Grape juice and wine yeast: happy marriages and how to avoid getting stuck with the wrong partner


Managing director Sakkie Pretorius.

By Simon A. Schmidt, Simon Dillon*, Radka Kolouchova, Paul A. Henschke, Anthony R. Borneman, Angus Forgan, Paul J. Chambers and Isak S. Pretorius
The Australian Wine Research Institute, PO Box 197, Glen Osmond , Adelaide, SA 5064, Australia
* The Yalumba Wine Company, PO Box 10, Angaston, SA 5353, Australia

There is a plethora of wine yeast strains available for winemakers to choose from. Some are more robust and reliable than others, and different strains will impart different sensory properties to wine; the choice is not always straightforward. Research at the AWRI is beginning to unravel the complexity of grape juice composition and how this impacts on wine yeast performance. In parallel with this, we are also unravelling the complexity of wine yeast genetic diversity. The convergence of these complementary approaches will pave the way for the generation of optimised wine yeast strains and provide a means for more informed choices to be made when winemakers are partnering a grape juice with a wine yeast.

INTRODUCTION

Partnering grape juice with a wine yeast can produce a match made in heaven or a disaster waiting to happen. For a perfect marriage, the juice will be a generous and nurturing partner, catering for all of a yeast cell's needs, with the yeast reciprocating by helping the grape juice reach its full potential. In such relationships, fermentations go without a hitch and produce top quality wines.

At the other end of the spectrum, when a juice is harsh and unforgiving, providing a hostile home, the yeast is required to be more robust and adaptable; many wine yeast strains fit this bill. However, some strains may not be able to cope with difficulties in the relationship, becoming stuck and requiring the intervention of a therapist (the winemaker).

Most vinification relationships probably fall between these extremes, requiring at least some attention from their therapist, without being excessively needy or becoming stuck. Scientists at the AWRI are beginning to explore the complexity of grape juices and wine yeasts and what makes a good match to produce wines reliably and efficiently for an ever more discerning and diverse marketplace.

The approach used for this work has been systems-based: instead of identifying individual components (e.g., a specific gene or metabolite) that impact on one characteristic of grape juice, yeast or the wine they produce, our experiments take a helicopter view. For example, in our yeast work we characterise the entire genome (all of the genes in an organism) for the yeast strains we are interested in. This comparative genomics strategy enables us to identify, at the level of DNA sequences, all of the differences among wine yeast strains. The next stage of this work will be to align these differences to determine which are important for desirable and undesirable traits in yeast. This knowledge will enable the development of more informed strategies for the generation of optimal wine yeast strains and it will provide a means of rapid screening of existing culture collections and natural isolates.

Similarly, with grape juice, we have taken a wholistic (so-called, omics) approach where we identified in juices as many elements as possible, rather than targeting one or two that we believe may be important. By then comparing the compositions of different grape juices and aligning these data with fermentation performance, we have identified features of grape juice that determine whether it will make a good partner for wine yeast; a dating service, if you like, with a high probability of success.

Given the potential scope of this research, a clear and focussed starting point was critical. Therefore, we chose a single grape variety, Chardonnay, for the juice component of the work. This variety was chosen because of its prominence in the Australian winemaking landscape.

Our aim was to identify the variation in Chardonnay composition as a whole and to understand better how, and to what degree, variations in composition affect fermentation outcomes. A survey of Chardonnay juice elemental composition was performed during the 2009 vintage. The influence of variation in juice composition on

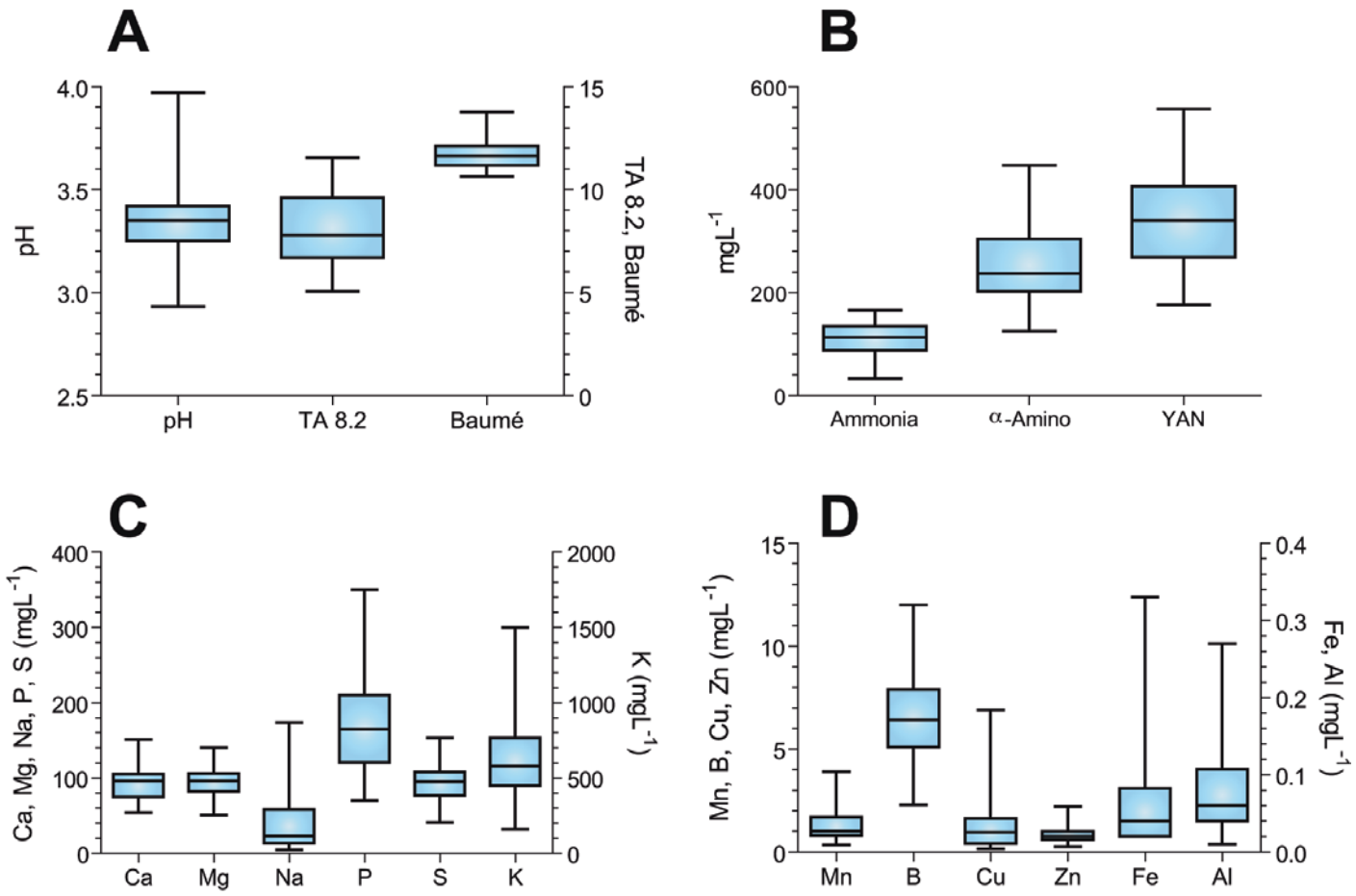

Figure 1. Compositional profile of Chardonnay juices surveyed during the 2009 vintage. Box and whisker plots showing the median values of pH, TA, Baume (A), nitrogen composition (B), macro-elemental composition (C) and trace elements composition (D) with whiskers showing the total range of concentrations observed for each analyte. Boxes show the upper and lower interquartile range in which 50% of all surveyed juices are found.

fermentation outcomes using a variety of yeast strains was explored using a defined medium, the recipe for which was based on Chardonnay composition. Observations made in defined medium experiments were substantiated using Chardonnay juices saved during the survey component of this work.

Our exploration of Chardonnay juice has identified juice pH and potassium concentration as critically impacting on fermentation performance and wine quality, and this was in a yeast strain-dependent manner. Much of the work discussed in this report is the subject of a recent publication (AWRI publication #1290).

HOW DOES CHARDONNAY GRAPE JUICE COMPOSITION VARY?

To answer this question, samples of 100 Chardonnay juices were collected. These juices were produced at The Yalumba Wine Company winery (Barossa Valley, SA) from grapes sourced from seven distinct Australian wine regions: Adelaide Hills (SA), Barossa Valley (SA), Eden Valley (SA), King Valley (Vic), Riverland (SA), Tamar Valley (Tas) and Wrattonbully (SA). These areas encompass a range of climatic and geological types - from the cool dolomite soils of the Tamar Valley, very warm red clay loam of the Barossa Valley to terra rossa soils of Wrattonbully.

Overall, the 2009 vintage was a dry one with below average rainfall and average temperatures. However, the vintage was punctuated by a two-week heatwave toward the end of January and into early February, in which daily maxima were above 35°C with consistently high night-time temperatures. All of the above regions were affected. The juices were sampled between January and April immediately prior to the commencement of fermentation. The ranges of major and minor juice component variation are shown in Figure 1.

The yeast assimilable nitrogen (YAN) levels for all juices exceeded the generally accepted risk threshold for

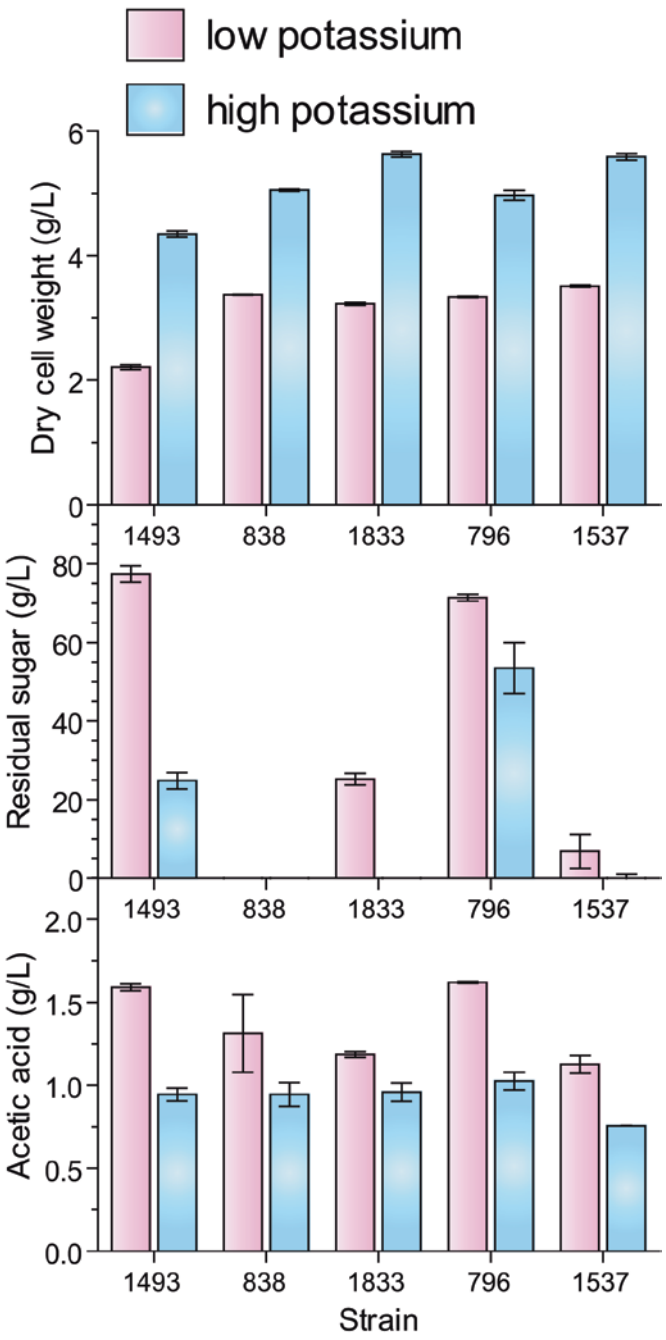

Figure 2. Screening wine yeast strains for performance and wine quality indicators. Five wine yeast strains were compared in defined medium (pH3.0) containing low and high potassium concentrations. Potassium concentration at low pH impacts clearly on fermentation outcomes. The residual sugar concentrations indicate that AWRI 1493 and AWRI 796 are particularly sensitive to 'low potassium' at low pH. Bars in graphs show average values of three fermentations. Error bars represent standard deviations from the mean.

suboptimal fermentation performance (AWRI publication #900) although some juices were at the lower end of what would normally be considered desirable. In general, elemental concentrations were also greater than previously reported as necessary for robust yeast growth. The juices sampled were destined for either still or sparkling wine, which may explain the wide range of both titratable acidity (TA) and pH observed here. It has long been understood that management of these parameters is key to obtaining a balanced wine style and ensuring wine stability in the bottle (Amerine and Joslyn 1970). Copper concentrations were relatively high but were not inhibitory to fermentation, even at the highest concentrations observed. Similarly, sodium concentrations were below levels known to be inhibitory.

HOW DOES ELEMENTAL COMPOSITION INFLUENCE YEAST FERMENTATION PERFORMANCE?

It has been suggested previously that an imbalance of magnesium with other cations, in particular calcium and zinc, is a potential cause of sluggish or stuck fermentations (Walker *et al.* 1996); management of zinc is critical in brewing. In addition, while it is known that limitation of some elements can impact on yeast growth and fermentation performance, mineral nutrient limitation has not been demonstrated in grape juice or grape juice-like media. Therefore, until now, we didn't know whether some mineral nutrients are potentially limiting in grape juice.

The effect of variations in juice elemental composition on yeast fermentation performance was evaluated systematically. This was done by spiking defined medium, prior to inoculation, with individual elements, such that their final concentrations matched maximum observed concentrations in juice while keeping all other elements at their minimum. This was then compared with defined medium in which all elements were at their minimum concentrations. We did not aim to vary either nitrogen or sugar profiles because the impact of these on fermentation performance is already well understood. However, the basal defined medium used in this work had a high initial sugar concentration (250g/L) and low initial YAN content (170mg/L) to match extreme values observed in the survey.

Of all the different elemental conditions evaluated, only low juice pH in combination with low juice potassium impacted negatively on fermentation performance (Figure 2), leading to reduced yeast growth, high residual sugar, higher volatile acidity and longer fermentation times than when the pH and potassium levels were in the normal range.

There is a well-known correlation between pH and potassium levels in grape juice (Boulton 1980, Rühl *et al.* 1988, Somers 1977). Potassium precipitates tartaric acid (producing bitartrate) thereby increasing pH; i.e., the more potassium present in grape juice the lower the tartrate and the higher the pH (Amerine and Joslyn 1970, Somers 1975).

It is interesting to note, in the context of potassium concentration in grape juice, that grafted Chardonnay vines tend to produce juice with lower potassium concentrations than vines grown on their own roots and this is contrary to most other winegrape cultivars (Rühl *et al.* 1988, Walker *et al.* 1998). In addition, lower potassium and pH profiles are usually associated with free run juice whereas increased pomace or skin contact (Test *et al.* 1986) or increased canopy density during ripening (Rojas-Lara and Morrison 1989) tend to generate juices with higher potassium and pH.

We did not observe any beneficial effects on fermentation performance from increasing calcium, zinc or magnesium concentrations to their maximum observed values, at least in defined medium. In fact, the addition of calcium exacerbated poor performance when potassium concentrations and pH were low. Given that both magnesium and calcium are in excess relative to yeast minimum requirements (Anraku *et al.* 1991, Dombek and Ingram 1986, Jones *et al.* 1981) the potential for calcium-mediated inhibition of magnesium uptake is extremely limited. Taken together, these observations suggest that imbalances of calcium and magnesium (or other divalent metal ions) are unlikely to play a role in fermentation outcomes in Australian Chardonnay production.

DO YEAST STRAINS DIFFER IN THEIR RESPONSE TO GRAPE JUICE COMPOSITION?

Our experiments show at least three different yeast strain-dependent responses to low pH: indifference; inhibition of fermentation progress that is responsive to potassium addition; and inhibition of fermentation performance that cannot be rectified by potassium addition alone (Figure 2). The degree to which fermentation was inhibited by low pH was demonstrated in defined medium experiments, comparing the performance of different wine yeast strains in a high sugar and low nitrogen environment. Yeast performance in this environment is characterised by slower fermentation rates, slower growth, lower biomass formation and increased acetic acid (Figure 2) and glycerol production (data not shown). Chardonnay fermentations exhibited many of the same features as those conducted in defined medium, although effect magnitudes were attenuated (data not shown).

DOES ELEMENTAL COMPOSITION AFFECT THE WAY A WINE SMELLS?

Volatile metabolites in yeast can account for much of the variation in wine style arising from the use of different yeast strains (see, for example, AWRI publication #1288). Using a fingerprinting method for volatiles we investigated how elemental composition contributes to variation in wine volatiles by assessing 68 fermentations with different combinations of medium composition and yeast strain. Principal component analysis of fingerprints of volatiles from these ferments provides a window into the relationship between medium composition and volatile production (Figure 3, see page 36).

The greatest variation in profiles of volatiles could be explained by variations in pH and potassium concentration (31%, by definition, principal component 1). In fact, pH and potassium gave rise to more variation in the volatile fingerprint than any other parameter including the yeast strain used, regardless of whether or not the ferment went to completion. This analysis excluded acetic acid (VA), which is a major contributor to sensory characteristics in its own right. These preliminary findings, while requiring more detailed investigation, indicate how influential juice composition can be on fermentation kinetics and wine sensory characteristics.

WHAT IS THE BASIS FOR DIFFERENT SENSITIVITIES OF DIFFERENT WINE YEAST STRAINS TO LIMITING POTASSIUM AND LOW PH?

Unravelling variation in grape juice composition is giving us a picture of what constitutes a good fermentation partner for wine yeast. This has enabled us to identify two components of juice, pH and potassium; the levels of which are critical for optimum fermentation efficiency. To understand better the relationship between fermentation partners we require knowledge of the genetic variation between wine yeasts. This is being achieved using comparative genomic analysis, i.e., we have compared the entire DNA sequences of several different wine yeast strains.

To date, we have published the

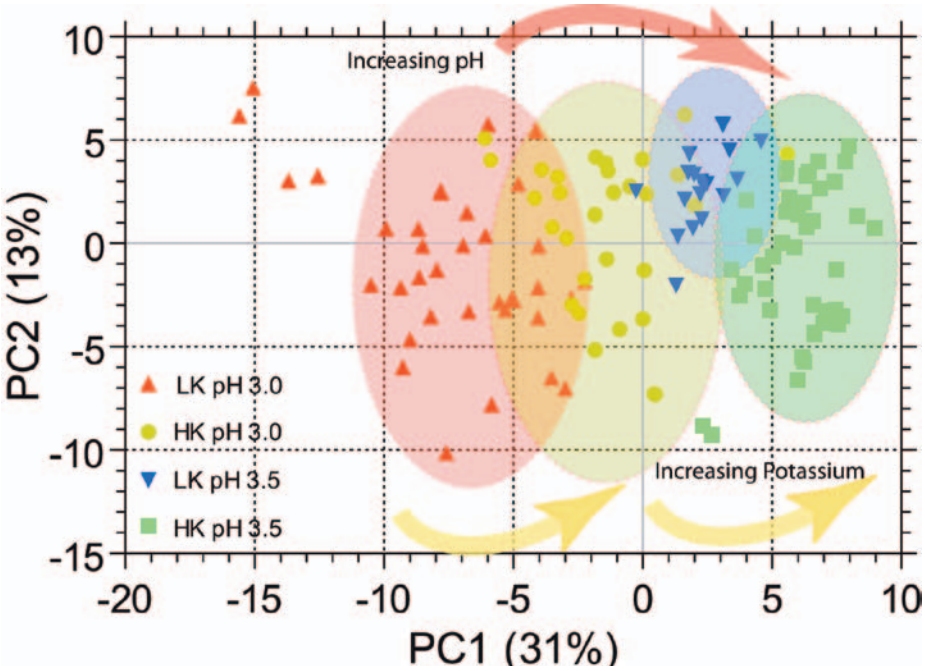

Figure 3. Influence of medium composition on production of fermentation volatiles. Analysis of 68 fermentations, with variations in both yeast strain and elemental composition, identified changes in pH and potassium levels as the predominant factors leading to variation in fermentation volatiles. This is shown in the above principal component analysis. The first principal component (on the X-axis) is a measure of the majority of variation in the sample (31% in this instance) and pH and potassium treatments (grouped by colour) separate samples in this axis.

genomic sequences of four wine yeasts commercially available: Lalvin QA23, AWRI796, Vin13 and VL3 (AWRI publication #1289), with several other strains sequenced and for which data are undergoing processing. Whilst it is early days, we can see that wine yeasts form a cohesive group and these are genetically different to yeast strains used in other industries (e.g., beer, biofuel and saké production). These genetic differences are often on par, and sometimes exceed, those that separate humans and chimpanzees. While the genetic differences that separate wine yeasts from each other are much fewer, the level of genetic variation is nonetheless generally greater than that which separates individual humans from each other. It is this variation that is ultimately responsible for the different behaviour of specific wine yeast strains during the fermentation process and, in the same vein as medical research which seeks to link human genetic variation with genetic diseases, we are now working to link wine yeast genetic variation with wine relevant traits (both positive and negative).

CLOSING COMMENT

Grape juice and wine yeasts are complex, and their coupling to produce wine reflects this. For example, predicting, with accuracy, the quality of a wine from its starting ingredients can be a rather 'hit and miss' affair, and the problem of suboptimal fermentations still takes winemakers by surprise from time to time. Results from work on grape juice composition and comparative yeast genomics, and the convergence of these approaches, will enable more informed strategies for winemakers so that they can make the best choice when coupling grape juice and wine yeast. We are already in a position to say that, at least for Chardonnay juice, if the pH is low, you can minimise the risk of a stuck or sluggish fermentation by choosing a robust strain.

ACKNOWLEDGEMENTS

This project was supported by Australia's grapegrowers and winemakers through their investment body, the Grape and Wine Research and Development Corporation, with matching funds from the Australian Government. We would also like to thank The Yalumba Wine Company for its support in this work. The AWRI is part of the Wine Innovation Cluster in Adelaide, South Australia. Systems Biology research at the AWRI is performed using resources provided as part of the National Collaborative Research Infrastructure Strategy, an initiative of the Australian Government, in addition to funds from the South Australian State Government. The authors wish to thank Rae Blair for her editorial assistance.

REFERENCES

AWRI publication #900. Bell, S.J. and Henschke, P.A. (2005) Implications of nitrogen nutrition for grapes, fermentation and wine. Aust. J. Grape Wine Res. 11:242-295.

AWRI publication #1288. Curtin, C.D.; Bellon, J.R.; Bartowsky, E.J.; Henschke, P.A.; Chambers, P.J.; Herderich, M.J. and Pretorius, I.S. (2011) Harnessing AWRI's yeast and bacterial research to shape 'next-gen' Chardonnay Part 1: 'Wild' and 'non-conventional' yeast. Wine Vitic. J. 26:15-20.

AWRI publication #1289. Borneman, A.R.; Desany, B.A.; Riches, D.; Affourtit, J.P.; Forgan, A.H.; Pretorius, I.S.; Egholm, M. and Chambers, P.J. (2011) Whole-genome comparison reveals novel genetic elements that characterize the genome of industrial strains of Saccharomyces cerevisiae. Plos Genet. 7:10.

AWRI publication #1290. Schmidt, S.A.; Dillon, S.; Kolouchova, R.; Henschke, P.A. and Chambers, P.J. (2011) Impacts of variations in elemental nutrient concentration of Chardonnay musts on Saccharomyces cerevisiae fermentation kinetics and wine composition. Appl. Microbiol. Biotechnol. DOI: 10.1007/s00253-011-3197-3.

Amerine, M.A. and Joslyn, M.A. (1970) Table wines. University of California Press, Berkeley, California.

Anraku, Y.; Ohya, Y. and Iida, H. (1991) Cell-cycle control by calcium and calmodulin in Saccharomyces cerevisiae. Biochim. Biophys. Acta 1093:169-177.

Boulton, R. (1980) The general relationship between potassium, sodium and pH in grape juice and wine. Am. J. Enol. Vitic. 31:182-186.

Dombek, K.M. and Ingram, L.O. (1986) Magnesium limitation and its role in apparent toxicity of ethanol during yeast fermentation. Appl. Environ. Microbiol. 52:975-981.

Jones, R.P.; Pamment, N. and Greenfield, P.F. (1981) Alcohol fermentation by yeasts - the effect of environmental and other variables. Process Biochem. 16:42-49.

Rojas-Lara, B.A. and Morrison, J.C. (1989) Differential effects of shading fruit or folliage on the development and composition of grape berries. Vitis 28:199-208.

Rühl, E.H.; Clingeleffer, P.R.; Nicholas, P.R.; Cirami, R.M.; McCarthy, M.G. and Whiting, J.R. (1988) Effect of rootstocks on berry weight and pH, mineral content and organic acid concentration of grape juice of some wine varieties. Aust. J. Exp. Agric. 28:119-125.

Somers, T.C. (1975) In search of quality for red wines. Food Tech. Aust. 27:49-56.

Somers, T.C. (1977) A connection between potassium levels in the harvest and relative quality in Australian red grapes. Aust. Wine Brew. Spirit Rev. 96:32-34.

Test, S.L.; Noble, A.C. and Schmidt, J.O. (1986) Effect of pomace contact time on Chardonnay musts and wines. Am. J. Enol. Vitic. 37:133-136.

Walker, G.M.; Birch, R.M.; Chandrasena, G. and Maynard, A.I. (1996) Magnesium, calcium, and fermentative metabolism in industrial yeasts. J. Am. Soc. Brew. Chem. 54:13-18.

Walker, R.R.; Clingeleffer, P.R.; Kerridge, G.H.; Rühl, E.H.; Nicholas, P.R. and Blackmore, D.H. (1998) Effects of the rootstock Ramsey (Vitis champini) on ion and organic acid composition of grapes and wine, and on wine spectral characteristics. Aust. J. Grape Wine Res. 4:100-110.

WVJ