown by their AWRI wine microorganism culture collection numbers and are preceded by a number indicating the batch in which the ferments occurred. Only chemical analytes with significant differences between strains within each batch of fermentations are included.

6.1.2.Evaluating variation in wine yeast aroma compound production during laboratory-scale fermentation of Shiraz grape juice

An independent set of experiments was conducted to evaluate flavour profile variation using a red juice as fermentation medium. Shiraz juice was prepared from freshly harvested grapes during the 2015 vintage. Initial chemical and microbial analyses were performed with the aim of evaluating juice sterilisation methods. In this regard DMDC (Velcorin) proved to be a useful stabilising agent for grape juice, allowing storage at least six months. Pilot fermentations were conducted with two strains and different rates of supplementation with a commercial grape phenolic extract, allowing final juice composition to be optimised prior to initiation of strain evaluations. Analyses of esters, alcohols and acids were completed but vinyl phenols analysis was not undertaken as fermentations were conducted in a red grape juice - these compounds form anthocyanin adducts and are reported at trace levels in red wine.

Of the 94 strains tested, 11 (~12%) did not complete fermentation (residual sugar > 4 g/L). Of these, two were commercial strains, two were environmental isolates, two were strains of *Saccharomyces uvarum* and three were winery isolates. A summary graph showing variation in volatile concentrations in wines fermented with the 94 yeasts is shown in Figure 2. Notable in the degree of variation between strains are some of the acetate esters such as 2-methyl butyl acetate, 2-methyl propyl acetate and hexyl acetate, for which 14, 20 and 31-fold variances were observed respectively between lowest producer and highest producer. Even greater variation was observed for acids and their ethyl esters, specifically butanoate and ethyl-2-methyl butanoate, which varied more than 60-fold. H_2S concentration in the finished wine ranged from undetectable to as much as 2 µg/L for some strains.

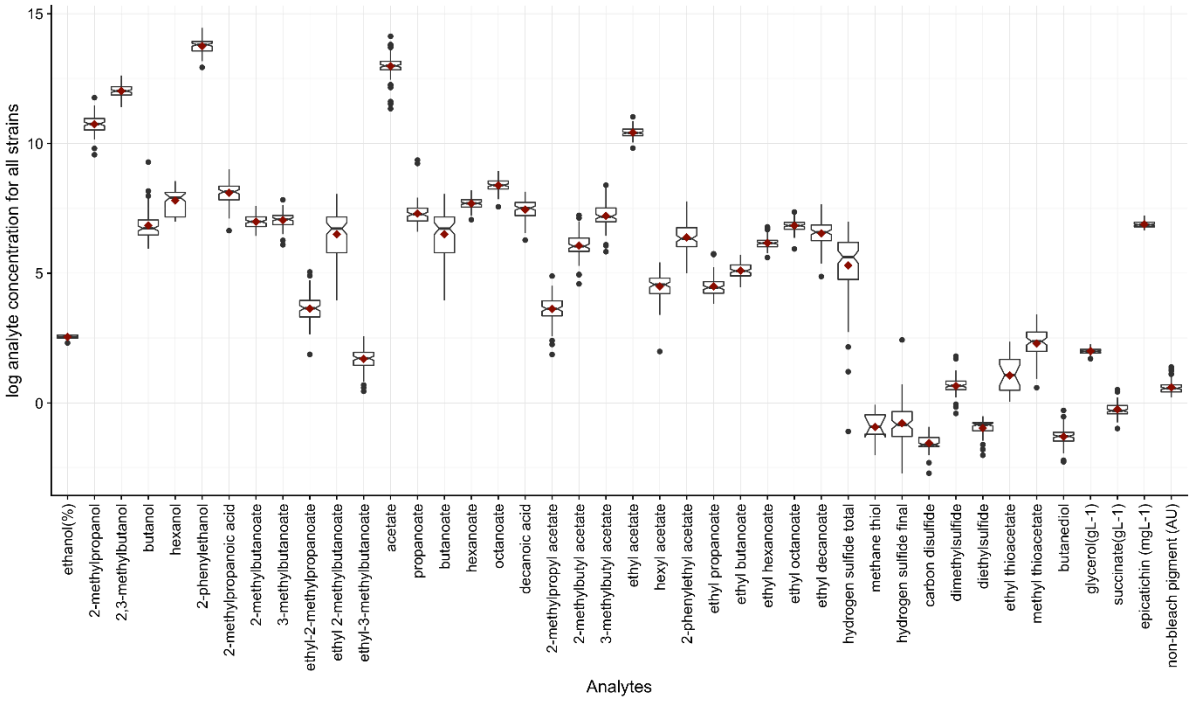


Figure 2. Boxplot showing variation in headspace volatile concentrations of wines fermented with 94 wine yeast. Box shows 25th and 75th percentiles with centre line showing the median. The whiskers show largest and smallest values with points plotting outliers. Red diamond shows the mean. Values are log (µg/L) except where noted.

6.1.3.Correlations of aroma profiles obtained in defined medium and grape juice

For metabolites measured in common for defined medium and grape juice fermentations, approximately 70% displayed statistically significant positive correlations (Figure 3). Some metabolites that were not correlated have previously been shown to be affected by juice precursor content, a key example being hexyl acetate (Dennis et al. 2012). It was also evident that several strains behaved quite differently in CDGJ and real juice.

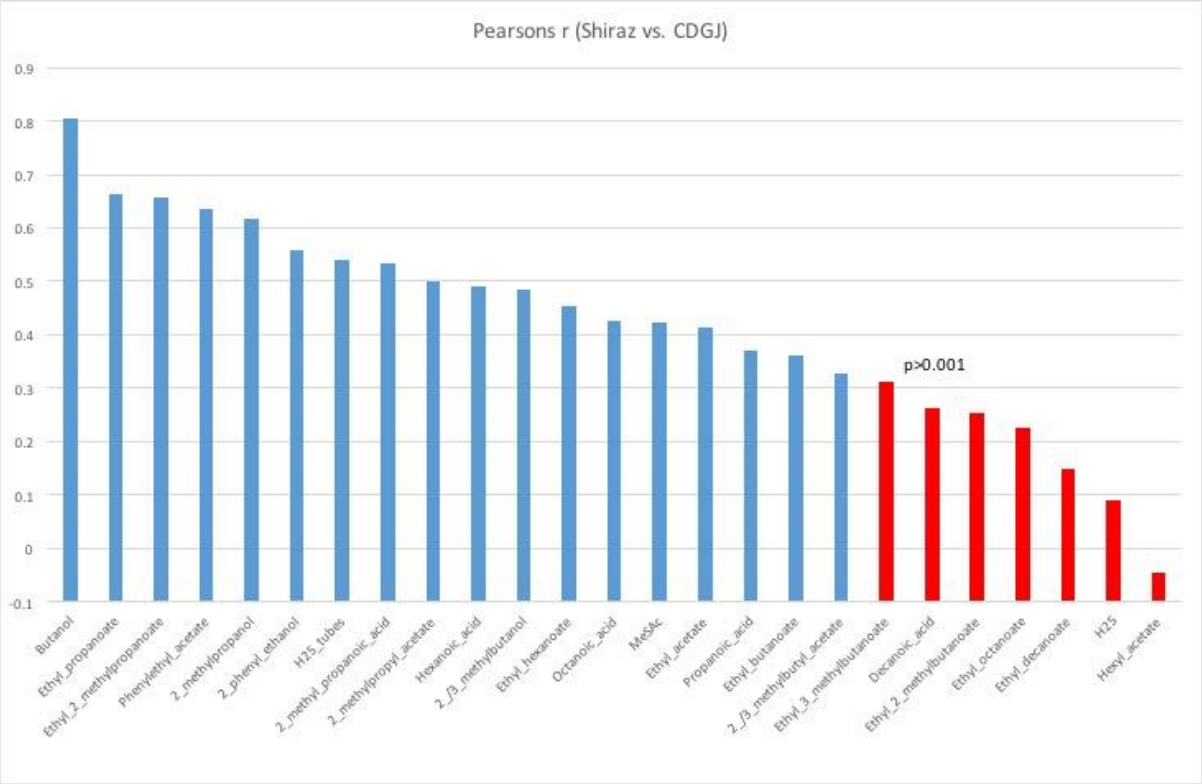


Figure 3. Pearson r correlation statistic for yeast-derived fermentation products measured following fermentation of defined medium and Shiraz juice.

6.1.4.Aromatic profiles of selected yeast determined at pilot scale

To better understand and contextualise the variation in wine observed in 100 mL laboratory-scale fermentations, pilot-scale experiments were performed over two vintages. In the 2014 vintage, a subset of strains was chosen that broadly represented the laboratory variation observed (RX60, F15, AWRI1503, AWRI 796, NT50, BDX, L2323, CLOS, EC1118 and UVAferm HPS). These strains were used to ferment 40 kg of Shiraz in triplicate.

All strains fermented to dryness, and produced wines with similar basic composition within commercially acceptable ranges. As observed at the laboratory scale, significant differences in tannin concentration were evident in wines sampled after alcoholic fermentation. Pre-filtration tannin concentrations varied by up to 30% depending on the strains used. Differences in total polysaccharide content were also seen, mainly due to differing levels of extraction of grape-derived polysaccharides. Strain L2323 is known for its pectinolytic activity (van Wyk and Divol 2010) and yielded the highest concentration of polysaccharides post-fermentation. Somewhat surprisingly, wines made with UVAferm HPS did not contain higher levels of yeast-derived polysaccharides.

Differences in the concentration of key aroma compounds from 1.5 to 2-fold were observed. Aroma compounds associated with 'dark berry' descriptors such as ethyl propanoate, ethyl 2-methyl propanoate and ethyl 2-methyl butanoate varied significantly, along with similar magnitudes of difference in 'red berry' compounds ethyl butanoate, ethyl hexanoate and ethyl octanoate. Wines made with BDX and 1503 contained higher concentrations of methanethiol, while those made with BDX also contained elevated concentrations of dimethylsulfide.

Sensory descriptive analysis of wines showed differences in 'dark fruit' and colour intensity as opposed to 'vegetal' and 'earthy', with 1503 and BDX, and to a lesser extent NT50 and UVAFERM, being low in 'dark fruit'/colour and higher in 'earthy' and 'vegetal'. BDX, 2323, RX60, EC118 and F15 were also lower in 'red fruit' flavour. Differences in astringency were not evident due to an apparent normalising effect of small-scale pad filtration. These observations are summarised in the Principal Component Analysis (PCA) presented in Figure 4.

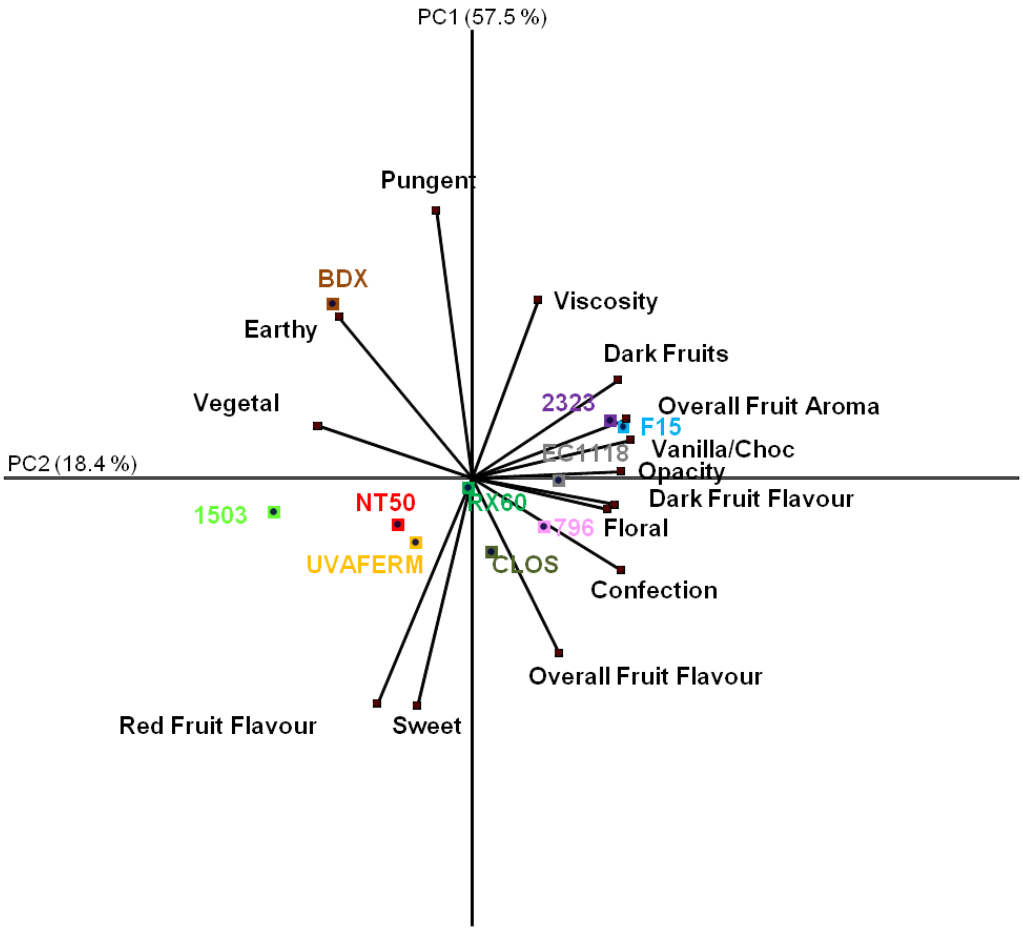


Figure 4. Scores and loadings bi-plot for PCA of attributes and mean values of treatments, showing PC1 and PC2

A second sensory assessment was performed on the 2014 vintage trial wines, providing a 24-month time-point to follow the initial ~9-month assessment. Wines retained some of the key aroma differences observed previously, predominantly separated by attributes such as 'floral', 'vegetal', 'dark fruit', 'herbal' and 'vanilla/chocolate', although the extent of aroma differences

between wines was less than observed at 9 months. Interestingly, the wines had developed significant differences between strains for mouth-feel attributes such as 'viscosity', 'sweet', 'salt', 'astringency', 'bitter' and 'hotness', not observed at the 9-month assessment.

Aromatic profiling of a broad range of yeast in defined medium (6.1.1) and red juice (6.1.2) identified cohorts of strains that produced wines with similar chemical profiles. Replicated pilot-scale winemaking trials were undertaken to determine whether the profiles observed in laboratory-scale screening work were representative of that which might be observed at a larger scale. Six strains were chosen that were representative of the cohorts previously identified. These strains were used to ferment 50 kg of McLaren Vale Grenache.

While these wines have not yet been bottled, post-fermentation analysis of the wines shows that all 6 wines display very different chemical profiles (both volatile and non-volatile) and the results are consistent with the trends observed in the red and synthetic grape juice (SGJ) small-scale ferments (Figure 5). For example, strains AWRI 778 and 1776 produced high and low amounts of acetic acid, respectively, in all three fermentation experiments. Strain AWRI 2260 produced high concentrations of different ethyl esters derived from medium chain fatty acids, while strain AWRI 2914 produced high concentrations of acetate esters and butanol. Finally, strain AWRI 1833 is characterised by a low flavour phenotype (low concentrations of 'fruity' esters). These results confirmed that model ferments using SGJ were good predictors of a strain's capacity to produce different chemical compounds important for the flavour and aroma of wine. Once sensory analysis of these wines has been performed it will, therefore, be possible to interpolate the sensory profiles of other strains used in laboratory-scale experiments based upon their volatile aroma compound profiles.

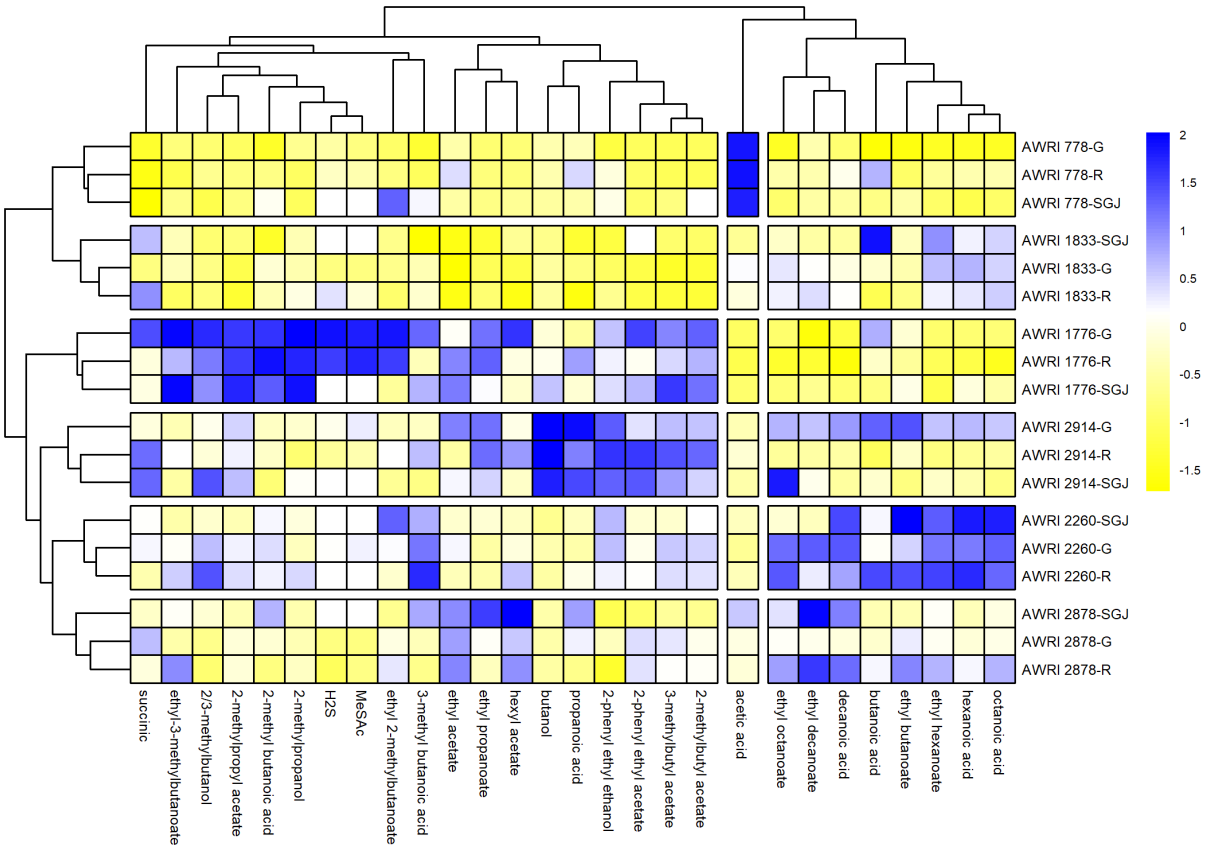


Figure 5. A comparison of aromatic profiles in wines made using six different yeasts used to ferment synthetic grape juice (SGJ), Shiraz juice without solids (R) or Grenache grapes (crushed and destemmed) in 100 mL (SGJ and R) or 50 kg (G) volumes.

6.2. Controlling the timing and strength of yeast flocculation

6.2.1.Measurement of flocculation behaviour

Cell to cell binding resulting in the formation of large clusters of cells that settle to the bottom of the fermentation vessel is known as flocculation. This behaviour involves a nonsexual, homotypic and reversible aggregation of yeast cells to form multicellular masses containing thousands of yeast cells. Yeast flocculation is highly complex in terms of phenotypes, adhesion mechanisms, signalling pathways, responsible genes and regulatory networks. In most biotechnological applications, yeast biomass is separated from the culture media after fermentation or production has finished. Specifically, in brewing and winemaking, yeasts are removed after fermentation by either filtration, flotation or racking, which add costs to the overall process. In theory, efficient yeast flocculation could make clarification and filtration easier and thus lower costs related to biomass separation benefitting these industries substantially.

At the initiation of this project a key challenge was to be able to measure the flocculation behaviour of many yeast strains. Two separate approaches were taken. In the first, a high-throughput assay based on monitoring kinetics of optical density change detected directly at the bottom of micro plate wells was developed to address this need. The assay involved monitoring the optical density of a culture in deep well plates by transferring a sample taken at a defined depth after a specific duration of sedimentation, to a fresh multi-well plate. The optical density of the new plate could then be assessed and combined with data from other samples to estimate sedimentation rates.

The second approach to measurement of flocculation behaviour involved using a barcoded wine yeast collection (produced as a component of Wine Australia project AWR1302) to compare the sedimentation rates of 87 strains in vertically extended fermentation tubes. Measurements for enrichment of strains in samples taken from the tops and the bottoms of the tubes over time was achieved through extraction of DNA and barcode sequencing as described by Robinson et al. (2014).

6.2.2.Profiling of yeast strain flocculation

Using the high throughput sedimentation assay described in 6.2.1, 99 yeast strains were screened, identifying several strains that flocculate heavily during fermentation in chemically defined wine, and a separate group that exhibit poor sedimentation behaviour. Additionally, employing a collection of barcoded wine yeast strains, the flocculation behaviour of 87 strains in response to several environmental conditions was determined. These environmental conditions included chemically defined grape juice (CDGJ) with decreased pH, increased sugar concentration and decreased fermentation temperature. Six of these strains were able to strongly sediment at the end of fermentation under the above-mentioned conditions and also in Chardonnay juice (Figure 6Figure 2). Two of these wine yeasts, AWRI1688 and AWRI1759, were selected for pilot-scale trials in vintage 2017 in order to evaluate filterability, lees density and sensory profile.

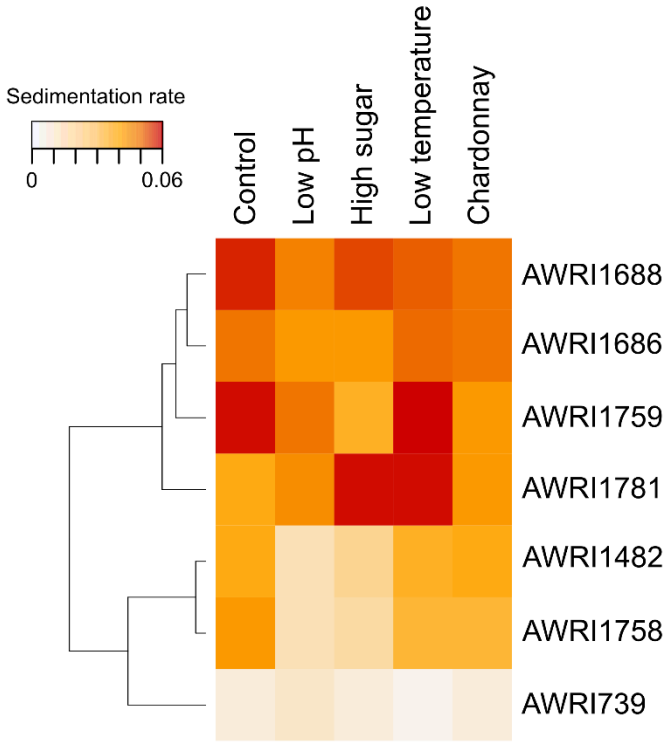


Figure 6. Flocculation behaviour of seven *S. cerevisiae* strains in different environmental conditions. Conditions include: CDGJ (control), CDGJ with lower pH, CDGJ with increased sugar concentration, CDGJ fermented at lower temperature, Chardonnay must. Colour indicates sedimentation rate calculated as ΔOD600/min.

6.2.3.Modification of flocculation behaviour through the application of adaptive evolution

As a proof-of-concept study, several gene modification approaches were evaluated to enable controlling flocculation onset. Successful strategies would enable induction of flocculation in wine yeast at any point during fermentation by modifying an external signal. The approach taken was to stimulate expression of a gene known to affect flocculation, *FLO1*. Ten promoters that had previously been shown to be responsive to environmental stimuli were evaluated but most were found to lack the tight control required of this system; that is, they stimulated flocculation in the absence of a stimulus. One was identified that had minimal background activity. The promoter of MF(alpha)1 linked to *FLO1* could stimulate flocculation on demand by addition of the commercially available yeast peptide hormone a-factor. This work demonstrated that the complexities of the flocculation process can be circumvented by a rational synthetic gene design.

A selection strategy capable of isolating non-flocculent variants of overly flocculent wine yeast was developed based on a variation of the screening strategy described in 6.2.1. This strategy made use of differential sedimentation rates in vertically extended fermentation tubes to capture genetically variant strains with altered flocculation traits. Fermentation in elongated tubes, initiated by inoculation with samples taken from the upper part of a previous fermentation (sequential batch fermentations), permitted the isolation of strains with modified flocculation behaviour.

The differential sedimentation strategy was employed to alter the flocculation behaviour of two highly flocculent strains, *S. cerevisiae* AWRI350 and *M. pulcherrima* AWRI1149. The strong flocculation characteristics that they exhibited negatively affected the kinetics of fermentation. Non-flocculent variants of both strains were obtained. That the strains ultimately derived from

the experiments were the same as those used to initiate the experiment was demonstrated using whole genome sequencing of parental and derivative strains. The non-flocculent isolates showed faster fermentation kinetics and similar production of primary metabolites, highlighting potential winemaking applications for these evolved isolates.

6.3. Interspecies yeast hybrids

6.3.1. Genomic structure and stability of interspecific hybrids

Mating in *Saccharomyces* species is typically between haploid cells of the opposite mating type. However, generation of interspecific hybrids between *cerevisiae* and non-*cerevisiae* species of *Saccharomyces* was performed using a diploid *cerevisiae* parent that had undergone a mating type switch at the mating type locus. These strains are homozygous (either a/a or α/α) at that locus allowing the diploid *cerevisiae* strain to mate with haploids of the opposite mating type. One consequence of this approach is that new hybrids will be initially polyploid, meaning that they will have a larger number of copies than the standard two homologous sets of chromosomes. This imbalance in gene copy number can potentially lead to genome instability to varying degrees with parts of chromosomes, whole chromosomes or large parts of one of the genomes being jettisoned because of incompatibilities that can occur during the mitotic and meiotic reproduction (Antunovics et al. 2005, Marsit et al. 2017). Large-scale genomic instability would have consequences for the stability of traits as they relate to winemaking and ultimately for the usefulness of the strain. It was therefore important to assess the genomic stability of strains generated as part of this work.

Hybrid stability was evaluated using PCR-RFLP with markers specific to the ends of each arm of all 16 chromosomes with specific restriction enzymes capable of distinguishing between chromosomes derived from *cerevisiae* and non-*cerevisiae* parents (Bellon et al. 2013). In total 150 end-of-ferment isolates from two independently generated *cerevisiae*/*mikatae* hybrid strains (CxM1 and CxM4) were evaluated in this way. In two out of 150 isolates of CxM1 and four out of 150 isolates of CxM4, chromosomal abnormalities were detected indicating loss or partial loss of chromosomes derived from *S. mikatae*. No isolate showed loss of DNA on more than one chromosome. Flow cytometry indicated no loss of ploidy for these hybrids.

A similar approach was used to assess the genomic stability of hybrids between *S. cerevisiae* and *S. bayanus*. In this work, hybrid stability was assessed after 200 mitotic generations to evaluate suitability of hybrids for commercial propagation. Again no loss of chromosomes was detected with PCR-PFLP and flow cytometry indicated a triploid genome complement (Bellon et al. 2015).

The genomic content of hybrids between *S. cariocanus* and *S. cerevisiae* strain AWRI947 (a *S. cerevisiae* wine yeast isolate outside the EC1118/PDM clade) was confirmed through molecular typing (ribosomal DNA, targeted PCR for all 16 yeast chromosomes) demonstrating the presence of the genome of both parent species. Flow cytometry analysis established that the hybrids had DNA content consistent with them being triploid. Targeted PCR analysis of both arms of all 16 yeast chromosomes (32 molecular markers) revealed some instability in the AWRI947 x *S. cariocanus* hybrids, whereby a small section on the left arm of chromosome 6 from the *S. cerevisiae* parent was lost in some individual clones during the initial hybridisation event. Subsequent analysis of their fermentation performance did not reveal changes to fermentation characteristics that could be associated with loss of that segment of chromosome 6.

In summary, interspecific hybrids generated between diploid *S. cerevisiae* and various non-*cerevisiae Saccharomyces* species are mitotically stable. Any genomic changes observed appear to occur during their initial generation. Poor performing hybrids are selected against in early rounds of screening. Hybrid strains that can grow and ferment efficiently have a low probability of phenotypic loss resulting from subsequent propagation.

6.3.2.Aroma profiling of interspecific hybrids

The aroma profiles of interspecific hybrids generated from *S. cariocanus*, *S. arboricola* and *S. eubayanus* species were evaluated in laboratory-scale Chardonnay juice fermentations. Differential production of flavour active compounds was observed to occur in hybrids between the different species. For instance, ethyl decanoate production showed an additive effect in *S. cerevisiae* x *S. eubayanus* hybrids, whereas a repressive effect was evident in *S. cerevisiae* x *S. arboricola* hybrids as these hybrid strains produced lower concentrations than either of the parent strains (less than half the concentration of the lower parent).

Industry trials in Tempranillo and Grenache (rosé) were completed using newly generated hybrids between wine yeast NT116 and several different *Saccharomyces* species: *S. kudriavzevii* (AWRI 2790); *S. cariocanus* (AWRI 2795); *S. arboricola* (AWRI 3054); *S. eubayanus* (AWRI 3058). Wines produced from these hybrids were showcased in a workshop at the 16th AWITC in July. While these trials were not conducted in a replicated format that permitted formal sensory descriptive analysis, informal commentary on the wines was sought from workshop participants. In Tempranillo wine, the *cerevisiae* parent was described as 'spicy' with a 'rich mid-palate'. To this background, the *cariocanus* hybrid was noted as being more complex with a softening of the palate and the *eubayanus* hybrid added 'confectionary' and 'chalky' attributes. These trials showed that hybrids of *S. arboricola* and *S. eubayanus* can perform in a winery context to an acceptable standard and that they generate wines with altered stylistic attributes.

6.3.3.Fermentation performance of interspecific hybrids in extremes of temperature

A small number of interspecific hybrid strains (*S. cerevisiae* x *S. kudriavzevii*, *S. cerevisiae* x *S. uvarum*, and *S. cerevisiae* x *S. eubayanus*) were shown to be able to grow robustly in laboratory assays on YPD agar plate at both high (37°C) and low (12°C) temperatures. In fact, together with ethanol tolerance, temperature-based selection is a key criterion enabling the selection of many of the hybrids generated in this and previous projects. The question was posed whether ability to grow at high (37°C) or low (12°C) temperature on plates was an indicator of fermentation capacity at those temperatures.

Despite being able to grow on plates at 37°C none of the hybrids could ferment Chardonnay juice to completion at that temperature. This was also the case for the *S. cerevisiae* parent. At 12°C some of the hybrids exhibited accelerated sugar consumption profiles compared to the *S. cerevisiae* parent. This was the case for both *kudriavzevii* and *eubayanus* hybrids although only data for the *kudriavzevii* hybrids are shown in (Figure 7). Not all hybrids exhibited the same fermentation capacity. Two of the three hybrids in the experiment shown in Figure 7 performed no better than the *S. cerevisiae* parental strain despite demonstrating robust growth on plates at than temperature.

These experiments demonstrated that hybrids provide a pathway for the development of enhanced fermentation attributes that can exceed the capability of performance driven *S. cerevisiae* yeasts in specific conditions. They also highlighted the need for condition-specific performance evaluations to ensure that sought-after traits are ultimately captured in the strains that make their way to production environments.

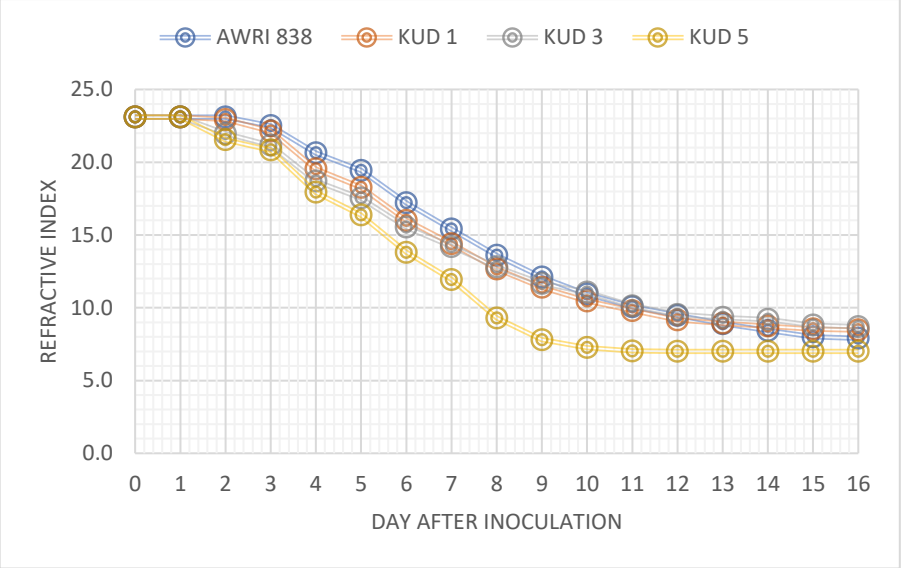


Figure 7- Fermentation kinetics of *S. cerevisiae* x *S. kudriavzevii* hybrid yeast at 12°C

6.3.4.Hybrids to reduce the volatile acidity of dessert wines

Sweet dessert wines are made from grapes with extremely high sugar content. When *S. cerevisiae* is in a high sugar environment it produces glycerol as a compatible solute. To balance the production of glycerol, acetic acid is also produced. The more glycerol that is produced, the more acetic acid is also produced. Previous work has shown that some strains of *S. bayanus* contribute less acetic acid to wines than *S. cerevisiae* (Castellari et al. 1994). However, *S. bayanus* generally lacks robust fermentation characteristics that would enable its direct industrial application. The combined traits of *S. bayanus* and *S. cerevisiae* suggested that progeny of a cross might have the potential to efficiently ferment high sugar juice while producing desirable sensory attributes.

Interspecific hybrids yeasts were generated (Bellon et al. 2011) and their ability to ferment high sugar juices was assessed. Trials were conducted in Chardonnay supplemented with glucose and fructose in equal amounts. At the highest sugar concentration assessed (355 g/L) the two hybrid strains produced wines with less residual sugar than either the *S. cerevisiae* or *S. bayanus* parent. Likewise, acetic acid concentrations in the finished wines were also lower (0.65 g/L), being almost half that of the *S. cerevisiae* parent and less than half that of the *S. bayanus* parent (Figure 8). Surprisingly, glycerol concentrations of the hybrids were higher than both parental strains (Bellon et al. 2015).

The experiments in chaptalised Chardonnay juice were replicated in botrytised Riesling juice with similar results. Acetic acid concentrations were again lower; however, glycerol concentrations were not different between any of the strains. Ethyl acetate concentration, the other key contributor to volatile acidity, was significantly less only for hybrid 1572. These hybrids provide an opportunity for winemakers to minimise acetic acid concentrations in wine styles that are traditionally fraught with volatile acidity issues.

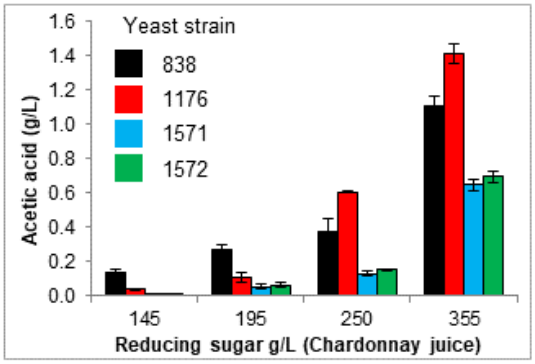


Figure 8. Acetic acid production of hybrids (1571 and 1572) in comparison to parental strains (838 and 1176) in juices with increasing sugar concentration.

6.4. Development of novel yeast strains using classical mutagenesis breeding

6.4.1. Minimising the production of succinic acid in high-succinic acid yeast

Succinic acid production during fermentation can lead to undesirable increases in total acidity (TA), and has the potential to increase perceived 'saltiness' of wine. The commercial strain Maurivin AWRI796 is known for its capacity to produce greater quantities of succinic acid, and this can be seen as a risk for some winemakers.

To isolate low-succinic-producing yeast derived from AWRI796, a classical mutagenesis technique was used based on the incubation of yeast cells with the chemical agent ethyl methanesulfonate. After mutagenesis, a large number of cells ($3x10^8$) were spread on glycerol solid plates containing the antifungal agent cerulenin, as selection pressure. Fifty-nine mutants that appeared after 5-6 days of incubation at 30°C were isolated.

Initial screening was performed in 96-well microplates (600 µL) in two synthetic grape juices and a Chardonnay juice. The fermentation performance of approximately 50% of the mutagenised strains was equivalent to the parent strain AWRI796. Of those, many produced succinic acid levels 20-40% lower than the parent. To confirm these results, laboratory-scale fermentations (200 mL) were performed in two Chardonnay juices with the selected strains (30 in total), and four of them displayed a consistent low succinic acid phenotype (on average 50% less than the parent) and similar fermentation performance to the parent.

One of these strains (AWRI 2955) was selected for a pilot-scale fermentation trial (50 kg) in Shiraz grapes (2016 vintage). In these conditions, strain AWRI 2955 produced 10% less succinic acid than the parent strain AWRI796 (2.34 vs 2.57 g/L). It should be noted that in this particular trial AWRI796 didn't display a high succinic phenotype in comparison to the other strains. Titratable acidity of the wine produced by AWRI2955 was also slightly less than the wine produced by the parent strain (5.5 vs 5.9 g/L, pH =7). These results show that AWRI2955 can be used to reduce succinic acid production relative to AWRI796, and under (yet to be elucidated) conditions where AWRI796 does overproduce this acid the reduction is expected to be more substantial.

6.4.2. Producing yeasts with enhanced floral attributes

While varietal 'floral' aromas in wine are typically the product of grape-derived terpenoid flavour compounds, products of yeast metabolism, 2-phenylethanol (2-PE) and 2-phenylethyl acetate (2-PEA), are considered important contributors to 'rose' aroma (Vilanova et al. 2013). The compounds can enhance the 'floral' sensory properties of wine and other fermented foods and beverages (Fukuda et al. 1990, Duenas-Sanchez et al. 2014). Generally, the concentrations of 2-PE and 2-PEA in finished wines are below their aroma perception thresholds (Vilanova et al. 2013), meaning their contribution to wine style is minimal.

To generate a novel yeast that imparts 'floral' aromas, a well-known and widely used wine yeast strain, AWRI796, was exposed to a chemical selection process previously applied to saké and baking yeast (Fukuda et al. 1990, Duenas-Sanchez et al. 2014). Chemically mutagenised and non-mutagenised populations of AWRI 796 were spread onto plates containing a toxic analogue of the amino acid phenylalanine. Only cells that carry mutations in key phenylalanine biosynthetic pathway genes (that enable the cell to make more of its own supply of this essential amino acid) can grow in the presence of this toxic analogue. A by-product of cells making large amounts of phenylalanine is that more is available for biosynthesis of the flavour-active compounds 2-PE and 2-PEA. All yeast derived using this and related protocols are not genetically-modified (GM) and are suitable for use by the Australian wine industry.

One-hundred and fifty-three AWRI796 mutants were screened for 'floral' aromas, and seventeen of those were used to conduct fermentations in the laboratory. The resultant wines were analysed for a suite of volatile aroma compounds. Ten mutants that produced significantly higher concentrations of 2-PE and 2-PEA had their whole genomes sequenced; importantly six of these were 'spontaneous mutants' that had been derived without use of the mutagenic agent, which means they carried a small number of changes (<10 nucleotides out of 12 million) in their genomes. This enabled identification of two genes, Tyr1 and Aro4, in the aromatic amino acid biosynthetic pathway that harboured possible causative mutations. Further evaluation of these genes and the mutations identified in them is discussed in 6.5.2.

6.4.3.Pilot-scale and industry trials of 2-phenylethanol overproducing strains

The 'rose' strain AWRI 2940 has been trialled in several pilot-scale ferments ranging from 20 L (whites and rosé) to 50 kg (red), for the last three vintages. Three different white wines (Chardonnay'15, Semillon'17 and Riesling'17), two rosé wines (Grenache'16 and Pinot Meunier'17), and three red wines (Tempranillo' 16, Shiraz'15 and Pinot Noir'17) have been produced. In all the trials, strain 2940 produced consistently higher levels of 2-PE and 2-PEA than the control strain AWRI796.

When compared to the control, the increase in the production of the 'rose' aroma compounds by strain AWRI 2940 was higher in the rosé wines, followed by the red wines, and lowest in the white wines. Formal sensory analysis of the Chardonnay'15 wines found that the wine made with the strain AWRI 2940 displayed pleasant and desirable 'floral' and 'rose' aromas (Figure 9). These wines contained 7- and 37-fold higher concentrations of 2-PE and 2-PEA respectively. This was not the case for the Shiraz'15 wine though, in this particular case, the high levels of the 'rose' aroma compounds seemed to mask the overall fruit ('red'/'dark') aromas of the wines.

An industry trial of two different 2-phenylethanol (2-PE) overproducing strains (AWRI 2940 and AWRI2965) was also recently completed in Riesling and Semillon. AWRI2965 wines had a slightly different aromatic profile to AWRI2940. Chemical analysis of the wines confirmed the 2-PE overproduction phenotype of both strains in white wines. In addition, an informal sensory of the wines confirmed the pleasant 'rose'/'floral' and 'honey' aromas imparted by strain AWRI2940 in both Riesling and Semillon, as observed previously for Chardonnay.

6.4.4.Additional traits of 2-phenylethanol overproducing strains

In addition to enhanced 'floral' characters, wines made with AWRI2940 were observed to be more bitter (Figure 9). Additional chemical analysis of the pilot-scale wines produced in the last three vintages showed that both strains AWRI2940 and AWRI2965 not only overproduce 2-PE but also a range of phenolic compounds derived from the metabolism of aromatic amino acids in yeast. Two of these compounds (tyrosol and tryptophol) are particularly interesting because they have been shown to contribute to the perception of bitterness. Informal assessment of different red and white wines indicated that the perceived bitterness of white wines produced with AWRI2940 is less than that of red wines.

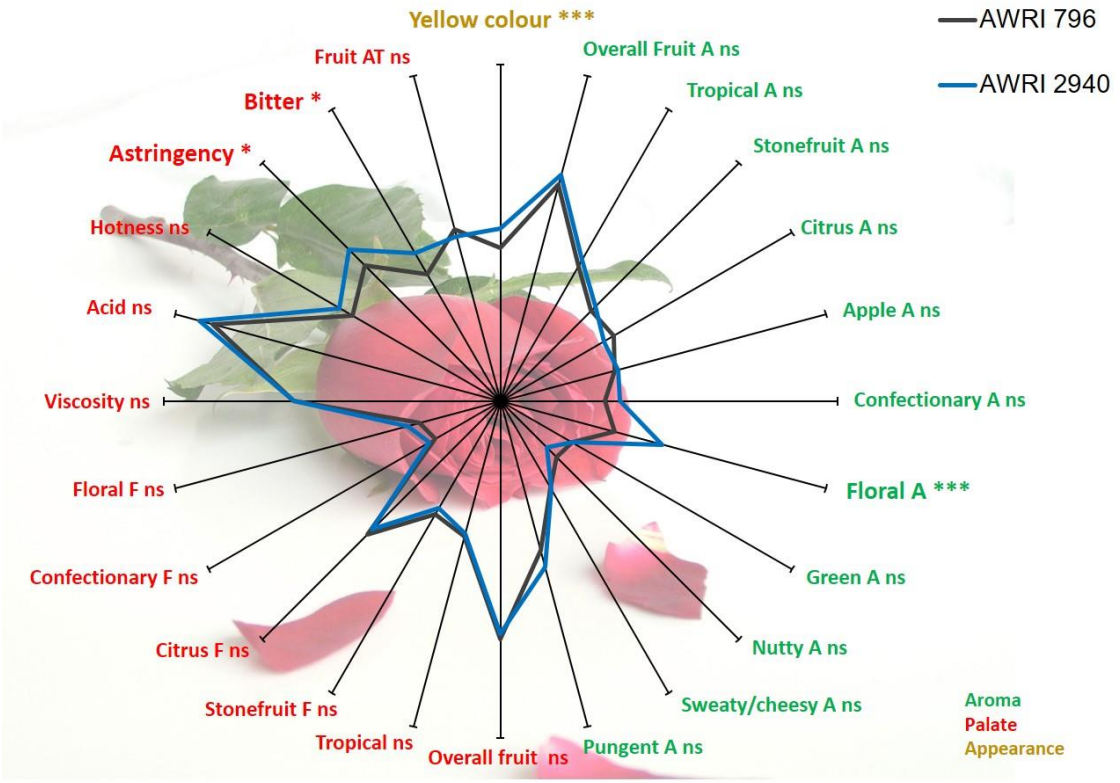


Figure 9. Mean ratings for aroma and flavour attributes for Chardonnay wines (20 L scale) produced using AWRI 796 and the 'rose' strain AWRI 2940 (n = three fermentation replicates × 10 judges). Aroma and flavour attributes are shown in green and red, respectively. ns, not significant; *p < 0.05; ***p < 0.001.

6.5. Genetic determinants of flavour profiles

6.5.1. Discovering potential genetic determinants of aroma compound production by genome wide association studies and quantitative trait locus analysis

Several candidate strains for QTL analysis exhibiting differences in VSC production, 2PE/2PEA production, and production of volatile fatty acids were tested for sporulation rate and spore viability. Due to the tendency of *S. cerevisiae* wine strains to switch mating types after sporulation and re-diploidise (mother-daughter mating), derivatives of candidate strains that have had this capacity disabled (by genetic modification) have been used. Some candidates displayed poor sporulation frequency and/or viability, or their spores were difficult to separate. The latter was resolved by troubleshooting the spore germination and separation protocol. Twelve strains with promising sporulation characteristics were re-sporulated and their stable haploid derivatives retained for further characterisation. Two batches of haploid derivatives (51 progeny from seven candidate strains for QTL) were evaluated in fermentation experiments in chemically defined grape juice, and individuals with appropriate production of volatiles (as observed for original diploid parent) selected.

Among the different volatile compounds analysed in the small-scale ferments, the production of 2-PE and 2-PEA displayed a huge variability between strains (30-fold difference). Therefore, a genome wide association study (GWAS) approach was considered appropriate to find the genetic determinant(s) involved in the formation of these 'rose' aroma compounds. For the GWAS study, a total of 20 wine strains were selected, half of them with a high 2-PE production phenotype, and the other half with the opposite phenotype (low 2-PE producers).

Three ‘gene’ candidates with high scores were identified on the basis that different versions of these genes are conserved between the high- and the low- 2-PE producers. In other words, for each of these three genes, one version is conserved amongst the high 2-PE producers, while a different version is conserved amongst the low 2-PE producers. Interestingly, only one of the three genes appeared to be associated with the pathway that leads to the formation of these aromatic compounds.

6.5.2.Demonstrating genetic determinants of phenylethanol production using CRISPR

Mutations in two genes, Tyr1 and Aro4, were identified following whole genome sequencing of PE overproducing isolates. A new technique for genomic editing (CRISPR-CAS9) was implemented to enable alteration of both gene copies in diploid industrial wine yeast strains to identify causative genetic factors in PE overproduction. Using this technique, a total of six allele swaps in the Tyr1 and Aro4 genes were successfully completed in two different yeast strain backgrounds (AWRI 1631 and AWRI 796). Both Tyr1 and Aro4 are involved in yeast amino acid metabolism, and previous findings indicated that mutations in these genes might be responsible for the enhanced ‘rose’ aroma (2-PE overproduction) of the non-GM wine yeast strains generated in this output. These allele swaps allowed evaluation of the relative contribution of different gene variants to the high 2-phenylethanol trait.

2-phenylethanol production by strains carrying mutations in either Tyr1 or Aro4 was assessed in small-scale fermentations. Chemical analysis of the wines produced confirmed that mutations in both genes contribute to the overproduction of 2-PE and 2-PEA. However, mutations in Tyr1 had a bigger impact than those in Aro4 with regards to the overproduction of both 2-PE and 2-PEA by yeast.

7. Outcome/conclusion:

7.1. Performance against planned outputs

7.1.1.Understand the potential value that can be derived from yeast genetic diversity to shape wine flavour profile and mouth-feel.

The approach of combining integrated laboratory and pilot-scale winemaking guided by genomic sequence data (project AWR1302) achieved the planned output.

Work performed towards this output has provided comprehensive volatile and non-volatile chemical datasets for laboratory-scale wines made with a significant cross-section of commercially available yeast strains. These datasets can be used to inform winemakers’ choice of yeast, and also as a reference against which to evaluate the potential novelty of newly developed strains.

Pilot-scale winemaking trials highlighted the extent of yeast impact on red wine aroma and mouth-feel, with some differences evident up to 24-months post-bottling. Reference strains shown at laboratory scale to impart divergent aroma compound profiles were incorporated into a vintage trial in 2017. Pending results of sensory analysis for these wines, it is anticipated that the data can be used to assign sensory profiles to a large number of strains currently used by the Australian wine industry.

7.1.2.*Understand the potential for more efficient processing of wine post-alcoholic fermentation through controlled timing and strength of yeast flocculation.*

This output was achieved through complementary high-throughput screening approaches to determine how many strains amongst the 94 studied in this project flocculate, and more importantly, how many flocculate late in fermentation in response to known wine-relevant parameters (low pH, high ethanol). The potential for some strains to flocculate strongly in response to a shift in temperature was also investigated.

7.1.3. *Develop knowledge of relative contributions of divergent genomes in interspecies hybrids to wine flavour profiles and winemaking outcomes, to support effective breeding and hybridisation methodology.*

Several new hybrids were generated in this project with the goal of building a collection where wine yeast parents are combined with each non-*cerevisiae Saccharomyces* parent (*S. arboricola, S. eubayanus, S. uvarum, S. kudriavzevii, S. paradoxus*). An additional focus was to incorporate strains that were unencumbered with third-party intellectual property rights.

While the AWRI was the first laboratory in the world to successfully generate hybrids between 'PDM'-clade wine yeasts and *S. arboricola* and *S. eubayanus*, it proved a significant challenge to develop hybrids with other *S. cerevisiae* parents. This slowed progress on planned investigations comparing relative contributions of *cerevisiae* and non-*cerevisiae* genomes across the intended multi-dimensional collection. Nevertheless, various hybrids were generated and their properties investigated through laboratory-scale, pilot-scale and industry fermentation trials.

7.1.4. Use genomics assisted breeding and interspecies hybridisation to develop novel yeast strains targeting traits identified as important for Australian winemakers by Wine Australia and through industry consultation, with initial priority targets of enhanced red wine flavour, production of 'rose' aromas, avoidance of faults (H_2S, VA, succinic acid), optimal flocculation behaviour, and stability in performance. Yeast strains identified in AWRI Project 3.2.3 as having desirable traits will be incorporated in strain development.

Plans to draw upon validated genetic markers by cross-breeding of IP-unencumbered strains were not fully realised due to deficiencies in mating fitness for many of the studied strains. Several potential markers were identified for production of H_2S and 'rose' aroma compounds, and potential breeding strains exhibiting high production levels for volatile compounds associated with 'red' and 'dark' fruit aromas in red wine were characterised.

A strength of this project was the implementation of multiple strain development approaches in parallel. Thus, while marker-guided breeding was not implemented, classical mutagenesis, breeding, adaptive evolution, and interspecies hybridisation were all effectively applied to yield novel strains.

Adaptive evolution was used to generate non-flocculent variants of a known heavy-flocculating wine strain, AWRI350, and a promising low-alcohol non-*Saccharomyces* strain *Metschnikowia pulcherrima* AWRI1149.

Interspecies hybrids generated in this project show promise in applications for low-temperature and high-sugar fermentations, along with sparkling wine production.

Classical mutagenesis and selection were successfully applied to isolate variants of popular wine yeast AWRI796 that either reduced production of succinic acid or increased production of volatile aroma compounds responsible for 'rose' aroma in wine.

7.2. Could changing the methodology/technology have improved the outcome?

Methodologies were adapted during the project to ensure robustness in large-scale screening of flavour compound production profiles, casting into doubt some recently published studies where insufficient replication and cross-batch validation were performed to enable comparison of large numbers of yeast strains.

Implementation of quantitative-trait-loci (QTL) methodology for optimisation of wine yeast traits was hampered by the relatively poor sporulation efficiency and spore viability of many industrial strains. This component of the project may have been more successful if all sequenced strains had been pre-screened for mating proficiency prior to choosing those to

use in fermentation screenings. To progress genetic marker validation, in parallel CRISPR-Cas9 based genome editing was implemented in years 3 and 4 of the project.

Similarly, generation of novel interspecies hybrids using different *S. cerevisiae* wine yeast parent strains proved challenging. This was partly due to apparent incompatibilities between some strains when used in rare-mating, in combination with incomplete data on parent strain traits that could be leveraged to select for hybrids. Choice of *S. cerevisiae* parental strains was guided by genetic differences (largely within or outside of 'PDM'-clade) and observed differences in flavour compound production. Screening of all strains for an expanded range of traits, including resistance to various inhibitors not relevant to winemaking, may have allowed design of more efficient hybrid-selection strategies.

7.3. Practical implications

While the impact of yeast strain on white wine style has been well established, in red varieties it has been assumed that choice of yeast strain is less important due to the time these wines spend in maturation prior to release. Results generated in this project demonstrated that young red wines made with different yeast strains differed in their aroma profiles 12 months post-fermentation and that some of these differences were evident 27-months post-fermentation. Furthermore, texture-related sensory descriptors became significantly different between yeast strains as the wines matured.

7.4. Benefits to the industry

Drawing upon datasets generated in this project and AWR1302, winemakers can make a more informed choice of yeast strain to match their winemaking goals. The observed redundancy in strain impact, where multiple strains displayed similar profiles, means that this choice can be made across yeast suppliers. This may enhance the ability of wine producers to negotiate with yeast suppliers and could thereby reduce costs of production.

Novel strains that are commercialised can be rapidly adopted by Australian winemakers, with the knowledge that support is readily available at the AWRI. In this project commercialisable strains were generated that can be used to shape wine style in unique ways, along with improved versions of existing strains (e.g. low-succinic AWRI796, non-flocculant AWRI350). Techniques developed in this project were also used to improve a low-alcohol non-*Saccharomyces* yeast strain, AWRI1149, by correcting its tendency to flocculate when inoculated into red grape must. Use of strains that impart unique flavour profiles (e.g. 'rose' aroma) can be used by winemakers to establish a point of difference in style, enhancing their ability to market their wine at a premium. The low succinic acid producing variant of AWRI796 can be used to reduce the risk of quality loss in some vintages due to acid-imbalance, thereby avoiding quality loss and downgrading.

Finally, the foundations have been laid for genetic marker guided breeding, which will enable the wine industry to rapidly draw upon available yeast germplasm to generate new strains for new purposes as they are defined.

7.5. Recommendations

The data generated in this project, and project AWR1302, provide an unprecedented foundation that can be further expanded. When suppliers introduce new strains into the Australian market, or new strains are developed in Wine Australia-funded projects, they can be benchmarked by sequencing their genomes and performing laboratory-scale fermentations alongside reference strains.

Various QTL analyses can be performed with a relatively small number of pairwise crosses, and now that CRISPR-Cas9 tools are available, downstream validation is achievable. Strains unencumbered by third party intellectual property constraints have been identified and will form the foundation of future strain development efforts. These strains, together with those

that will be identified in bioprospecting projects that are already underway, will provide the next generation of wine yeasts featuring much broader genetic and phenotypic diversity compared to wine yeasts available today.

In addition to harnessing untapped sources of genetic diversity, mutagenesis and selection have demonstrated their capacity to introduce phenotypic diversity into existing strains. This should be an ongoing approach to diversifying wine yeast phenotypes in the absence of acceptance of GM technology. The extent to which the Ehrlich pathway can be manipulated to alter or enhance wine yeast flavour profiles has not yet been fully explored and opportunities remain to produce yeast with aromatic attributes not present in the market today.

8. Appendix 1: Communication:

8.1. Communication of the outcomes

Outcomes and knowledge generated during the progress of this project have been communicated to peers through peer-reviewed publications and conference presentations. In addition, annual meetings were held with researchers at the University of Adelaide and representatives of Wine Australia to discuss updates on project progress outside of the regular six-monthly reports that were provided for the duration of the project.

Information has been extended to industry stakeholders through articles in trade journals, presentations in the AWRI roadshow program, webinars, and workshops at both the 15th and 16th Australian Wine Industry Technical Conferences.

8.2. Journal articles written during project

Solomon, M., Francis, L., Pearson, W., Barker, A., Kassara, S., Bindon, K., Smith, P. 2015. The influence of yeast strain on Shiraz wine composition and sensory properties. *AWRI Technical Review* (216): 11-14.

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9. Appendix 2: Intellectual Property

Various new yeast strains were generated during the course of this project, and those that have been used in pilot- and industry-scale winemaking trials are sufficiently characterised for evaluation of commercial application. A list of developed yeast strains is presented in the table below.

Category	Characterised strains	Parental strains
'Rose' aroma variants	AWRI2940, AWRI2965	AWRI796[1]
Low succinic acid producers	AWRI2955-2963	AWRI796[1]
S. eubayanus hybrids	AWRI3058	AWRI1540 (NT116)[2] AWRI2759 (*S. eubayanus*)[3]
S. arboricola hybrids	AWRI3051	AWRI1540 (NT116)[2] AWRI2280 (*S. arboricola*)[3]
S. cariocanus hybrids	AWRI2794	AWRI947 AWRI1528 (*S. cariocanus*)[3]
Reduced flocculation strains	AWRI3050 AWRI3878	AWRI1149[3] AWRI350[4]

[1]AWRI796 is a commercial strain licensed to AB Mauri from AWRI

[2]NT116 is a commercial strain developed by Infruitec-Nietvoorbij (South Africa) and licensed by Anchor Yeast

[3]These strains are publicly available from the CBS-KNAW (Netherlands) yeast collection and were obtained under their standard MTA

[4]AWRI350 is a commercial strain licensed to AB Mauri from AWRI

10. Appendix 3: References

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11. Appendix 4: Staff

Chris Curtin, Toni Cordente, Cristian Varela, Jennifer Bellon, Caroline Bartel, Markus Herderich, Paul Chambers, Simon Schmidt

12. Appendix 5: Supplementary Data

Table 1. AWRI numbers of yeast strains screened

	Batch 1	**Batch 2**	**Batch 3**	**Batch 4**
1	**796**	170	81	228
2	**838**	213	266	350
3	1116	722	793	**796**
4	1483	724	**796**	**838**
5	1488	735	**838**	1082
6	1490	739	1429	**1116**
7	1491	740	1497	1176
8	1493	763	**1688**	1375
9	1501	778	1709	1482
10	1502	**796**	1712	1631
11	1503	833	1736	1684
12	1504	**838**	1742	**1688**
13	1505	896	1756	1759
14	1537	934	1757	1833
15	1638	947	1758	1899
16	**1688**	1001	1776	1918
17	2078	1017	1778	2255
18	2079	1432	1795	2526
19	2776	1436	1910	2825
20		1485	2013	2826
21		1486	2077	2828
22		1487	2851	2829
23		1686	2858	2830
24		**1688**	2860	1787
25		1716	2861	2832
26		1722	2863	2852
27		1729	2865	2880
28		1781	2867	2881
29		2260	2874	2910
30			2878	2913
31				2914

13. Appendix 6: Budget reconciliation

The project's budget reconciliation statement will be submitted separately.