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Development of tools to verify origin and varietal nature of wines



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1. Abstract

This project was successful in developing tools to identify the provenance of Australian wines in an international context, based on statistical analysis of selected isotope ratios naturally found in wine. The models developed were able to identify Australian wines when applied across different vintages and varieties of wines, demonstrating their robustness. The project also made important strides in understanding why some methods that have been previously promoted as tools for identifying provenance (particularly those involving analysis of lead isotope ratios and trace metal concentrations) have not been successful.

2. Executive summary

Verifying the origin of wines by analysis relies on the use of parameters that not only reflect the geology and water source of the location where grapes are grown, but which are also not altered during winemaking. The project set out to identify analytical parameters based on isotope ratios to verify the geographic origin – or provenance – of Australian wines within the framework of the international packaged and bulk wine trade. Multi-dimensional statistical tools were employed, in particular Orthogonal Projection of Latent Structure-Discriminant Analysis (OPLS-DA) to analyse isotope ratios of boron, lithium, strontium and oxygen, which were determined using appropriate Inductively Coupled Plasma Mass Spectrometry (ICP-MS) methodology and Isotope Ratio Mass Spectrometry (IRMS). A discriminant model was developed using data from 292 Australian wines (from 16 production zones) and 94 international wines (from Europe, South America, Canada, New Zealand, South Africa and USA). The model had a successful classification rate of 97.3% for Australian wines, with a successful classification rate of 92.5% on average for all wine analysed. The main drivers of the model were isotopic ratios of boron, oxygen and strontium; however, some concentration data from selected trace elements including nickel, lithium and chrome was required to achieve these levels of discrimination. Identification of the region of origin of Australian wines, mostly through the impact of subsoil strata on strontium isotopes, was also achieved with a classification success rate of approximately 60%. The lower levels of successful classification for regions is strongly linked to the underlying similarity of geology that contributes to the subsoil composition of many Australian grapegrowing regions. This information will provide important baseline data for any future studies of analytical methods to determine regional origin of Australian wines.

Several potentially confounding factors on these isotopic ratios and their ability to differentiate Australian from overseas wines were investigated:

Vintage variation was measured using vertical series of three single-vineyard wines from very different regions across Australia over a period of ten years. This temporal variance was tested against that measured for all study samples and found to be smaller for all isotopic parameters with the except of two lead isotope ratios. This allowed the majority of isotope ratios to be used.

Grape variety variation was tested in ten cultivars, both red and white, grown in several locations of South Australia subjected to controlled micro-vinification. Differences in the isotope ratios of Li, O, and Sr differed by below 10% due to grape cultivar and were therefore useable.

Neither vintage nor variety showed a significant negative effect on the use of the models to classify if a wine was of an Australian origin.

Bottle source and type were considered to have potential to influence the boron isotopic ratios found in packaged wine. However, analysis demonstrated that the bottle did not make a significant contribution in comparison to the differences introduced by wine origin.

Bentonite clay fining is a common winemaking practice which can introduce significant amounts of lead into wine. This fact makes the use of lead isotopic ratios unsuitable for classification of white wines. Analysis demonstrated that the lead isotopes were more useful at indicating the regional source of the bentonite used, an internationally traded commodity, than the wines origin.

The success of this project in using combined isotopic ratios and statistical analysis to categorise a wine's origin will allow the provenance of a wine that claims to be Australian to be analytically determined. While by its nature this technique is not suitable for rapid in-market applications, it will provide an important addition to technologies such as smart labelling and methods that compare direct retention samples of known wines. Significantly, unlike many other technologies used in authentication of wine, the use of isotopic ratios can link a wine's provenance to the underlying physical attributes of its place of origin. This is important in cases where rather than being a direct forgery of an existing Australian product, a wine is simply making claims of Australian provenance. Such cases can expose the consumer to, at best, a disappointing quality outcome and at worst possible health impacts from the use of unapproved additive or ingredients, with additional obvious negative outcomes for the reputation of all Australian wines.

While this work identified the overall potential of using a combined groups of isotopic ratios to provide information on wine origin, further work is needed to clarify nature of the links between the final values of these components in wine and the underlying sources in the geology, soil, water and air. Such an understanding will allow the much larger data sets compiled in environmental and geological studies to be used in the determination of the provenance of wine as well as other agricultural products.

3. Acknowledgements

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4. Background

Determination of the geographical origin of agricultural produce became important once it was realised that consumers would pay a premium for agricultural products coming from specific areas. Legally binding laws that had existed in France since the 1930s under the Appellation contrôlée laws were then enshrined in national and European laws which ultimately became the 'Protected Denomination of Origin' (PDO), although most were designed to protect the winemaking traditions in place at the time in terms of grape cultivar, yields and geographical boundaries. Australia only used this last criterium in the establishment of its framework of Geographical Indications (Anon 2013). Instances of economically motivated adulteration in food and beverages (Everstine et al. 2013) have been a constant for many centuries but some of the biggest have significantly cost the reputation of the country's industry – and their suppliers – involved. One major example of this was the terrible consequences of added melanin in infant formula sold in the People's Republic of China (Shi Xiuhua and Shiguo 2018). The risk associated with a similar incident embroiling the Australian wine industry remains high given the large proportion of Australian wine shipped in bulk and protected only by administrative paper trails.

The first authors to specifically analyse trace elements for the determination of geographic origin in wine recognised that counterfeiting was also of great economic importance (Siegmund and Bächmann 1977). Since then, innumerable studies have demonstrated their utility, sometimes tenuously, for differentiating diverse wine regions or subregions within one country, as highlighted in some salient reviews (Suhaj and Korenovská 2005, Versari et al. 2014). Acknowledging the inter-regional limits for geographical discrimination of biogenic stable isotopes (^{2}H, ^{13}C, ^{18}O) and site-specific quantitative ^{2}H-NMR parameters, trace element data were used as complements in order to achieve superior discrimination (Day et al. 1994, Day et al. 1995, Martin et al. 1999). Analytically and statistically this approach was successful but it disregarded the many changes that occur in absolute

trace element concentrations during winemaking, therefore casting some doubt on this simplistic approach (Bekker et al. 2019, Castiñeira Gómez et al. 2004). Rare earth elements (REEs) were proposed as suitable candidates (Aceto et al. 2018) as they were thought not to be modified by fermentation. The use of bentonite clay, however, to protein stabilise white wine was shown to negate this (Mihaljevič et al. 2006, Mihucz et al. 2006, Rossano et al. 2007). For this reason, the use of isotope ratios of heavy elements such as lead (Almeida and Vasconcelos 1999, Barbaste et al. 2001, Larcher et al. 2003, Rosman et al. 1998) or strontium (Almeida and Vasconcelos 2001, Barbaste, Robinson, et al. 2002, Horn et al. 1993, Lurton et al. 1999) were suggested, based on a long understanding of geochemical chronology, and having the benefit of potentially being not influenced by winemaking. Although the use of lead isotope ratios was initially short lived, the suitability of strontium isotope ratios subsequently generated a large body of work, often comparing small differences within European PDOs in isolation to other countries (Durante et al. 2015, Durante et al. 2013, Florin-Dumitru et al. 2017, Marchionni et al. 2013, Martins et al. 2014, Petrini et al. 2015, Victor et al. 2015). Strontium isotope ratios have now been shown not to be influenced by the use of barrels, bentonite or nanofiltration (Almeida and Vasconcelos 2004, Catarino et al. 2019, Catarino et al. 2008, Catarino et al. 2016, Moreira et al. 2017). Notwithstanding this information, Green et al. (2004) cautioned their use as they argued the isotope signature was carried in the irrigation water supply; this study, however, was very restricted in the sample numbers used to draw this conclusion.

Epova, Berail, et al. (2019) successfully demonstrated the classification of authentic Bordeaux wines from fraudulently mislabelled wines in an overseas market using $R^{87}Sr/^{86}Sr$. However, the outcome from this method is often not as unequivocal and other parameters that are unrelated, or physically orthogonal, need to be explored. Coetzee and Vanhaecke (2005) and subsequently Vorster et al. (2010) demonstrated that $R^{11}B/^{10}B$ showed good separation of regions in the Western Cape of South Africa (Robertson, Stellenbosch, Swartland) which also differentiated from Bergerac in France and Valpolicella in Italy. Recently, $R^{11}B/^{10}B$ was used to differentiate wine from the northern and southern wine regions of Brazil (de Almeida et al. 2017) and combined with $R^{87}Sr/^{86}Sr$ has been used to characterise four tea-producing regions of Taiwan (Chang et al. 2016) and several coffee-producing regions of Africa, America and Asia (Liu et al. 2014). The use of $R^{7}Li/^{6}Li$ is becoming an important tracer for studying water-sediment interaction particularly when integrated with B and Sr isotopic ratios (Meredith et al. 2013). The known sensitivity of the water isotope ratios, such as $\delta^{18}O$, to temperature, rainfall, altitude and distance from the sea would make them a good candidate within an orthogonal data system (Rossmann et al. 1999). Together these orthogonal parameters should better define the geographical provenance of Australian wines and differentiate them from overseas wines.

Vintage variation

Annual variations in isotopic ratios are well known in Europe and necessitate the collection of annual reference data such as for $\delta^{18}O$ (Christoph et al. 2015); but the extent of similar variations in Australian wines has never been demonstrated in wine. Seasonal variations to long-term climate conditions characterise each vintage. These include incident solar radiation, precipitation and temperature, although the last two have been shown to play the biggest role, with precipitation being the most important (Hollins et al. 2018). In addition to increased precipitation having a direct effect on water isotopes, water availability may have an effect on trace element concentrations through vine uptake as well as evapotranspiration. In the example given by (Christoph et al. 2015), the standard deviation of $\delta^{18}O$ permil (‰) on a set of wines over 22 vintages from Franconia in Germany is 1.8 around an average value of -1.0 ‰.

Varietal variation

The effect on analytical parameters used in provenance testing brought about by grape variety has been less clearly demonstrated in isotope ratios. Differences in organic compounds, particularly aroma molecules (Springer 2018, Springer et al. 2014, Welke et al. 2013) or phenolic compounds (Mayr et al. 2018, Villano et al. 2017) have been proposed as a way of determining cultivar. Basic experimental design is important in trying to demonstrate this, as varietal differences are often confounded by stronger differences in origin (Makris et al. 2006).

5. Project aims and performance targets

Although the use of strontium isotope ratios is now a mature technique, to date there is little data on strontium isotope ratios in Australian produce, with only one study on milk (Crittenden et al. 2007) and a small sample set of wines from McLaren Vale in an unrelated study on airborne lead contamination (Kristensen et al. 2016). The variations due to year of production, grape cultivar, use of bentonite or even influence from bottle glass need to be understood if these parameters are to be used for the purpose of this study. These were therefore explored in a series of small-scale winemaking trials.

The overall objectives for the project were to:

- measure the isotope ratios of boron, lithium, oxygen and strontium using ICP-MS and IRMS in a wide selection of red and white commercial wines from across Australia, drawn from most of the wine-producing zones over several vintages.
- determine which parameters, if any, were able to discriminate Australian wines from those produced overseas.
- ensure vintage variation was within acceptable limits.
- investigate any obvious confounding factors that might render these analytical parameters redundant such as:
    - grape cultivar
    - the use of bentonite, a processing aid in stabilisation and fining
    - the use of bottle glass produced overseas.

6. Methods

6.1 Materials

6.1.1 Commercially finished wines for survey

(i) General geographical survey

Examples of finished, bottled, commercial Australian wine (n=292) were collected under the authority of the Wine Australia export provisions or directly from producers. Samples were collected in two sets during 2015 and 2017. Details of the samples collected (which represented 16 production zones from all wine-producing Australian states except Queensland) are given in Table 1. Wines produced in overseas countries sourced for the project (n=94) are detailed in Table 2 and represent 14 countries that contribute to bulk wine movements. These wines were obtained either by purchase directly from their Australian importers or from reputable wine stores. The overall split between red and white varieties was 226 and 160. The majority of Australian samples in this study (97%) were made from the *Vitis vinifera* cultivars Cabernet Sauvignon, Chardonnay, Pinot Noir, Riesling and Shiraz. Because of the much greater diversity of cultivars grown outside Australia, the overseas wines were made from 29 different varieties with the highest number (64%) coming from Chardonnay, Cabernet Sauvignon and Pinot Noir. A breakdown is given in Table 3. Most samples were produced between 2012 and 2017 although some of the overseas samples were from earlier production years.

Table 1. Breakdown of sample numbers of Australian wines included in the survey, identified by production zone

Zone† name	Code	State	Count		
			Total	Red varieties	White varieties
Big Rivers	19	NSW	4	4	0
Central Ranges	20		21	9	12
Hunter Valley	21		15	5	10
Southern New South Wales	25		18	7	11
Barossa	6	SA	20	12	8
Fleurieu	8		17	9	8
Limestone Coast	10		24	19	5
Lower Murray	9		13	8	5
Mount Lofty Ranges	11		15	9	6
Tasmania	64	TAS	28	19	9
Central Victoria	13	VIC	4	3	1
North West Victoria	16		4	3	1
Port Phillip	17		32	16	16
Western Victoria	18		15	9	6
Greater Perth	3	WA	12	9	3
South West Australia (excluding Margaret River)	4		31	17	14
South West Australia (Margaret River only))			19	10	9

† Zone information sourced from Wine Australia (2017)

Table 2. Breakdown of sample numbers of non-Australian wines in survey identified by country

Country	Continent	Count		
		Total	Red varieties	White varieties
Argentina	America	6	3	3
Canada	America	3	3	-
Chile	America	8	5	3
France	Europe	13	9	4
Greece	Europe	2	-	2
Italy	Europe	12	6	6
Lebanon	Asia	1	1	-
Macedonia	Europe	2	1	1
New Zealand	Australasia	18	12	6
Romania	Europe	2	1	1
South Africa	Africa	3	2	1
Spain	Europe	12	6	6
Uruguay	America	4	2	2
USA	America	8	7	1

Table 3. Breakdown of sample numbers in survey identified by grape cultivar, where n > 5

Grape cultivar	Count		
	Total	Australia	Overseas countries
Chardonnay	124	104	20
Cabernet Sauvignon	113	92	21
Pinot Noir	56	37	19
Shiraz	33	30	3
Riesling	17	17	-
Merlot	6	4	2
Others	37	8†	29‡

† 7 cultivars; ‡ 24 cultivars

(ii) Assessment of annual variation in isotope ratios

Three sets of vertical series of Australian wine were analysed to determine annual variation in the isotope ratios of boron, lithium, oxygen, lead and strontium.. The wines were monovarietal, all came from the same vineyard, and were vinified in a consistent style. A time span of 10 years was chosen for the selection of wines in the hope of reflecting recent winemaking techniques. However, because of the difficulty in accessing such samples not all vintages were available, and therefore some sets contained non-contiguous vintages. The three sets were also chosen to come from different regions situated on different geophysical domains as designated by their Australian Crustal Elements. Wines were donated or provided at favourable rates. Details of the vertical series analysed are given in Table 4.

Table 4. Details of wines in the vertical series used to assess annual variation

Cultivar	Years available	Geographical Indication	Geophysical domain crustal element
Riesling	2005, 2007, 2009-2010, 2012-2016	Clare Valley, SA	AD 17, 27
Semillon	2006-2009, 2011-2016	Hunter, NSW	MN 32 32
Chardonnay	2008-2016	Margaret River, WA	LU 25 25

6.1.2 Grapes used for assessing variation due to cultivar

Grapes of different cultivars were manually picked from two separate blocks, subjected to laboratory-scale winemaking and analysed to determine any varietal differences. To overcome potential geographic variations that might confound variations due to those from varietal variation, an experimental trial was executed which minimised differences in geographical origin while observing variations from a commercially relevant range of cultivars. Grapes were harvested during the 2017 vintage from two different germplasm collections located in separate regions of South Australia (Nuriootpa and Monash). The collections – SARDI Nuriootpa Viticultural Station and the Riverland Vine Improvement Committee, Monash – supplied 4 kg of grapes of each cultivar at commercial maturity. Vines were grown on their own roots. The cultivars selected were: Chardonnay, Riesling, Semillon, Cabernet Sauvignon, Merlot, Pinot Noir and Shiraz. Samples were immediately frozen after picking and transported to the AWRI where they were stored at -18°C until ready for winemaking.

6.2 Analytical methods

All wine samples were digested in acid-cleaned 'Savillex' PFA vials. using concentrated HNO_3 with heating for 12h at 60 °C (for B samples) and 80°C for other elements, followed by addition of H_2O_2 to achieve sample clarity. Sample handling was conducted in fume cabinets in the CSIRO trace analysis facility operating to clean room practices. Column separations were carried out with resins beds prepared in Bio Rad Poly-Prep® gravity flow columns (Bio Rad, Gladesville, NSW, Australia).

The concentrations of trace elements Al, As, B, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, Pb, S, Se, Si, Sr and Zn were determined by Inductively-Coupled Plasma–Optical Emission Spectrometry (ICP-OES) using an Agilent 5100 without digestion, with wine samples receiving only a 1+9 (by volume) dilution with Milli-Q water. The Sr and B data were used to calculate digestion volumes for column preparation clean-up for the MC-ICP-MS isotopic analysis. The diluted samples were further analysed using an Agilent 7700 ICP-MS to determine Li and Pb concentrations.

After digestion, specific methodology was followed for the determination of the isotope ratios:

- **Boron**: After digestion with HNO_3–H_2O_2, samples were evaporated and buffered to pH 9-10 with NH_4OH solution prior to purification on Amberlite 743 or PWA10 (Dow Chemical, USA) resins (granularity: 106 -125 µm) for separation of B; $\delta^{11}B$ was determined using a Neptune MC-ICP-MS against the standard reference material (SRM) 951 (H_3BO_3)
- **Lead**: Stable isotope ratios for Pb ($R^{206}Pb/^{204}Pb$, $R^{206}Pb/^{207}Pb$, $R^{208}Pb/^{207}Pb$) were determined in digested solutions using an Agilent ICP-MS 7700 with SRM 981 as certified reference material (CRM).
- **Lithium**: $R^{7}Li/^{6}Li$ was determined using an Agilent 7700 ICP-MS with IRMM-016 Li_2CO_3 (Institute for Reference Materials and Methods, Geel, Belgium) as CRM.
- **Oxygen-18**: Oxygen-18 determination was made using a Continuous-Flow Isotope Ratio Mass Spectrometer (CF-IRMS; automated preparation system GasBench II, ConFlo IV interface and

Delta V Advantage IRMS; ThermoFisher Scientific, Waltham, MA). Samples were supplied for analysis in sealed ampoules. A 200 µL aliquot was removed and added to a 12 mL Vacutainer which was then sealed. In batches, vials were flush-filled with a prepared standard mix of 1000 mg L^{-1} CO_2 in He using the 'GasBench II' preparative system. Vacutainers were transferred to a water bath at 30˚C and allowed to equilibrate for 18 hours. Multiple external in-house standards were analysed across the analysis runs including Waite Distilled Water (WDW, $\delta^{18}O$ = -2.3‰), Ice Core Melt (ICM, $\delta^{18}O$ = -24.9‰) and Median Lab Standard (MLS, $\delta^{18}O$ = -3.5‰). These in-house standards were calibrated multiple times previously against V-SMOW, SLAP and GISP (NIST, Gaithersburg, MD, USA) and represent long-term averages.

- **Strontium**: Sr was separated from Rb in the digestate using a specific Sr resin containing 4,4'(5')-di-t-butylcyclohexanol 18-crown-6 in 1-octanol on an inert polymeric support (particle size 50-100 µm; Eichrom Technologies, Lisle, IL, USA). The ratio $^{87}Sr/^{86}Sr$ ratio was determined on the column-purified solutions using a Neptune MC-ICP-MS, (Thermo Fisher Scientific, Bremen). The CRM used for method validation and quality control was SRM 987 ($SrCO_3$).

6.3 Quality control protocol

The quality assurance scheme for B, Li, Pb and Sr isotopic analyses employed:

- procedural blanks with each batch of wines to monitor background concentrations.
- measurement of mass bias and instrumental drift using the CRMs SRM 951 (B), IRMM-016 (Li) , SRM 981 (Pb) and SRM 987 (Sr) per measurement session.
- duplicate analysis of two to three unknown wine samples per batch.
- analysis of an in-house standard of red wine spiked with known amounts of trace elements to provide an expected concentration and isotope ratio to monitor the precision of isotope measurements over time.

Reproducibility (R) and repeatability (r) as measures of precision were calculated for ^{18}O in the water isotopic standards (WDW, ICM, MLS), the in-house mix wine standard, and duplicate analyses of unknowns. Additionally, due to the CF methodology used with up to 10 inlets per equilibrated sample, a measure of instrument repeatability of individual unknown samples was determined.

6.4 Data management and statistics

All elemental concentrations which were reported as below limit of detection (LOD) were left as blank values and the missing data algorithms of the statistical packages were invoked. An initial data cleansing of isotopic ratios or elemental concentrations screened for outliers by calculating the Grubb's G for upper or lower outlier values using Minitab, release 18 (Minitab Pty Ltd., Sydney, Australia). The data were split into two sets: Australia or overseas, and further split by production zone for Australian wines, or country for overseas wines. For those values identified as outliers using Grubbs G, the calculated arithmetic mean of a parameter for the group in question was substituted back into the data set and used for subsequent statistical analyses. Analysis of Variance (ANOVA) was carried out using Minitab with critical limits set to 99%. Principal Component Analysis (PCA) and Orthogonal Projection of Latent Variables (OPLS) was carried out using SIMCA, release 16 (Umetrics, Sartorius Stedim Biotech, Umeå, Sweden). Prior to multi-dimensional analyses, all data were pre-treated using a $\log_{10}$ transformation followed by mean centring and SD scaling.

6.5 Investigation of confounding effect of cultivar

A small stainless-steel benchtop vertical screw press was powder-coated with white pigment. It was washed with 2% HNO_3 before use. Grapes (nominally 4 kg) were defrosted at 25°C and pressed to a set torque of 30 Nm to produce 2 L (nominal) juice which was cold settled in 2 L acid-washed HDPE bottles (Azlon). The supernatant (1.6 L) was supplemented with diammonium phosphate (270 mg L^{-1}), inoculated with EC-1118 yeast (250 mg L^{-1}) and allowed to ferment at 25°C until dry (<2 g residual reducing sugars) with twice-daily resuspension of yeast solids. The wine was racked using acid-

washed silicon tubing into 1 L acid-washed HDPE bottles and potassium metabisulfite (200 mg L^{-1}) was added and the samples stored at 0°C prior to analysis.

6.6 Investigation of confounding effects from bentonite use

Three white wines produced in South Australia of known heat instability having high protein content were selected. Six commercial bentonite clays were chosen to reflect a diversity of production origins (Australia, France, Germany, Italy, USA) and forms (Na-, Ca-, mixed). Each bentonite sample was hydrated by mixing (50 g L^{-1}) in Milli-Q water at 50°C for 2 hours and left to swell overnight. The three wines were each treated with the six bentonites in acid-washed (2% HNO_3) 250 mL HDPE bottles at a rate of 1.5 g L^{-1}. They were mixed for 10 minutes and centrifuged at 3000 rpm for 10 minutes and the supernatant added to 50 mL low element background PP tubes (Varian Inc., CA USA) for storage and subsequent analysis of isotope ratios and mineral elements.

6.7 Investigation of confounding effects from different bottle glass on boron-11

A red wine (100 L) was adjusted to pH 3.2 with tartaric acid and filtered using a cross-flow device (AMS Filtration, Adelaide) under inert gas cover. Bottles (750 mL) of different colour (flint or antique green) and origin (Australia, China, New Zealand, UK, USA) were purged with nitrogen and the wine admitted using a four-head gravity filler (Framax, Italy). Bottles were sealed with a saran-tin screw capsule (Vinpac, Australia). The wine was also filled into 250 mL high density polyethylene (HDPE) bottles. All bottles were stored for five months at the University of Adelaide bottled wine store at 15 °C and then analysed as a finished wine.

7. Results and discussion

7.1 Differentiating Australian wines from overseas production

7.1.1 Data exploration

The pooled variance across all samples measured in this study was compared with the achieved long-term reproducibility from the QC sample for all isotope ratios using ANOVA and is presented in Table S1. The Fisher statistic, $F_{ALL/QC}$, shows that the variance between the samples is greater than the analytical precision and they can be considered a useful parameter for further analysis.

(i) *Boron isotope ratio data*

In their review of analytical techniques to measure B isotope ratios, Aggarwal and You (2017) suggest that MC-ICP-MS is currently the best method, with a suggested precision of 0.2–0.4‰. They highlight this in contrast to the large spread of $\delta^{11}B$ found in the natural world from -75 to +60‰, noting that seawater is a relative constant around 40‰. Values of $\delta^{11}B$ for Australian wines (Table S2) ranged from 8.9‰ to 47.2‰, while for overseas wines the range was -9.1–36.2‰. The data are consistent with those measured in wine by several authors particularly for the Western Cape, South Africa 33.8–43.6‰ (Coetzee et al. 2005); South Africa & Australia 40.2–46.6‰, South America 2–10.2‰ (de Almeida et al. 2017); USA 10.5 – 16.7‰, Europe 20–26‰ (Guo et al. 2016).

(ii) *Lithium isotope ratio*

There are no published reports of determination of $^{7}Li/^{6}Li$ in wine samples, but this fast-growing technique is used on rock samples and natural waters as a geochemical tracer for understanding such matters as near-surface weathering or crust/mantle recycling (Tang et al. 2010) or silicate lithologies and clay formation (Burton and Vigier 2012). These authors illustrate wide variations of $\delta^{7}Li$ between -40 and +50‰ but suggest that the achievable 2σ reproducibility of ICP-MS is of the order of 0.5 ‰, which conforms to the results for IRMM-016 in this study. A similar, if not wider, range was observed in wine samples in this study from -67 to +85‰ with an average of 21.7‰ (Table S2). The $F_{ALL/QC}$ value is significant at α=0.01 and can be considered for further analysis.

(iii) *Lead isotope ratios*

Early work on lead isotope ratios cast doubt on their utility as geographic markers (Stockley et al. 1997). The authors stated that there was significant variation in in both soil and grape lead concentrations between and within locations. This was not observed for varietal differences and it was concluded that contributions from the soil or environment is low, although no correlation was found between the lead isotope value in the wine and the capsule (Gulson et al. 1992). Early vintages of Bordeaux wine (1950–1980) gave $^{206}Pb/^{207}Pb$ ratios between 1.152 and 1.173, while later vintages displayed significantly lower concentrations and a smaller range of isotopic ratios (Rosman et al. 1998).

The low spread of values for $R^{206}Pb/^{204}Pb$ and $R^{206}Pb/^{207}Pb$ observed in Uruguayan wines (cultivar Tannat) 18.164–18.218 and 1.165–1.161 respectively (Celio et al. 2018) appear in agreement with data from that country in this work (16.83–17.46; 1.113–1.178) as do values for $^{206}Pb/^{207}Pb$ in Romanian wines (1.0417– 1.1079). However, the differences between Australian wines and those from overseas in wines of this study (Table S2) is only significant at probability levels > 99% and average values show low influence from environmental lead contamination from leaded petrol and confirm a geogenic origin (Kristensen et al. 2016) although values at the low end of the range would suggest a residual influence. Values in this study for Italian wines also agree with Larcher et al. (2003) who found differences across several regions within that country despite concluding that lead isotopic ratios do not seem to be a very effective tool for authenticating the origin of wines.

(iv) *Oxygen isotope ratio*

Within the Australia dataset (Table S2), two upper outliers (the higher being 14.47‰) were identified by Grubb's test in the Barossa and Tasmania zones and were replaced with zone averages. The vintage-averaged (2012-2017) $\delta^{18}O_{V\text{-}SMOW}$ value across all Australian wine regions was 6.22‰ (SD 2.43, n=292) with a very broad spread of values from 0.28 to 11.38‰. A plot of individual $\delta^{18}O$ values (with average, median values and 95% confidence interval bars) by production zone is given in Figure 1 for those zones where there were more than four samples. The lower decile values (<2.95‰) occur in the lower distributions of Canberra District in 2017; Central Ranges in 2012 and 2013; Port Phillip (Yarra Valley region only) in 2012 and 2016; South West Australia (Great Southern region only) in 2015 and Tasmania in 2012, 2013 and 2015. In contrast, the Barossa, Fleurieu (McLaren Vale region), Greater Perth, Lower Murray and Mount Lofty Ranges (Clare Valley region) all have average values above the 3rd quartile. This is consistent with the behaviour of isotopomers of water as they pass through the meteorological system and then through the vine into the wine (Martin et al. 1989). The $\delta^{18}O$ value of rainwater depends on monthly averages of temperature, T (°C) as well as precipitation, P (mm month^{-1}), altitude and latitude. Specifically for wine, $\delta^{18}O$ of wine water follows the equation $\Delta\delta^{18}O_W{}^{Q} = 0.8\Delta T - 0.05\Delta P$ (Martin and Martin 2003). More recent modelling from California data incorporated the maximum mean monthly temperature, T_{maxSO}, and monthly temperature dew point temperature T_{dmnSO}, for the months of September and October (or more generally the last two months before harvest): $\delta^{18}\ O_W{}^{Q} = 8.70 + 0.588T_{maxSO} - 0.568T_{dmnSO} + 1.37\ \delta\ ^{18}O_{precip}$ (West et al. 2007).

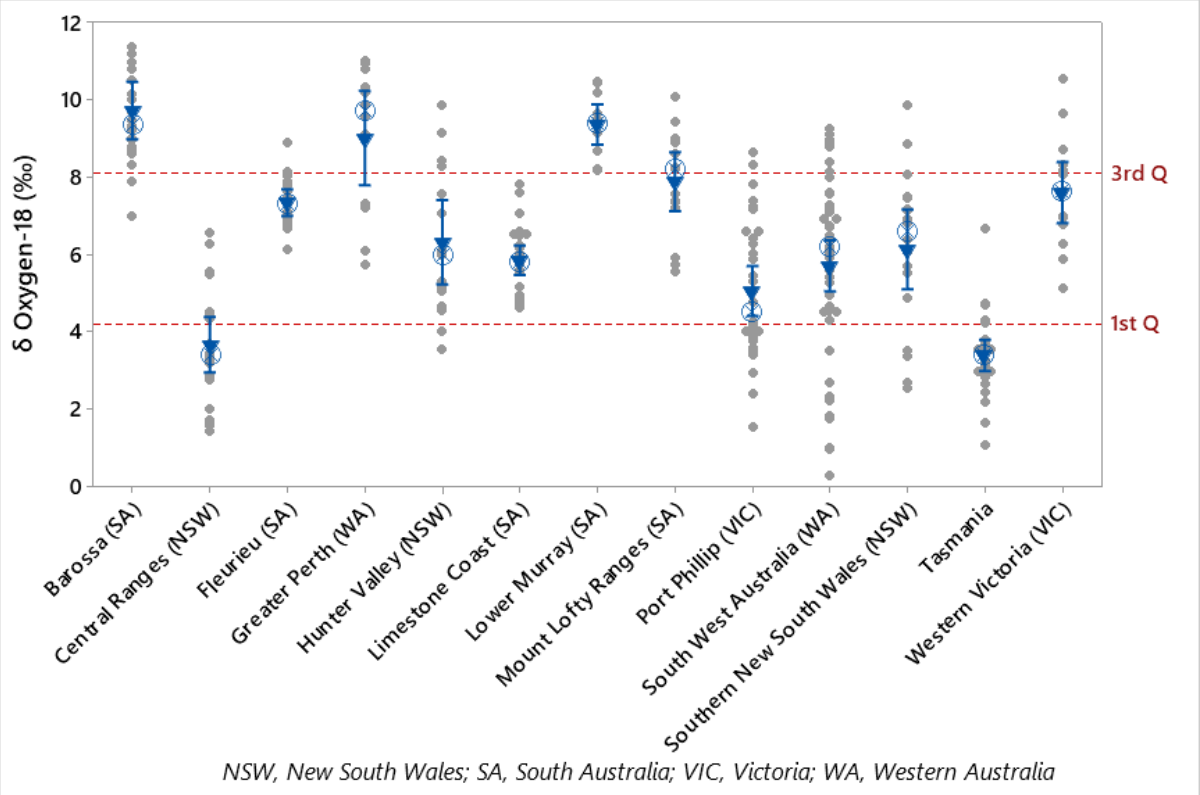


Figure 1. Plot of individual $\delta^{18}O$ values with average (▼), median (⊕) and ± 95% confidence interval bars of Australian wines by production zone (state) where N_{ZONE} >4

The average $\delta^{18}O$ value of the consolidated overseas countries, in contrast, is 2.74‰ (SD 2.62, n=94) which is very similar to the average value of 2.33‰ (n = 1,188; no SD; range -5.70–12.60‰) reported by Smeyers-Verbeke et al. (2009) using data from Hungary, Czech Republic, Romania and South Africa over three vintages (2001-03). Countries with low $\delta^{18}O$ values measured in this study were Argentina (average -0.70‰, SD 0.99, n=6, vintages 2012–13), Canada (-2.07‰, SD 0.61, n=3), New Zealand (+0.59‰, SD 1.99, n=18, vintages 2015–17) while the highest values were for South Africa (7.87‰, SD 1.84, n=3, vintages 2012–13), and Spain (4.71‰, SD 1.4, n=12, vintages 2011–16). Countries in between were Uruguay (2.45‰, SD 0.96, n=4, vintages 2012–13), France (2.55‰, SD 1.32, n=13, vintages 201, 2012–16), Italy (2.84‰, SD 1.74, n=12, vintages 2007, 2013–17), Chile (3.72‰, SD 1.38, n=8, vintages 2010-12), and USA (3.79‰, SD 1.80, n=8, vintages 2008–11, 2013, 2015). These data agree (average difference 0.47‰) with a recent paper which presents results from a survey of multiple countries across multiple recent vintages (Wu et al. 2019).

(v) *Strontium isotope ratio*

The average $^{87}Sr/^{86}Sr$ ratio for Australian wines is 0.71189 (SD 0.00300; n=292; range 0.70544–0.72460) across 16 production zones (Table S2). Although the data were tested for outlier points by zone using Grubb's test with none reported, the probability plot indicated five dislocated data points (0.7217–0.7246) above 98.2%. The average values by production zone for $R^{87}Sr/^{86}Sr$ are given in Figure 2. For some zones, such as Southern NSW or Central Victoria, the spread of data (± 95% confidence interval) is bigger than others. This is explained by the fact that these zones are made up of several regions with different subsoils or bedrock types (e.g. the Greater Perth zone includes Perth Hills, on basement designated Y 2 in the Australian Crustal Elements scheme (Shaw et al. 1996) and Swan District on the younger NH 22 22; Tumbarumba in Southern NSW is on WO 30 30 while other regions are sited on LE 30).

There is a paucity of literature data on strontium isotope ratios in Australian wine or more generally in agricultural produce. Crittenden et al. (2007) characterised Australian milk using $^{87}Sr/^{86}Sr$, determined by Thermal Ionisation–Mass Spectrometry (TIMS), along with stable isotope ratios of C, N, O and S. Although these latter parameters were useful in indicating a geographical origin, the authors noted that measurements of $^{87}Sr/^{86}Sr$ did not correlate with expected values from bedrock composition. More recently $^{87}Sr/^{86}Sr$ was measured in a small number of wines from McLaren Vale in South Australia (Kristensen et al. 2016). These values match very closely those determined in this study.

Values of $R^{87}Sr/^{86}Sr$ for overseas wines measured in this study ranged from 0.70473 to 0.71598 (mean 0.70916, SD 0.00021, n=94) (Table S2). A summary of the data, for countries in which there were more than five samples, is given in Table S3, and the values agree with results from the literature. No data for Spain or New Zealand appeared to be available in the literature and as such these data are the first recorded average values for these countries. A two-way ANOVA indicates the mean value for Australia is significantly different from that of the overseas wines analysed; variance due to cultivar colour was not significant.

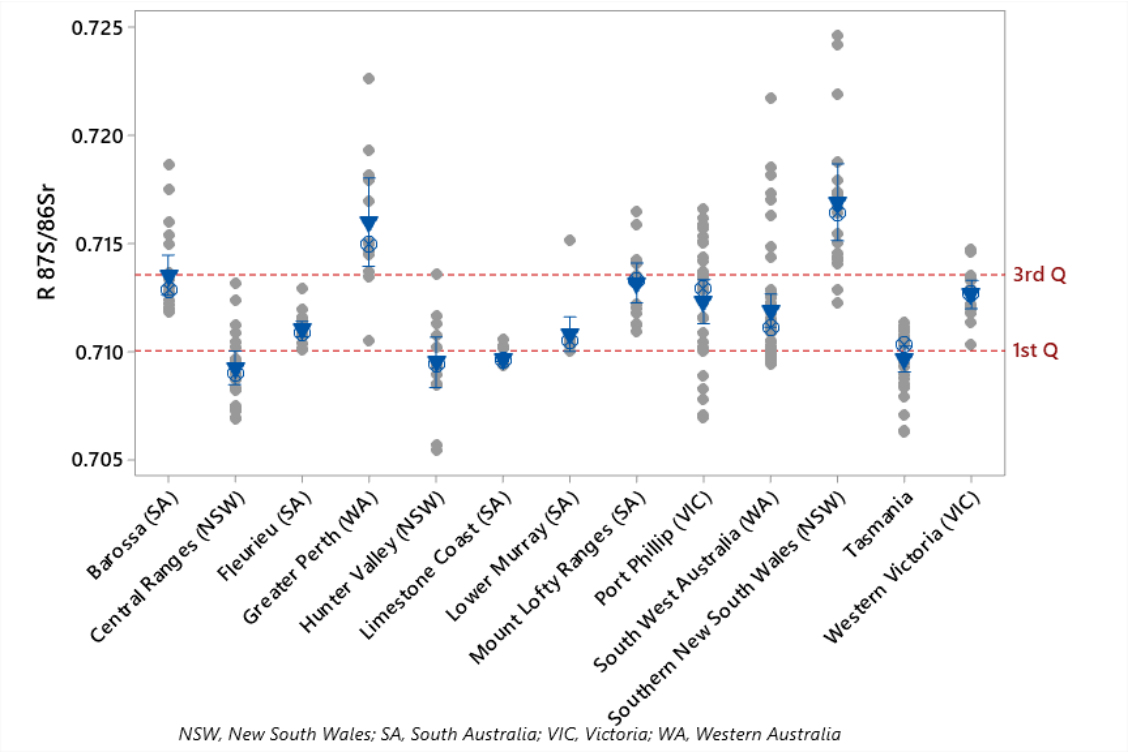


Figure 2. Plot of individual R $^{87}Sr/^{86}Sr$ values with average (▼), median (⊕) and ± 95% confidence interval bars of Australian wines by production zone (state in parentheses) where N_{ZONE} >4

7.1.2 Multivariate analysis to differentiate Australian from overseas wines

Principal Component Analysis

The isotope ratio data was assessed to see whether the variance between samples from Australia and those from overseas countries was greater than the variance within those groups, using an ANOVA test; the results are given in Table S2 where the data is partitioned to include all samples or just those from red grape varieties to account for the effect of long skin contact. The parameters $\delta^{11}B_{NIST\ 951}$ (‰), $\delta^{18}O_{V\text{-}SMOW}$ (‰) and R $^{87}Sr/^{86}Sr$, were found to have the highest probability of being different and can be assumed to show the greatest potential discriminating power.

A standard principal component analysis (PCA) of all the isotope data explained 73% of the original variance across three factors. Eight samples were previously removed from this model as their Hotelling T^2 values were above the 99% critical limit. It can be seen from the loadings plot Figure 3 (top) that the lead isotopes correlate with the principal component PC-1 along which most white wines separate as seen from the scores plot (middle figure). The bottom plot indicates the origin of the wine samples, identified by continent for clarity. This demonstrates that the isotope ratios of boron, oxygen and strontium which are correlated with PC-2 are instrumental in effecting a separation between wines from Australia and overseas. The parameter R $^{7}Li/^{6}Li$ is strongly correlated with the PC-3 and appears to partially separate wines from New Zealand. Because most of the variance along PC-1 is attributed to grape colour, as a proxy for differences in winemaking practices between white or red grapes, this confounds the separation that occurs along the minor axes. Most commercial white wines, particularly in Australia, are treated with bentonite clay to confer heat stability by removing excess pathogenesis-related proteins (Pocock et al. 2011). This procedure is known to leach relatively large amounts of lead into the treated wine (Stockley et al. 2003) with a concomitant effect on the lead isotope ratios. It is therefore of importance to take this behaviour into account when using lead isotope ratios for provenance testing (Epova, Bérail, et al. 2019) and possibly preclude them.

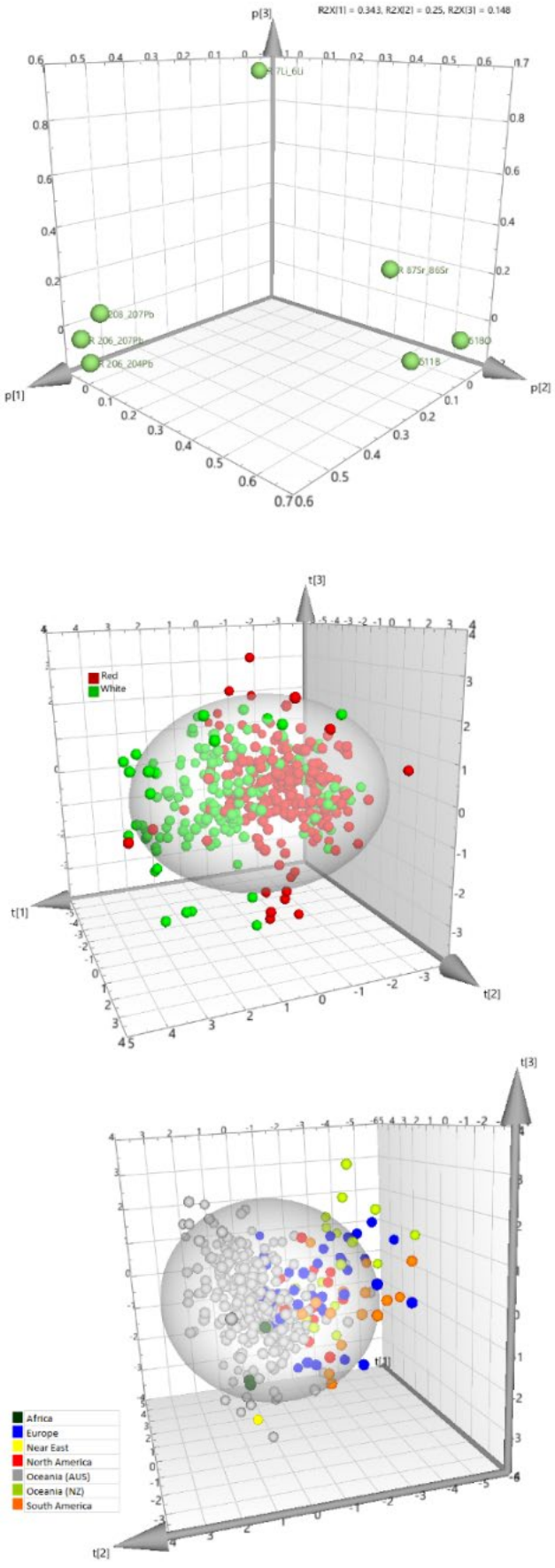


Figure 3. Principal component analysis of all isotope ratios (B, Li, O, Pb, Sr) of commercial wines from Australia and other countries. Plots of loadings (top) and scores identified by grape colour (middle) or continent of origin (bottom). Eigen values for 1st three factors: λ_1=2.4; λ_2=1.7; λ_3=1.0.

Upon removal of the three lead isotope ratios from the model, the total variance over three principal components increased to 88%. Again 12 samples were removed as their Hotelling T^2 score exceeded the 99% critical value. The loadings in Figure 4 (top) show that $\delta^{18}O$ and $\delta^{11}B$ are highly correlated with PC-1 (45% total variance), R $^{7}Li/^{6}Li$ with PC-2 (26% variance) and R $^{87}Sr/^{86}Sr$ with PC-3 (17% variance). Examination of the scores plots in Figure 4 (middle) shows there is now a random distribution of the red and white wines throughout the projected space but that the discrimination between Australian and overseas wines (coloured by continent) is maintained along PC-1 (t[1] in Figure 4, bottom).

Using this simple non-directed multi-dimensional analysis, the latent ability of just four isotopic parameters ($\delta^{11}B$, $\delta^{18}O$, R $^{7}Li/^{6}Li$, R $^{87}Sr/^{86}Sr$) to distinguish Australian wines from overseas wine has been demonstrated. The correlation of the different parameters with several orthogonal axes in the multi-dimensional space of the PCA is a confirmation of the underlying influence from different physical/chemical factors.

Orthogonal Projection of Latent Squares – Discriminant Analysis

The limiting influence of winemaking practices on the utility of lead isotope ratios to discriminate all types of wine requires a different statistical approach. A further method to remove the influence of the grape colour influence is to choose a different, more directed form of multi-dimensional analysis. Principal component analysis is an unsupervised model and separation, where groupings occur based on the variance in the original data. Other unrelated latent factors (such as provenance, vintage) are then placed on subsequent PCs. The PCA model constructs the first PC following the greatest variance and may not necessarily reflect the required latent factor, in this case differences in provenance. By using a directed or supervised method this instructs the algorithm to find and maximise the initial variance according to the desired parameter; this is the case with discriminant analysis. More recent algorithms have sought to further improve this by looking to place the supervised latent factors on the predictive axis/axes but to search out other strong orthogonal latent factors so that they do not infer with those predictive axes. A suitable algorithm for this dataset is the Orthogonal Projections of Latent Structure (OPLS) also referred to as Orthogonal Partial Least Square. When the supervisory classifier vector/matrix is continuous data, this analysis is a regression format but when discrete classes are used this is OPLS-DA.

All the isotope ratios that were significant with $P_{ANOVA}<0.05$ ($\delta^{11}B$, R $^{7}Li/^{6}Li$, $\delta^{18}O$, R $^{206}Pb/^{207}Pb$ and R $^{87}Sr/^{86}Sr$) were subjected to OPLS-DA using the classifier whether the samples were from Australia or overseas. One predictive and one orthogonal component were found, with 31% and 18% variance expressed respectively. Figure 5a shows the loadings (top) and scores (bottom) plots; the predictive axis is horizontal, and the orthogonal axis is vertical. The scores plot is coloured to show Australian red wines (AR), Australian white wines (AW), overseas red wines (OR) and overseas white wines (OW). Overall, using isotope ratios, 89.6% of samples were correctly classified but more importantly 98% of all Australian wines were correctly classified. The model is less efficient for classifying wines from overseas countries. This may be due either to the disparity in sample numbers for each class (292 Australian compared to 94 from overseas) or limitations in the inherent power of the isotope ratios alone.

To assess this possibility, trace element concentration data was added to the isotope ratio data. A large suite of elements was analysed using ICP-MS for elements typically found in wine at lower concentrations. Many elements change during winemaking (Bekker et al. 2019) and the choice of elements for provenance testing is fraught with difficulty because of this. The higher concentration elements and those that change with differences in redox potential such as Cu and Fe were therefore not considered. Several elements (Al, Ag, Be, Cd, Sb, Tl and V) occurred in concentrations below the quantification limits for more than half the dataset and were eliminated; the number of samples with concentrations below the LOD is given in Table S4.

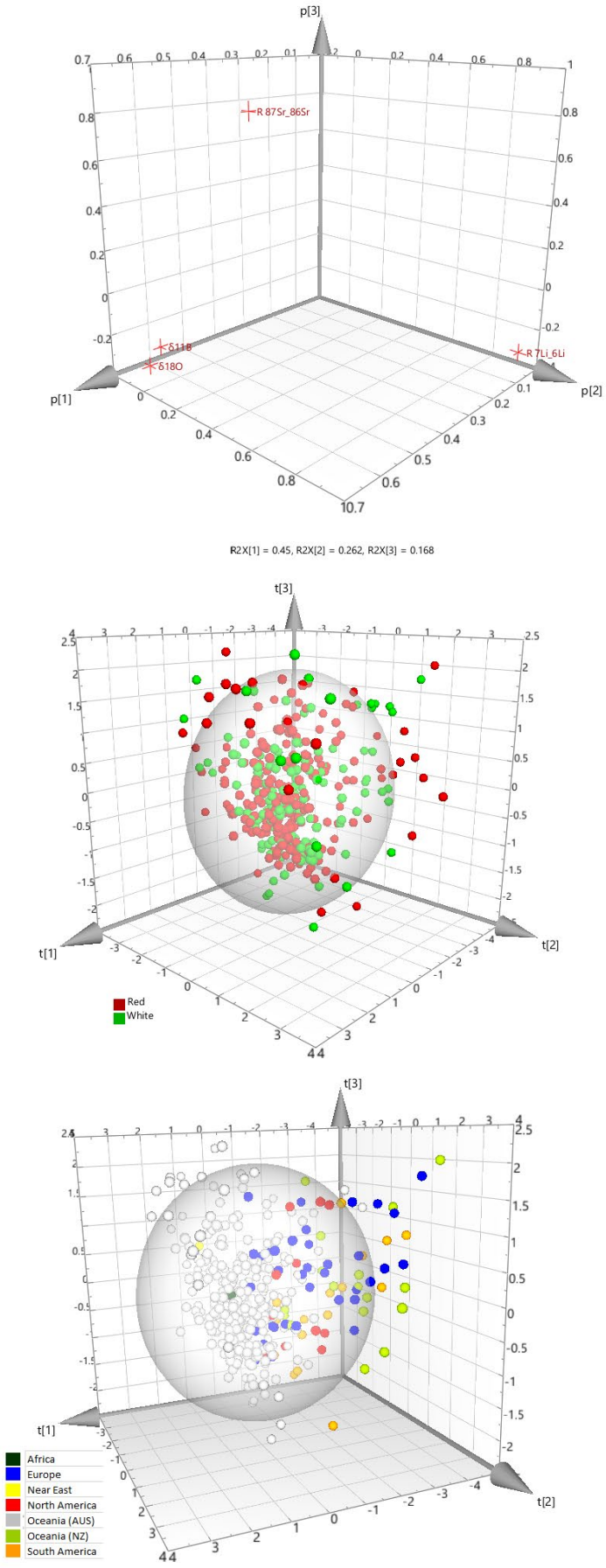


Figure 4. PCA of all isotope ratios except lead of commercial wines from Australian and other countries. Plots of loadings (top) and scores identified by grape colour (middle) or continent of origin (bottom). Eigen values for 1st three factors: λ_1=1.8; λ_2=1.0, λ_3=0.7

For the element tin, although more than 60% of samples had reportable values, the greater proportion of the overseas wines were sealed under cork and potential leaching from damaged screwcaps (Hopfer et al. 2013) might provide false discrimination; these data were not included in PCA or OPLS analyses.

The results of a one-way ANOVA are given in Table S4 which also lists the number of samples per class where the concentration was below detection. Only the elements B, Mn and Rb showed no significant difference ($P<0.05$). This is a notable result as many earlier reviews on wine authenticity using trace elements cite these elements as being powerful discriminating parameters (Suhaj and Korenovská 2005). Therefore, the concentrations of elements As, Ba, Co, Cr, Li, Ni, Pb, Sr, Zn were added to the isotope ratios $\delta^{11}B$, $R^{7}Li/^{6}Li$, $\delta^{18}O$, $R^{206}Pb/^{207}Pb$, $R^{87}Sr/^{86}Sr$ and the OPLS-DA calculated. Six samples had Hotelling T^2 values greater than the 99% critical value and were eliminated before the model was recalculated; the DModX value of the last two samples also exceeded 2x the Dcrit ($P=0.05$). One predictive and one orthogonal component were found, with 15% and 11% variance expressed respectively. The cross-validation metric, Q2 (0.60), was close to the prediction variance of 0.62 indicating a strong model. Figure 5b gives the loadings plot (top), scores plot (bottom). From the scores plot the horizontal predictive axis separates Australian wines to the left and overseas wines to the right. The vertical orthogonal axis is instrumental in separating the red wines at the top half of the plot and whites to the bottom. The tighter grouping of the Australian wines compared to the overseas wines may just be due to the greater number of samples in the dataset.

Examination of the Variable Importance to Projection (VIP) plots for the predicative axis in Figure 6 (top plot) show that $\delta^{11}B$, $\delta^{18}O$, $R^{87}Sr/^{86}Sr$ as well as Li and Ni were important for the prediction because they had a VIP > 1. From the VIP values given on orthogonal axis Figure 6 (bottom plot) three orthogonal parameters were important: Ba, Pb, $R^{206}Pb/^{207}Pb$. This is consistent with the orthogonal axis explaining variance due to the differences in winemaking practices between red and white wines, either through the influence of bentonite used in white wine production or possibly effects of extended maceration in red wines. In terms of classification rate, 97.3% of Australia samples were correctly assigned and with 77.7% of the overseas samples correctly classified; the overall classification rate was 92.5%.

In summary, the isotopic ratios of boron, oxygen and strontium have been demonstrated to show the greatest discriminating power in differentiating Australian wines from those produced overseas. The addition of trace elements, particularly Ni, Li and Cr, to the data matrix only improved the overall classification rates. With the inherent problems of relying on trace element data which can be strongly influenced by viticultural and winemaking practices, the strength of the tripartite isotope parameters $\delta^{11}B$, $\delta^{18}O$, $R^{87}Sr/^{86}Sr$ as principal discriminators of Australian wine is preferable.

The influence of white winemaking practices – particularly the use of bentonite clay for protein stabilisation – on the isotope ratios of lead, in particular $R^{206}Pb/^{207}Pb$, would seem to preclude the utility of this isotopic parameter. The positive correlation of the concentrations of barium and strontium with ‘wine colour’ as demonstrated in the OPLS-DA score plots, suggests a relationship with extended skin maceration. This again reinforces the point that use of absolute concentrations of trace elements in a discrimination protocol would lead to error.

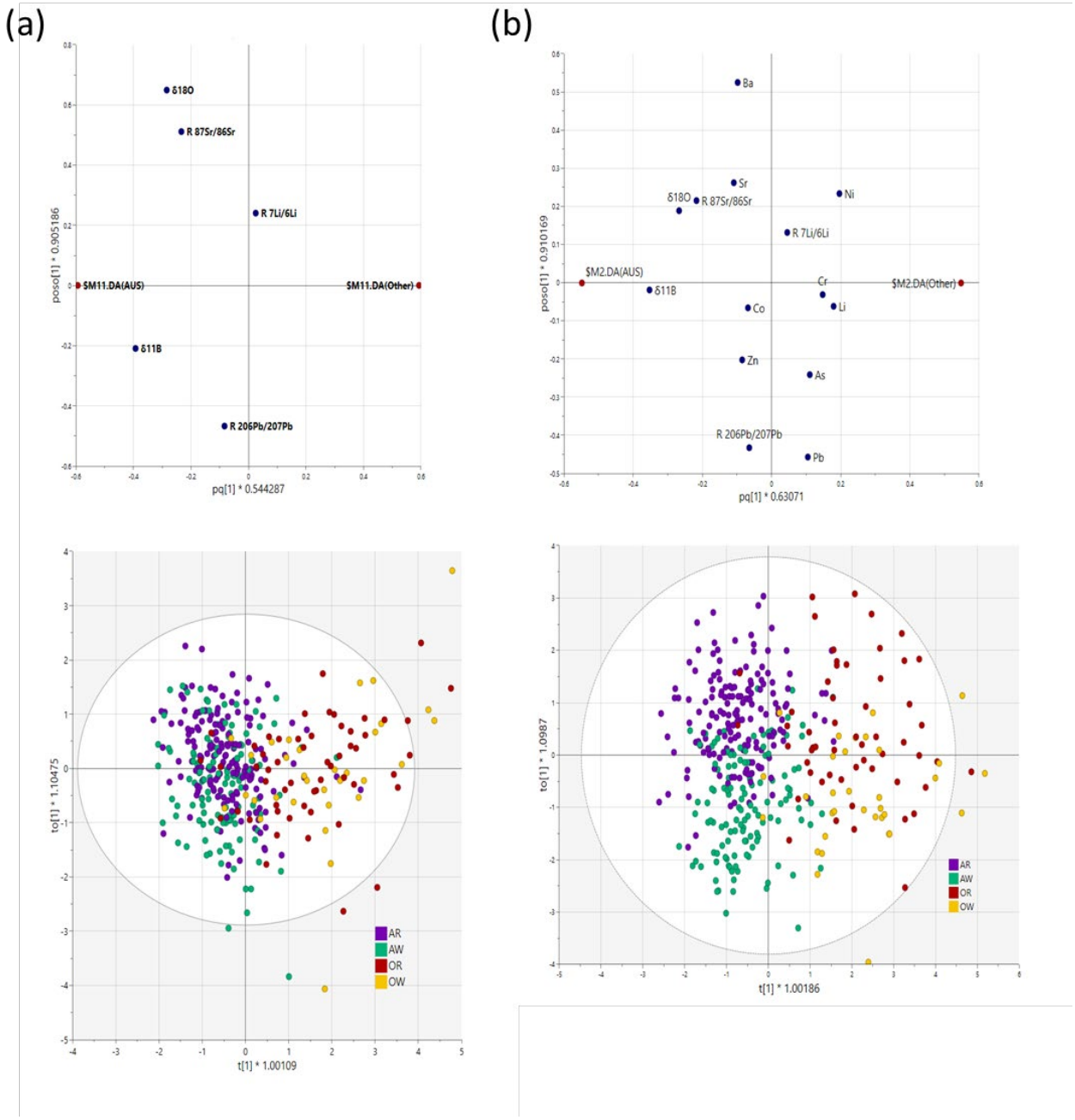


Figure 5. OPLS-DA of commercially produced wines from Australian and other countries using (a) significant isotope ratios where P_{ANOVA} <0.05 (eigen values λ_{P1}=1.6; λ_{O1}=0.9) and (b) also including trace elements where P_{ANOVA} <0.05 (eigen values λ_{P1}=2.1; λ_{O1}=1.6); plots of loadings (top) and scores (bottom) identified by origin and grape colour (AR: Australia-red; AW: Australia-white; OR: Overseas-red; OW: Overseas-white).

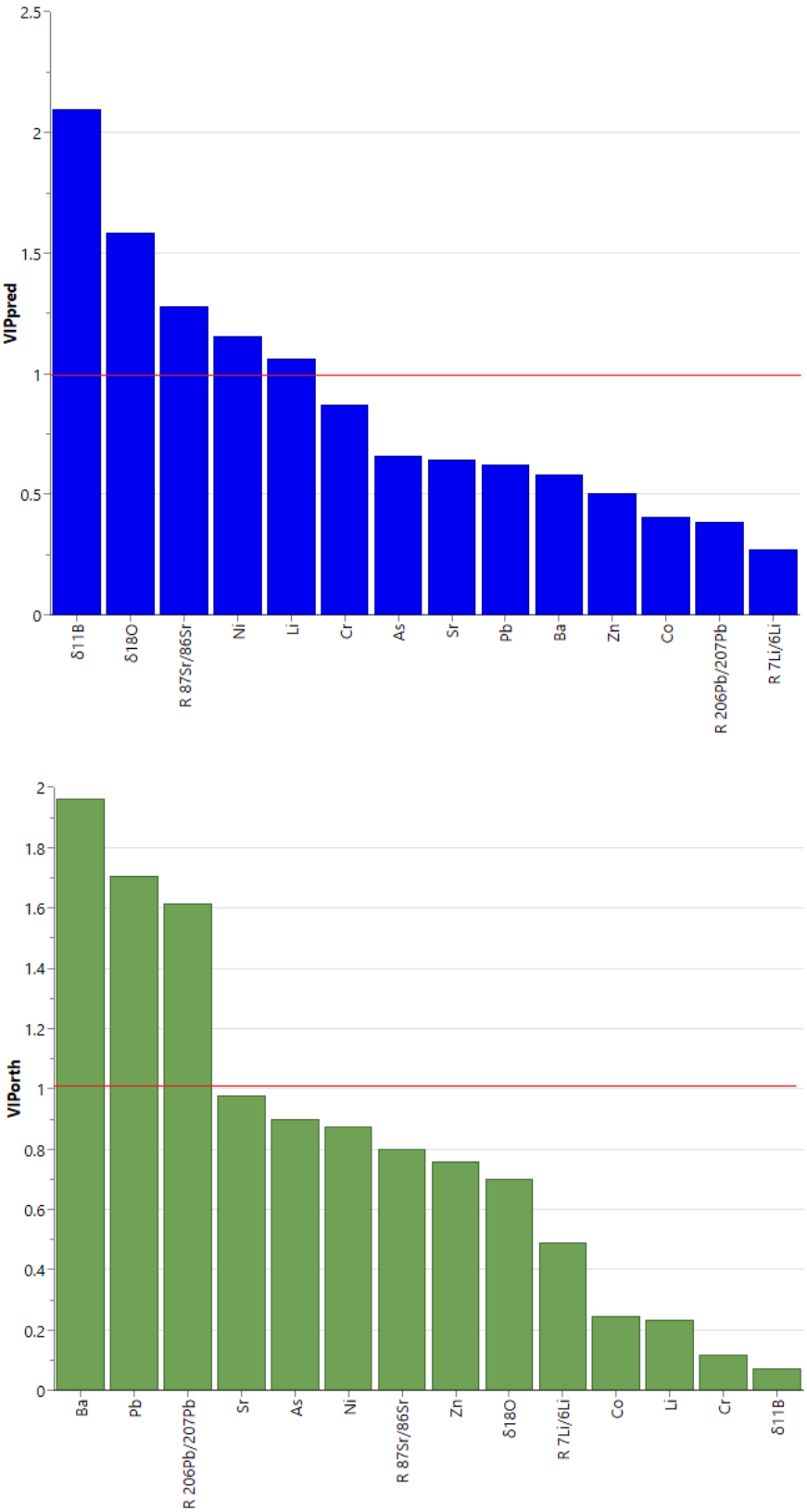


Figure 6. Variable Importance on Projection (VIP) values of the predictive (top) and orthogonal (bottom) axes for OPLS-DA of commercial wine from Australian and other countries using significant (P_{ANOVA} <0.05) isotope ratios and trace elements

7.2 Differentiating Australian wines by designated production zones or state

To ascertain whether the isotope ratios of boron, oxygen and strontium had similar discriminating power within Australia, the dataset was subdivided for Australian wines only and reanalysed using ANOVA before using OPLS-DA. There are several ways in which the dataset can be classified based on the hierarchy of the Australia Geographical Indication: by state, zone, region. All wine-producing states, except Queensland (Southern Burnett and Granite Belt regions), were represented in the dataset (Table 1) and these covered 16 of the 26 official production zones and in turn represent 34 of the 65 official regions. The results of the ANOVA explained by state, zone and region are listed in Table S5. All isotopic parameters are significant ($P < 0.001$), whether partitioned by state, zone or region. Given the large number of regions or zones represented in this dataset, discriminant analysis based on such high numbers of classes can be problematic.

By state using isotope ratios only

Initially the data were subjected to OPLS-DA with state (m=5) as the classifier using only the isotopic ratios of B, Li, O, Sr. Isotope ratios of Pb were not included to avoid discrimination by bentonite use. Three significant predictive components (λ_{P1}=1.46, λ_{P2}=0.77, λ_{P3}=0.87) were returned by the OPLS-DA modelling representing 77.5% of the initial variance. No significant orthogonal component could be calculated. The classification matrix is shown in Table 5 from which it can be seen only 42% of the samples were correctly classified. Nearly all samples from South Australia were correctly attributed to the that state, although 100 samples from other states were also assigned to SA! The best attribution rate is 31% for NSW although no correct attribution was made for Victorian samples. This model, based solely on the isotope ratios of B, Li, O, and Sr, is clearly not suitable for differentiating among samples from wine regions in the various states of Australia.

Table 5. Classification rate of samples discriminated using OPLS-DA (B, Li, O, Sr isotope ratios only) differentiated by state

Designated state		Assigned state					
	Count	Correct	NSW	SA	TAS	VIC	WA
NSW	58	31%	**18**	22	4	0	14
SA	89	97%	0	**86**	0	0	3
TAS	28	14%	18	3	**4**	0	3
VIC	55	0%	10	37	0	**0**	8
WA	62	24%	8	38	1	0	**15**
Total	292	42%	55	114	3	38	82

By state using isotope ratios and trace element concentration

Despite the potential problems using trace element concentration data to differentiate wine samples, their contribution to isotope ratios was evaluated by adding selected trace elements to the dataset. The results of the ANOVA of the trace element concentration data explained by state, zone and region are listed in Table S5. When partitioned by state, only Be, Cd, Cr, Pb and Zn were shown not to be significant, and these parameters were not included in the OPLS-DA. All data was subjected to a $\log_{10}$ transformation prior to OPLS-DA. Four predictive and one orthogonal functions were calculated, expressing 57% of the original variance. The Variable Importance for Projection (VIP) values for the predictive and orthogonal functions, given in Figure 7, demonstrate that the most important predictive parameters (VIP >1) were, in order, Rb, $\delta^{18}O$, B, Li, Sr, $R^{87}Sr/^{86}Sr$, Ni, Co, Mn and $\delta^{11}B$.

The score projection is given in Figure 8 as a 3D projection of the OPLS space as well as 2D projections of the first two predictive functions for each state. A reasonable separation of wine samples from several states can be seen. Examples include full differentiation of SA and TAS, NSW and WA. The classification success, indicating the percentage of samples correctly assigned, is given in Table 6. In total only 63 % of samples were correctly classified, with wine samples from SA and WA achieving the highest classification rates (84% and 69%, respectively). The OPLS-DA model predictive function coefficients (Figure 9) show which variables (X) and categories (Y) are correlated; those which are significant have error bars which do not straddle '0'. The first predictive function is correlated with $\delta^{18}O$(-), $\delta^{11}B$(-), As(-), B(-), Ba, Li(-), Rb Sr and with SA(-), TAS and VIC. Function 2 correlated $R^{87}Sr/^{86}Sr$, $\delta^{11}B$, $R^{7}Li/^{6}Li$, Co(-), Li(-), Mn(-), Ni(-),Rb, Sn with NSW(-) and WA.

Although improved by adding trace element concentrations, this degree of classification is still not at a suitable level for regulatory use. One reason for this low classification rate based on state is simply the diversity of regions within any one state. Ideally a discrimination based on region would allow modelling to account for the diversity of origins.

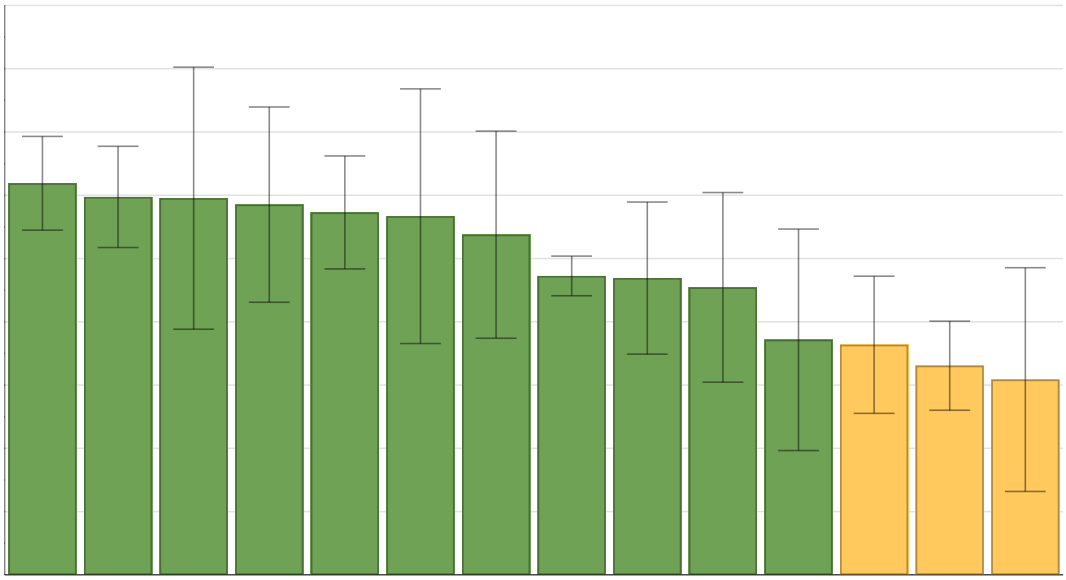


Figure 7. *Variable Importance for Projection* values for OPLS-DA of Australian wines classified by State (stable isotope ratios of B, Li, O, Sr and selected significant trace elements). Eigen values: λ_{P1}=2.1, λ_{P2}=1.7, λ_{P3}=1.3; λ_{O1}=2.3.

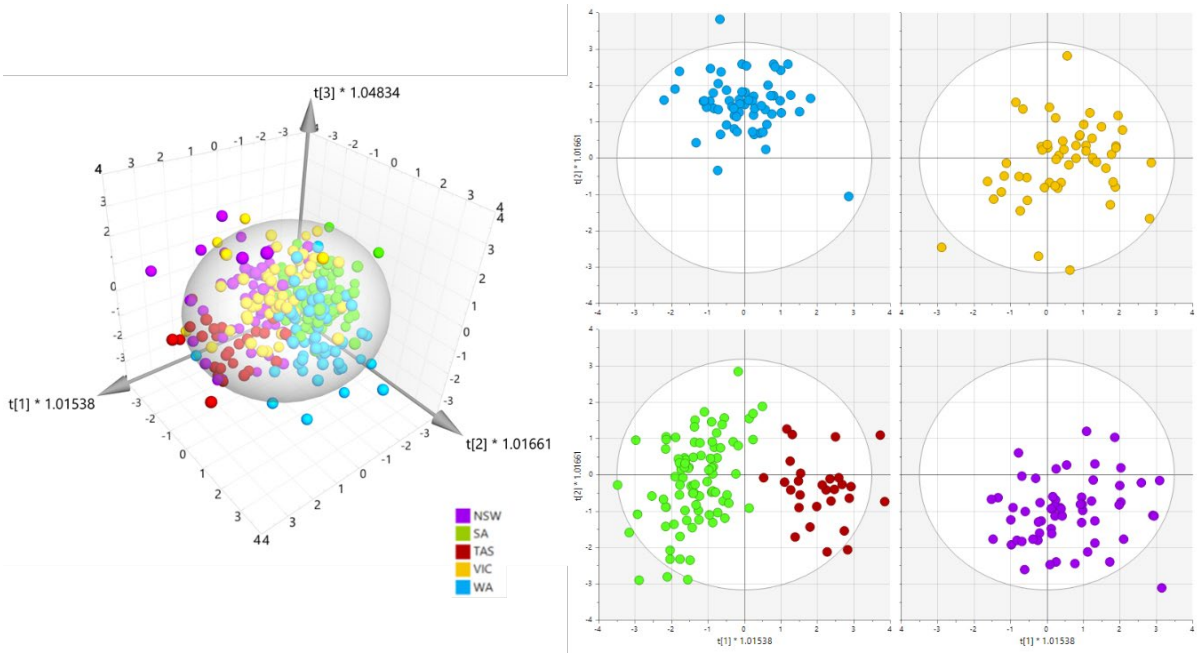


Figure 8. 3D score plot of OPLS-DA classified by state (isotope ratios and significant trace elements).

Table 6. Classification rate of samples discriminated using OPLS-DA (isotope ratios and significant trace elements) differentiated by state

Designated state		Assigned state					
	Count	Correct	NSW	SA	TAS	VIC	WA
NSW	58	52%	30	20	2	3	3
SA	89	89%	0	79	0	0	10
TAS	28	50%	8	0	14	3	3
VIC	55	51%	5	14	1	28	7
WA	62	79%	1	8	0	4	49
Total	**292**	**68%**	44	121	17	38	72

By zone

Discriminant analyses are limited in the number of groups that the model can contain by the number of analytical parameters; it is not possible to subdivide the dataset according to region. There are 38 individual regions represented in the dataset of this study out of 64 actual Australian regions. This can be reduced to 15 production zones, as defined by Wine Australia.

Differentiation based on zone was carried out using OPLS-DA with isotope ratios of B, Li, O, Sr and concentrations of all trace elements except Be and Pb (to avoid bentonite bias as variables). All variables were $\log_{10}$-transformed before analysis. Because there were insufficient individual numbers in some zones, samples from these zones were deleted: Big River, Central Victoria and North West Victoria. The total variance explained by 10 predictive functions was 85%, with one orthogonal function (Eigen values: λ_{P1}=2.8, λ_{P2}=2.3, λ_{P3}=1.7…; λ_{O1}=0.8). The VIP values indicated that all four isotope ratios as well as all trace elements were important for the model. Figure 10 shows the 3D and 2D scores for individuals, colour coded by state for clarity. The closeness of spread in some cases is reduced compared to the previous model (e.g. for WA). In the case of individuals from zones in NSW there were two distinct clusters, with the upper comprising largely wines from Tumbarumba. The correlations between variables and zones is given in Table 7. The main variables

involved were $\delta 1^{8}O$, $R^{7}Li/^{6}Li$ As, B, Co, Cr, Li, Mn, Ni, Rb, Sn, Sr, Zn**.** There was no correlation with $R^{87}Sr/^{86}Sr$ in this model.

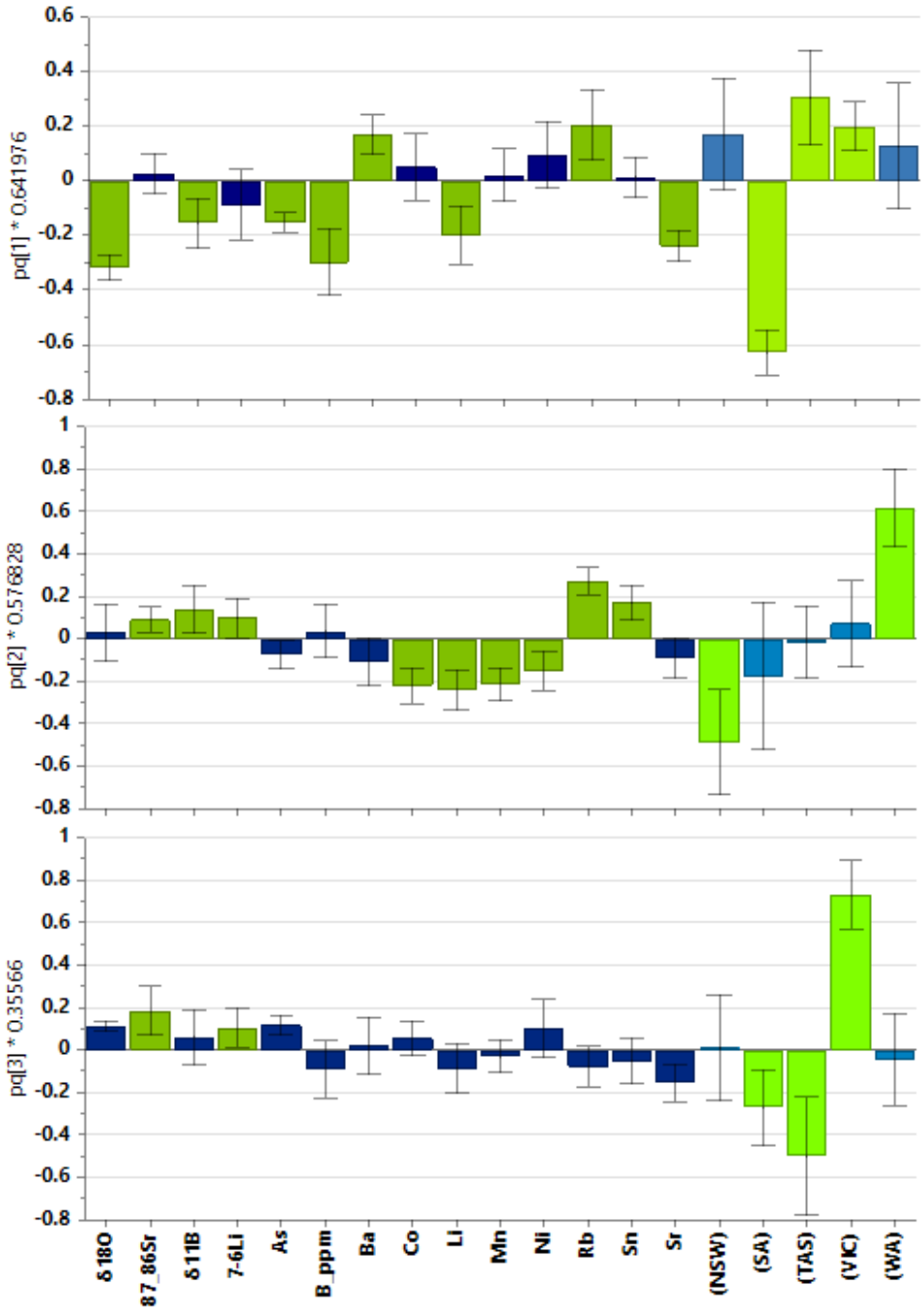
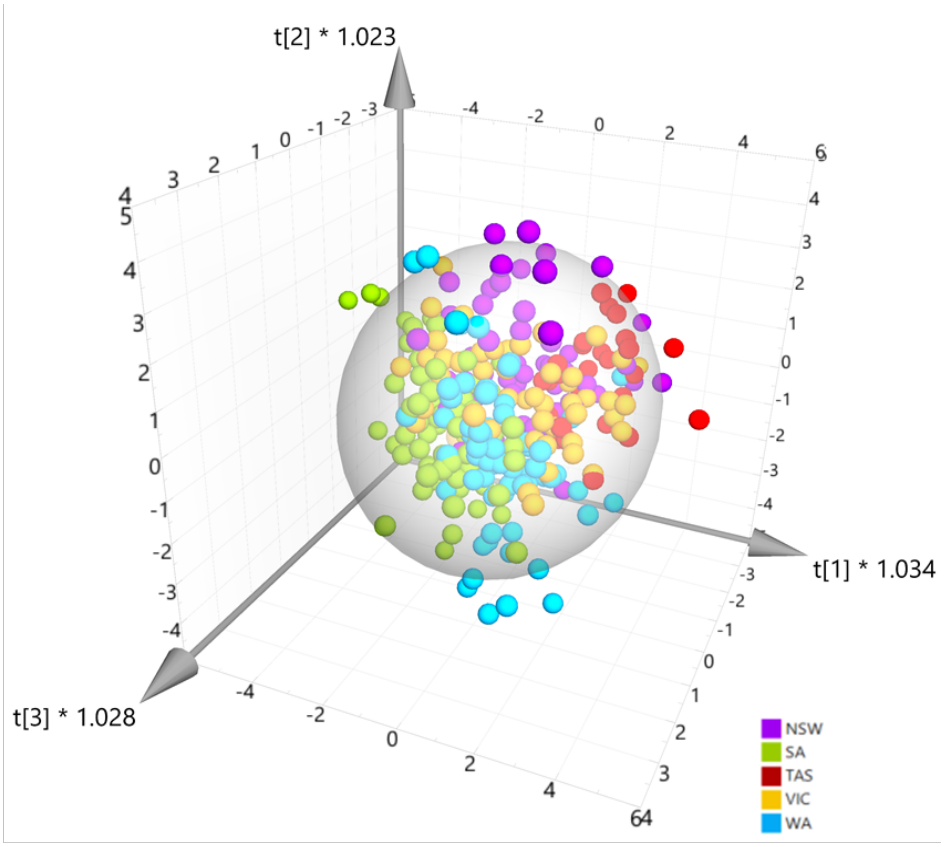


Figure 9. X and Y coefficients for OPLS-DA by state



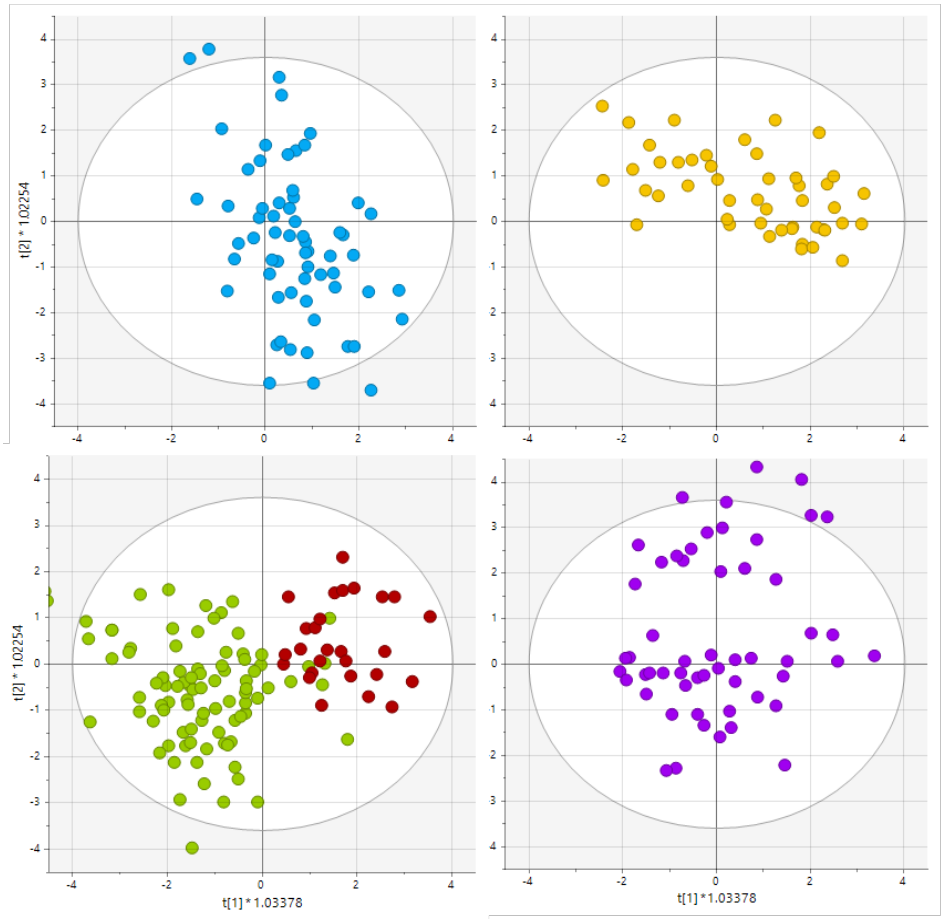


Figure 10. Score plots from OPLS-DA (isotope ratios and all trace elements except Be and Pb) differentiated by zone

Table 7. Summary of significant X and Y coefficients in OPLS-DA based on zone categories and the wine regions which constitute them.

Predictive function	Variables	Zone names
1	$\delta^{18}O$(-)†, As(-), B(-), Cr(-), Li(-), Mn(-), Rb, Sr, Zn	South West Australia, Lower Murray, Port Phillip, Western Victoria
2	B(-), Co, Ni, Zn	Greater Perth, Western Victoria, Southern NSW
3	$\delta^{18}O$, R $^{7}Li/^{6}Li$, Sn	Mount Lofty Ranges, Central Ranges

†designates a negative correlation

The overall classification rate was 66% (Table 8) although nine zones out of 17 had rates >50% with six >70%. Samples from Central Victoria were 100% correctly classified (with no other wines misclassified to this group), as were those from Southern NSW. Wines from SW Australia were 92% correctly classified, although a similar number of samples were misclassified here from other groups.

Defining discriminant models based on more refined geographic boundaries has demonstrated that isotope ratios and selected trace elements have an inherent discriminating power. Many of these boundaries (Geographic Indications) were, however, defined without reference to their geological characteristics.

Table 8. Classification rate of samples discriminated using OPLS differentiated by zone (where N_{ZONE} >4) using isotope ratios (excluding lead isotope ratios) and all trace elements except Be and Pb

Designated zone			**Assigned zone**												
Zone name	**Count**	**% Correct**	Greater Perth	SW Australia	Barossa	Fleurieu	Lower Murray	Limestone Coast	Mount Lofty Ranges	Port Phillip	Western Victoria	Central Ranges	Hunter Valley	Southern NSW	Tasmania
Greater Perth	12	8	1	6						1				3	1
SW Australia	50	92		46				1	1	2					
Barossa	20	65		3	13	2		1						1	
Fleurieu	17	65		3	2	11	1								
Lower Murray	13	100					13								
Limestone Coast	24	75		4		1		18							1
Mount Lofty Ranges	15	27		8			2	1	4						
Port Phillip	32	56		3		1				18	1			1	8
Western Victoria	15	33		4			1	2		2	5			1	
Central Ranges	21	57		1		1						12			7
Hunter Valley	15	33		2	1		3					3	5		1
Southern NSW	18	100												18	
Tasmania	28	82		2				1		2					23
Not included	12				3	4	1				1	1		2	
Total	**292**	**67**													

7.3 Differentiating Australian wines based on geology – Classification by Australian Crustal Elements (ACE) designation

Another approach to choosing a class for the wines is to consider the geology and geophysical aspects on which the vineyards are sited. Given the extreme complexities of soils across Australia it would be difficult to classify the soil type of the soil horizons because of the fine level of detail this would give. By taking a broader picture and looking at the bedrock or subsoil types a much coarser classification can be adopted. The continent of Australia has been divided into eight mega-elements which are "magnetic and gravity domains, or crustal elements having some cohesion and unifying features, such as similarity in gross geophysical character and enclosure within common crustal boundaries" (Shaw et al. 1996). Within that framework, the Australia Crustal Elements (ACE) are generalised geophysical domains that considered to represent individual pieces of upper crust formed from the Archean until the Paleozoic period (Shaw et al. 1996).

There are 19 ACEs which lie under wine-growing regions (Figure 11); however, 94% of the Australian crush in 2017 was produced from vineyards sited on just three crustal elements (Table 9)! This is largely due to the predominance of bulk or low-cost wine made in the Murray Darling-Swan Hill, Riverina, Riverland and Limestone Coast regions. The Australian wine dataset used in this study encompasses 15 ACE designations, which, although only representing a low volume of the Australian crush, may represent a higher value product.

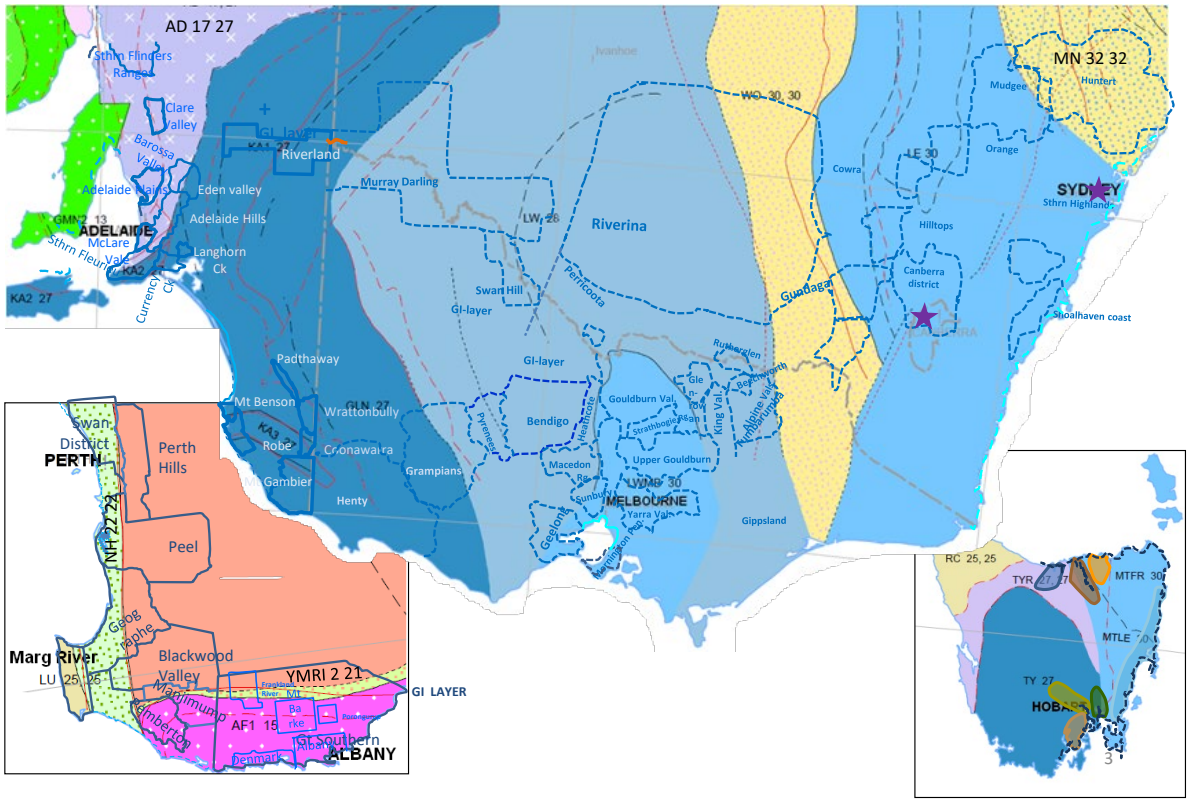


Figure 11. Map of major Australia wine regions superimposed over the Australia Crustal Elements.

Table 9. Australian Crustal Elements (ACE) on which vineyards grow listed in order of % total crush.

ACE symbol	No. wines/ ACE	State	Geographical areas per ACE	% weight of total (V17 crush)
LW 28	22	VIC	NE Victoria zone, Murray Darling-Swan Hill, Riverina, Perricoota, Pyrenees, Heathcote, Bendigo, Macedon Ranges, Sunbury (NW corner), Grampians (SW corner), Geelong	56%
KA1 27	16	SA	Eden Valley, Riverland, Limestone Coast	29%
AD 17,27	49	SA	Adelaide Hills (W side), Barossa Valley, McLaren Vale, Clare Valley, Southern Flinders Ranges, Adelaide Plains	9%
KA2 27	0	SA	Adelaide Hills (E side), Fleurieu (SE corner),	3%
KA3 27	24	SA	Coonawarra	2%
LWMB 30	28	VIC	Goulburn Valley, Upper Goulburn, Strathbogie Ranges, Yarra Valley, Mornington Peninsula, (Gippsland zone)	1.2%
LU 25, 25	19	WA	Margaret River	1.1%
LE 30	29	NSW	Central Ranges zone, Cowra, Hilltops, Canberra, Gundagai, Southern Highlands, Shoalhaven	1.0%
MTFR 30	15	TAS	Tasmania: 'Tamar Valley',' North East'	0.5%
MTLE 30		TAS	Tasmania: 'East Coast', 'Coal River Valley'	
AF1 15	17	WA	Great Southern, Manjimup, Pemberton	0.3%
GLN 27	9	VIC	Grampians (NE corner), Henty	0.20%
MN 32, 32	15	NSW	Hunter zone, Northern Slopes zone	0.17%
NH 22, 22	16	WA	Geographe, Swan District	0.26%
WO 30, 30	10	NSW	Tumbarumba, central Gippsland zone, non-regional NE Victoria	0.06%
NE32	0	NSW /QLD	Granite Belt, Hastings River, New England, South Burnett	0.04%
MN 2, 13	0	SA	The Peninsulas (Yorke) zone	0.011%
Y 2	10	WA	Peel, Perth Hills, Blackwood Valley, Great Southern: Frankland River	0.003%
TYR 27	13	TAS	Tasmania: 'Huon/Channel', 'Derwent Valley', 'Coal River Valley' (W side)	
TYR 27, 27		TAS	Tasmania: 'North West', 'Tamar Valley' (W side)	

To establish whether discrimination can be achieved using such geological provinces, the dataset was reanalysed by ANOVA using ACE as classifier; the results are given in Table S6. When ACE was used as the classifier, all parameters were significant ($P < 0.01$) except for Cr. Given the high number of samples whose Be concentration was below LOD the significant ANOVA results were disregarded and Be was excluded from analysis. Although there are several identified crustal elements identified in Tasmania given the small number of samples available these were included as one ACE type.

The first six factors of the OPLS-DA using ACE as classifier explained 62% of the original variance. Because of the higher number of classes involved in the analysis, nine significant predictive functions were required, although the VIP values of all parameters was within the limits of significance (95%). Figure 12 shows a 3D plot of the OPLS scores of the first three predictive functions with individuals colour coded by state for clarity. There is evidence of individuals grouping tightly within state groupings. Because of the higher number of predictive functions required in this model, however, it is difficult to visually assess how the model reflects the initial data. The significant correlations of the

predictive functions with the variables and explicative groups are summarised in Table 10. The variables which correlated with most functions were the isotope ratios of B, O and Sr, and the trace element concentrations B, Ba, Co, Mn, Ni, Rb, Sr and Zn. Only 10 of the 15 ACE groups were well represented in the OPLS model. This is further borne out by the mediocre 60% overall classification rate of all ACE types designated (Table 11). The classification rate was >75% for only five ACE groups with only nine groups in total.

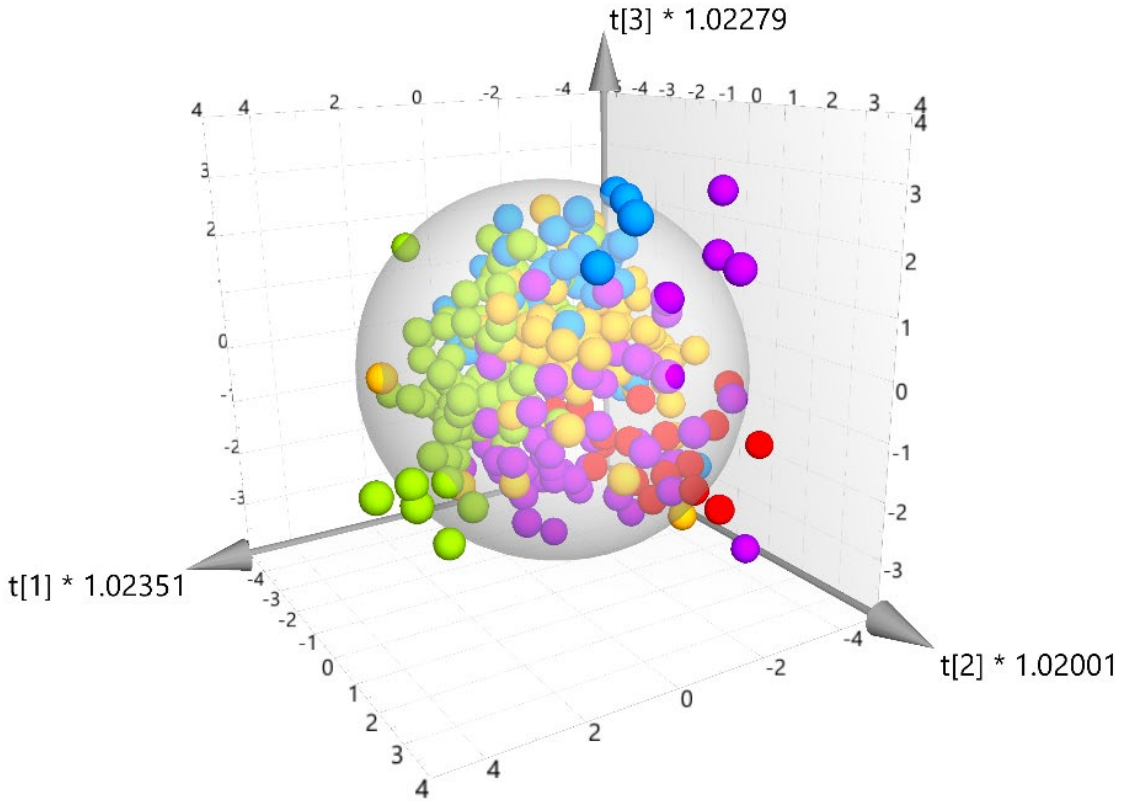


Figure 12. 3D OPLS-DA score plots categorised by ACE type identified by state

If it is the influence of physical/geological/pedological/climatic factors – all that sums up the notion of 'terroir' – that defines the chemical provenance markers of a wine, then the key parameters explored in this study should be effective in correctly classifying wines through discriminant analysis. It was posited that combining isotope ratios of boron, lithium, oxygen and strontium would explain both basement/rock characteristics and the water source, modified by climate, soil type, water retention etc. Bringing these data together with concentration data from carefully selected dissolved trace elements has indeed allowed a good proportion of the variance to be explained using ACE categories. Oxygen, strontium and boron isotope ratios are the most prevalent with the trace elements B, Ba, Co, Mn, Ni, Rb, Sr and Zn. However, higher levels of correct classification would be expected for a truly robust and valid model. Even when groups have been fully assigned to the originally designated group, the models elaborated have affected other individuals in that group. This is illustrated in Table 11.

Table 10. Summary of significant X and Y coefficients in OPLS-DA based on ACE groups and the wine regions that constitute them

Predictive function	Variables	ACE groups	Regions
1	$\delta^{18}O$, B, Li, Rb(-)†, Sr	LWMB30(-) KA1 27	Mornington Peninsula, Yarra Valley (VIC) Riverland, Eden Valley (SA)
2	Ba, Co, Mn, Ni, Zn	LU 25 25(-) MLTE30 KA3 27(-)	Margaret River (WA) Tasmania (TAS) Coonawarra (SA)
3	Li(-)	LE30(-) GLN 27 NH 22 22	Orange, Mudgee, Hilltops, Canberra District (NSW) Grampians (VIC) Swan District, Geographe (W) (WA)
5	R $^{87}Sr/^{86}Sr$(-), B, Ba, Rb, Sr	WO 30 30(-)	Tumbarumba (NSW)
6	$\delta^{18}O$(-),$\delta^{11}B$, Cd(-), Co, Zn	AF15	Great Southern, Pemberton (WA)

†designates a negative correlation

Table 11. Classification of Australian wines using isotope ratios and selected trace elements calculated by OPLS-DA with Australia Crustal Elements as category.

Designated ACE		Assigned ACE group														
		Correct	LW 28	LE 30	MN 32 32	WO 30 30	AD 17,27	KA1 27	KA3 27	TYR 27 (27)	MTLE 30	LWMB 30	GLN 27	NH 22 22	Y 2	LU 25 25	AF1 15
	Count																
LW 28	20	20 %	**4**	2	1		8		1			1		2		1	
LE 30	29	62 %		**18**	1	2	3	1				2		1			1
MN 32 32	15	20 %	1	3	**3**		4	2	1					1			
WO 30 30	10	50 %		2		**5**		1				1		1			
AD 17,27	47	87 %					**41**		1					2		2	1
KA1 27	16	63 %			1		4	**10**	1								
KA3 27	24	79 %		1			2	0	**19**								2
TYR 27	13	31 %		3						**4**		5				1	
MTLE 30	6	0 %		6							**0**	0					
LWMB 30	28	61 %	1	4			2					**17**		1			3
GLN 27	9	0 %	4				3						**0**				2
NH 22 22	16	81 %	1			1								**13**			1
Y 2	10	0 %					5		1			1		2	**0**		1
LU 25 25	19	89 %			1		1									**17**	
AF1 15	17	94 %							1							0	**16**
Not incl.	13			4			4					2		1		2	
Total	**292**	60 %	42	9	7	76	14	23	5	0	29	0	25	0	23	27	42

7.4 Assessment of annual variation in isotope ratios

Individual isotope ratios for all wines in the three vertical series studied are depicted in Figure 13 and listed in Table S7. The average and standard deviation (SD) for each of the three vertical series are given in Table 12 along with a pooled SD for each isotopic ratio. As a first approximation of validity, the variance due to annual vintage differences over the 10 years examined was compared the natural variance of all Australian analysed samples. This was assessed using the Fisher statistic, $F_{AUS/ANN}$ and compared to the critical value F_{CRIT} (291, 23). At P=0.01 the significant isotopic ratios were those of ^{11}B, ^{7}Li, $^{206}Pb/^{204}Pb$ and ^{87}Sr, while at the lower level, P=0.05, the ratio for $\delta^{18}O$ was also significant, meaning that the variance between samples from across Australia was greater than that observed in three separate vineyards over a decade. The extent of these variations, however, needs to be considered if specific value ranges are to be used to define the authenticity of a particular region.

The SD on the $\delta^{18}O$ value for the Australian data is commensurate with the literature value for Germany (Christoph et al. 2015). This implies that Australian wine regions are just as subject to annual variations in weather as Europe and that it may well be necessary to establish a similar database for Australian wines. The higher $\delta^{18}O$ SD for the Hunter Valley Semillon wine is driven by three years returning values < 1.5‰. These low values were determined not to be outliers using Dixon-Q. The SD on $\delta^{11}B$ was equally high for this region; negative values (-4.9 and -7.1 ‰) were measured for two vintages (2015 and 2016) and these were deemed as Dixon r_{20} outliers and were excluded from data (Table S7). However, overall $\delta^{11}B$ annual variance was less than that expressed across all Australian samples, indicating this parameter could be meaningful. This was also the case for $R^{206}Pb/^{204}Pb$ and $R\,^{87}Sr/^{86}Sr$.

Table 12. Average values for isotope ratios of three single-vineyard wines over a period of 10 years

	Chardonnay **Margaret River, WA**	**Riesling** **Clare Valley, SA**	**Semillon** **Hunter Valley, NSW**	**Pooled SD_{ANN}**	**$F_{AUS/ANN}$[§]**
$\delta^{11}B_{\ NIST951}$	31.3 (2.2) [†]	35.2 (2)	13.7 (3.9) [‡]	3.6	2.59
$R^{7}Li/^{6}Li$	12.15 (0.2)	12.33 (0.11)	12.27 (0.11)	0.21	2.25
$\delta^{18}O_{\ V\text{-}SMOW}$	5.2 (0.8)	8.7 (1)	3.9 (2.7)	1.8	1.88
$R\,^{206}Pb/^{204}Pb$	17.3 (0.4)	17.4 (0.2)	17.3 (0.3)	0.5	2.59
$R\,^{206}Pb/^{207}Pb$	1.189 (0.01)	1.14 (0.016)	1.124 (0.012)	0.03	1.73
$R\,^{208}Pb/^{207}Pb$	2.355 (0.02)	2.326 (0.023)	2.334 (0.03)	0.04	1.56
$R\,^{87}Sr/^{86}Sr$	0.71037 (0.0003)	0.71201 (0.00082)	0.71073 (0.00018)	0.0014	4.35

[†]standard deviation in parenthesis; [‡]excludes values for 2015 and 2016 as these were identified a r_{20} outliers.
[§]F_{CRIT} (P=0.01/0.05) = 2.297/1.78; $df_{AUS,\ ANN}$ = 291,23

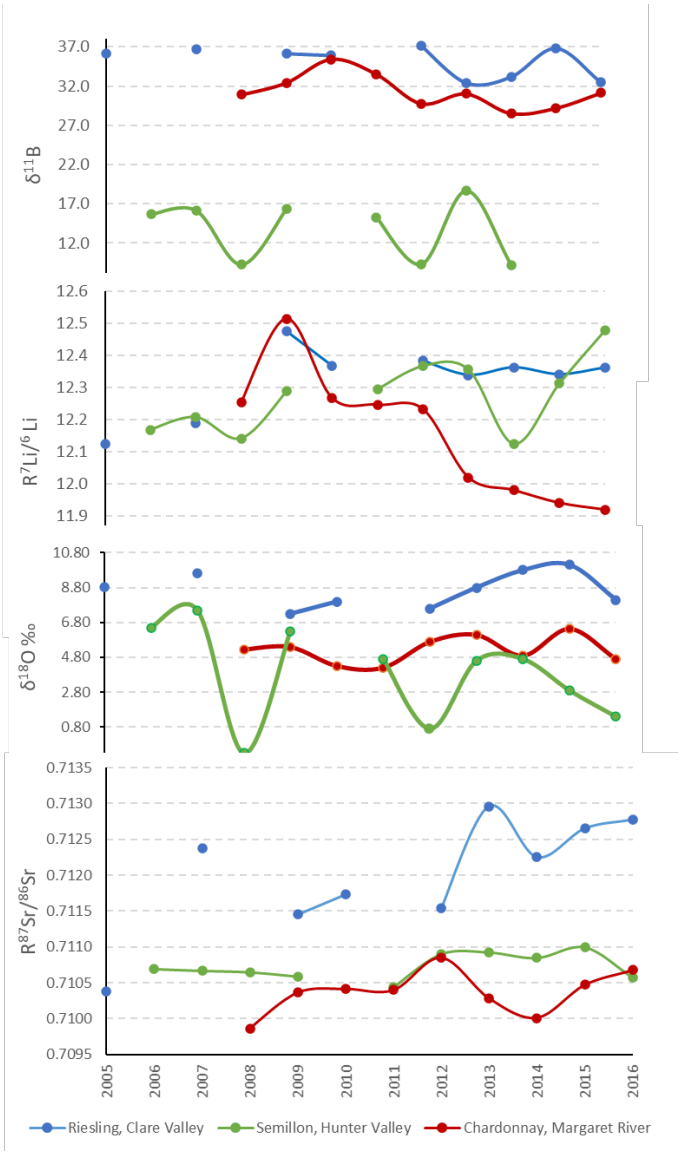


Figure 13. Annual variation in isotope ratios of boron, lithium, oxygen and strontium of three vertical series of wines

7.5 Assessment of potential confounding factors

7.5.1 Effect of grape cultivar

Grapes from seven common cultivars of *V. vinifera* were gathered from two separate vineyard blocks, vinified and analysed. The individual results of isotope ratios and element concentrations for the different grape cultivars are given in Table S8. The vineyard block average (overall, white and red cultivars) was calculated for each parameter and the SD across both blocks was pooled.

The pooled variance – calculated from the difference between the isotope ratios of each varietal wine sample and the block average separated by colour (red or white varieties) – was compared to that arising from all Australian wine samples, using a Fisher test (Table 13). The values of $F_{AUS/CULT}$ were significant for all parameters except for $\delta^{11}B$ for which large variations from the Nuriootpa block average were observed. These % differences are shown in Figure 14. Cultivar appears not to have an influence on any Li or Sr isotope ratios where the difference from the block average by colour is < 1.1 %. Values of $\delta^{18}O$ also appear to vary considerably up to 12% with Shiraz and Pinot Noir showing the largest negative and positive differences, respectively. Such differences in $\delta^{18}O$ would be ascribable to differences in evapotranspiration.

In summary the isotope ratios of Li, O, and Sr differ due to grape cultivar by below 10% and are therefore useable. More care is required when using the $\delta^{11}B$ data from this experiment, as the

variation for this parameter is much greater than other isotope parameters. A repeat of this experiment with an additional vineyard site would allow this larger variation to be investigated.

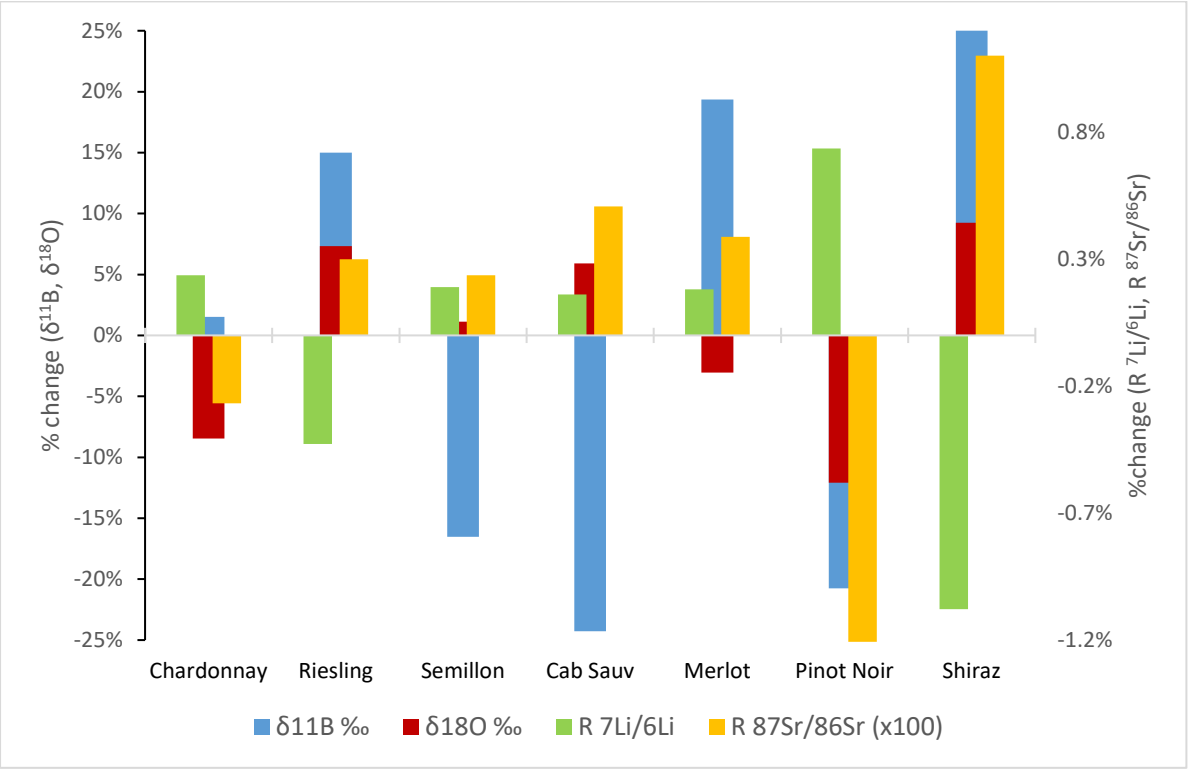


Figure 14. Percentage change from block average for different cultivars grown on two different sites

Table 13. Variation due to grape cultivar - pooled standard deviation, Fisher statistic

	Mean of all samples	**Pooled SD†**	**F AUS/CULT‡**
$\delta^{11}B_{NIST951}$	21.6	6.1	1.0
$R^{7}Li/^{6}Li$	12.49	0.10	10.2 ***
$\delta^{18}O_{V\text{-}SMOW}$	8.28	1.28	3.7 ***
$R^{206}Pb/^{204}Pb$	17.310	0.19	18.1 ***
$R^{206}Pb/^{207}Pb$	1.140	0.0120	8.8 ***
$R^{208}Pb/^{207}Pb$	2.346	0.03	2.1 *
$R^{87}Sr/^{86}Sr$	0.71124	0.00008	1,337 ***
Σelements§	2194	348	na

† 12 DF; ‡ F AUS/CULT (291,12 DF);

Significance represented by *** 99%, **95%, *90% §B, Ca, K, Mg, Na, P, S, Se, Si

7.5.2 Effect on lead isotope ratios from bentonite use

The widespread use of bentonite in white winemaking to remove pathogenesis-related protein that causes heat instability (Waters et al. 2005) can be considered one of the biggest confounding winemaking factors in looking for an analytical tool to determine wine provenance. For example, analysis of rare earth elements (REE) has also been proposed as a powerful authentication tool (Aceto et al. 2018, Barbaste, Medina, et al. 2002, Capron et al. 2007, Galgano et al. 2008), despite the analytical challenges associated with very low concentrations. The masking effect of bentonite on REE profiles used as provenance markers has been demonstrated (Catarino et al. 2008, Jakubowski et al. 1999, Rossano et al. 2007). It was also demonstrated several years ago that adding bentonite to a wine increase the concentration of Pb and several other metals in finished wine (Stockley et al. 2003).

It can be hypothesised that using bentonites from different origins would contribute Pb expressing the isotope ratios of the bentonite origin, thus rendering the isotope ratios useless in provenance testing. This was tested by treating three protein-unstable wines with bentonite clays manufactured in five different countries covering the different forms available. From a one-way ANOVA of isotope ratio (results in Table S9) and mineral elements (concentrations given in Table S10), significant differences ($P < 0.01$) between bentonites were only found for the three Pb isotope ratios. Two groups of wines, each treated with three bentonites, gathered together and were significantly different (Tukey post-hoc test) from each other and the untreated wines. These groupings also correlated with the residual Pb concentration as seen in Figure 13 where concentrations ranged from 1.7 µg L^{-1} in the control wines (light green) and 6–15 in group 1 to 18.8–28.7 µg L^{-1} in group 2 (blue, pink, red). The same trend was followed by Li and Na and the inverse for Al concentration (summary data not shown). These data suggest that it would not be judicious to rely on any of the Pb isotope ratios studied here when attempting to define the provenance of a white wine without knowing its processing history. Although the majority of white and rosé wines require bentonite treatment, its use in commercial red wine production is not common as the polyphenol content complexes with the protein during fermentation, avoiding the requirement for later removal (Waters et al. 2005).

Given this information it is recommended that lead isotope ratios are not used in generalised discriminant models.

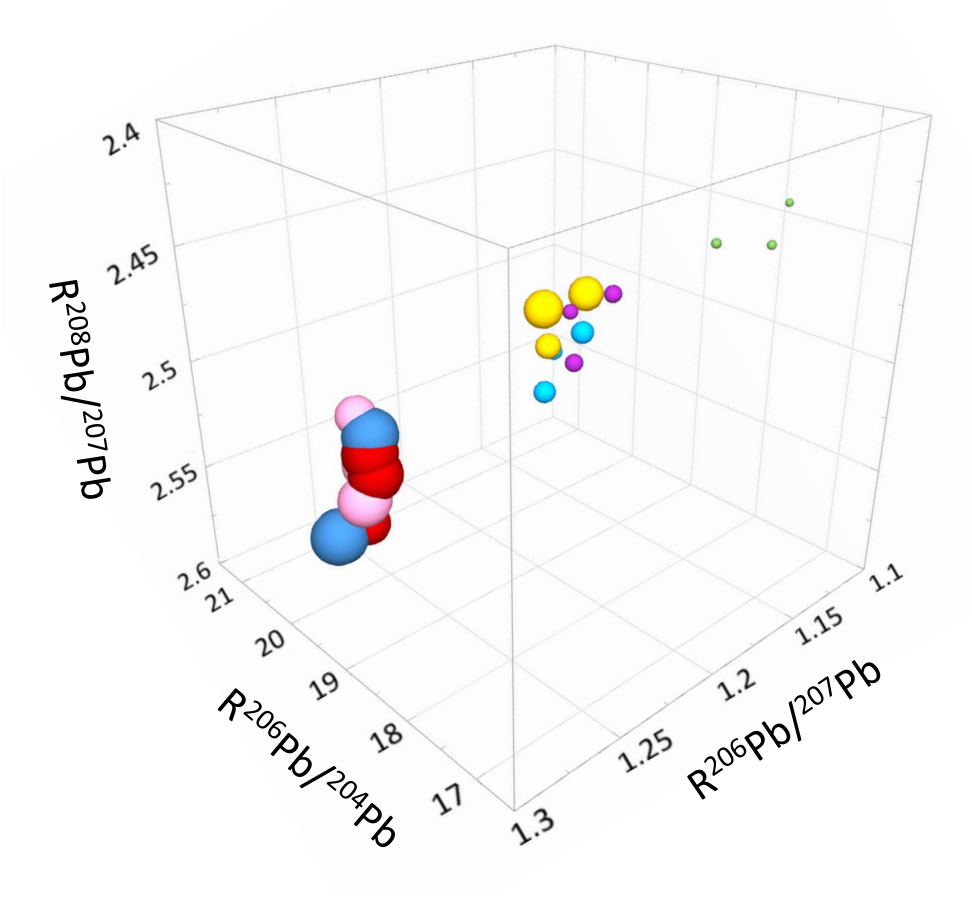


Figure 15. Variations in lead isotope ratio in wines treated with different bentonite clays; residual lead concentration is indicated by sphere volume (0 – 29 µg L^{-1})

7.5.3 Effects from different bottle glass on boron-11

With the suggestion that $\delta^{11}B$ may allow differentiation between Australian and overseas wines careful consideration of confounding factors is required. There are two potential explanations for differences in ^{11}B arising from exogenous supplementation. Vineyard foliar supplementation with boron is often used in certain regions (Christensen et al. 2006), including in Australia, to counteract known low levels. There is evidence that boron foliar sprays can influence the bulk $^{11}B/^{10}B$ ratios in horticultural produce, although in this example, the foliar sprays were applied directly to the vegetables analysed rather than requiring transport from the vine canopy to the grapes (Vanderpool and Johnson 1992).

The other possible influence is the impact of bottle glass, as increasing amounts of recycled glass or cullet are added during glass manufacture, which in Australia can amount to 35-60% (Anon. 2017). If this cullet is contaminated with borosilicate glass from domestic glassware it could result in a higher ^{11}B in wines bottled in Australia, but not necessarily in Australian wines. This latter theory was tested by filling a set of wine bottles manufactured in five countries with a commercially produced red wine. A coloured glass, commonly referred to as Antique green, and a colourless flint glass from each country were analysed after five months of storage. The average $\delta^{11}B$ value was 31.4 ‰ (SD 0.6) and no statistical difference between country of origin or colour was determined (Table 14). Until other possible sources of influence come to light it can be considered that the differences in $\delta^{11}B$ reported here are indeed due to geographical provenance.

Table 14. Variation in $\delta^{11}B$ as a function of bottle glass origin and colour

Bottle origin	**Colour designation**	**$\delta^{11}B_{NIST951}$**		**sum metals**
		Mean	**SD**	**(mg L^{-1})**
Australia	Antique green	32.0	1.1	2767
	Arctic Blue	31.6	1.3	2724
	Flint	30.8	1.7	2786
China	Antique green	31.1	1.3	2658
	Flint	30.8	0.7	2626
USA	Antique green	30.7	1.9	2640
	Flint	31.5	1.2	2644
NZ	Antique green	31.4	1.4	2684
	Flint	32.8	0.8	2661
UK	Antique green	31.3	0.8	2656
	Flint	31.0	1.8	2673
-	Plastic	31.5	3.0	2717
	Mean	31.4		2686
	SD	0.6	1.9% =>	51

8. Outcomes and conclusions

The project was successful in developing tools to discriminate Australian wines from overseas wines using isotopic analysis and OPLS-DA statistics, specifically isotopes of strontium, oxygen, boron and lithium. The model developed using 292 wines from Australian regions and 94 international wines demonstrated an overall successful classification rate of 92.5 % for all wines and 97.3% for Australian wines. The main driver for this success was demonstrated to be boron isotopic ratios, with significant inputs from oxygen and strontium isotopic ratios. Some success was also achieved in using isotopic ratios for the identification of the region of origin of Australian wines, mostly through the impact of subsoil strata on strontium isotopes, with a classification success rate of around 60%. The lower levels of successful classification for regions is strongly linked to the underlying similarity of geology that contributes to the subsoil composition of many Australian grapegrowing regions. This information will provide important baseline data for any future studies of analytical methods to determine regional origin of Australian wines.

Investigation of the impacts of vintage and variety on the use of these isotopic ratios showed they did not have a significant negative effect on the use of the models to classify if a wine was of Australian origin. A study of the impact of bottle source and type on the boron isotopic ratios found in packaged wine demonstrated that the bottle did not make a significant contribution in comparison to the differences introduced by wine origin.

The project also made important strides in understanding why some isotopic and elemental analyses that have been previously promoted as tools for identification of provenance have not been successful. A specific example is the use of lead isotopic ratios, which were clearly shown in this project to be unsuitable for classification of white wines due to the significant amounts of lead introduced by the use of bentonite during winemaking. Analysis demonstrated that the lead isotopes were more useful at indicating the regional source of the bentonite used, an internationally traded commodity, than the wines' origin. Similar influences were noted for efforts to use relative trace metal concentration ratios to identify the provenance of Australian wines. Overall it was clear from the statistical analysis of the data for trace metal concentrations that they did not contribute significantly to differentiation of wines from Australia and other wine-producing regions from around the world, despite these being the basis of a number of commercial offerings to determine provenance.

9. Recommendations

The success of this project in using combined isotopic ratios and statistical analysis to categorise a wine's origin will allow the provenance of a wine claimed to be Australian to be analytically determined. While by its nature this technique is not suitable for rapid in-market applications, it will provide an important addition to technologies such as smart labelling and methods that compare direct retention samples of known wines. Significantly, unlike many other methods used in authentication of wine, the use of isotopic ratios can link a wine's provenance to the underlying physical attributes of its place of origin. This is important in cases where rather than being a direct forgery of an existing Australian product, a wine is simply making claims of Australian provenance. Such cases can expose the consumer to, at best, a disappointing quality outcome and at worst possible health impacts from the use of unapproved additive or ingredients, with additional obvious negative outcomes for the reputation of all Australian wines.

The publication of the most effective isotope ratios identified in this work and the underlying data describing their typical content in Australian wines compared to wines from outside Australia will allow the adoption of this approach, in conjunction with the statistical protocols identified, by any organisation with appropriate equipment and capabilities to carry out the testing.

Another outcome of the study is the understanding gained about why some previously promoted technologies for authenticating wine have been less than successful, especially in their ability to deal with samples that have undergone different winemaking processes such as the use of bentonite. It is recommended that before adoption of any authentication approach that it should not only show a reasonable correlation with origin for the calibration data set (through methodologies such a cross validation), but also demonstrate that it can achieve similar results with a significant number of blind samples that are not part of the original sample set. Such an approach is necessary to ensure that confidence in the technology is well founded and that unseen biases are not introduced.

While this work identified the overall potential of using a combined groups of isotopic ratios to provide information on wine origin, further work is needed to clarify nature of the links between the final values of these components in wine and the underlying sources in the geology, soil, water and air. Such an understanding will allow the much larger data sets compiled in environmental and geological studies to be used in the determination of the provenance of wine as well as other agricultural products.

10. Appendices

Appendix 1: Communication

Conference presentation:

Day, M.P. Measuring up authentication: analytical tools to test wine provenance. Presented at the 16th Australian Wine Industry Technical Conference, 27 July 2016, Adelaide, SA.

Posters:

Day, M.P., Wright, C. Warwyk, C., Kirby, K., Wilkes, E.N. 2019. Australian wine provenance testing using novel isotope ratios: differentiation of production regions and country. *Poster presented at the 17th Australian Wine Industry Technical Conference*., Adelaide, 21-24 July. (available at https://awitc.com.au/wp-content/uploads/2019/07/133-New-Isotope-parameters-for-Wine-Provenance.pdf)

Day, M.P., Wright, C., Kirby, J., Wilkes. E.N.. 2019. Does bentonite fining in white wines compromise the utility of lead isotope ratios in wine provenance testing? *Poster presented at the 17th Australian Wine Industry Technical Conference*., Adelaide, 21-24 July. (available at https://awitc.com.au/wp-content/uploads/2019/07/137-Bentonite-fining-and-Pb-isotopes-in-authentication.pdf)

Day, M.P., Warwyk, C., Wilkes, E.N. 2019. Does the type or origin of bottle glass affect boron isotope-ratios used for wine authentication? –*Poster presented at the 17th Australian Wine Industry Technical Conference*., Adelaide, 21-24 July. (available at https://awitc.com.au/wp-content/uploads/2019/07/116-Does-bottle-glass-affect-Boron-isotope-ratios.pdf)

Day, M.P., Wilkes, E.N., Wright, C., Warwyk, C., Kirby, J., Schmidtke, L. 2018. New isotopic markers for provenance authentication of Australian wines. *Poster presented at the Crush Symposium,* Adelaide, 25-26 September 2018.

Day, M.P., Wilkes, E.N. 2016. Determination of Australian geographic indication by strontium isotope and selected trace element analysis. *Poster presented at the 16th Australian Wine Industry Technical Conference*., Adelaide, 24-28 July 2016.

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Day, M.P., Wilkes, E.N. 2017. Measuring up authentication: analytical tools to test wine provenance. Beames, K., Robinson, E., Dry, P., Johnson, D. (eds.) *Proceedings of the 16th Australian Wine Industry Technical Conference*, 24-28 July 2016, Adelaide, SA: The Australian Wine Industry Technical Conference, Inc.: 141-143.

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Appendix 2: Intellectual Property

Nothing to report.

Appendix 3: References

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Appendix 4: Staff

AWRI staff:
Dr Martin Day
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Collaborators:
Industry partners at Xanadu (Margaret River, WA), Taylors (Clare Valley, SA), Tyrrells (Hunter Valley, NSW); SARDI Nuriootpa Station (Barossa, SA); Riverland Vine Improvement Committee Inc (Monash, SA); Dr Mark Farrell (CSIRO).

Appendix 5: Supplementary data

Table S1. Figures of merit and QC data for metal isotope ratios of B, Li, Pb and Sr and comparison of sample variance to analytical reproducibility

		$R^{11}B/^{10}B$	$R^{7}Li/^{6}Li$	$\delta^{18}O$ (‰)	$R^{206}Pb/^{204}Pb$	$R^{206}Pb/^{207}Pb$	$R^{208}Pb/^{207}Pb$	$R^{87}Sr/^{86}Sr$
Reference Materials								
Reference used		NIST 951	IRMM-016	V-SMOW	NIST 981			NIST 987
Certified value		4.04362 ± 0.00137	12.1769 ± 0.00012	0	16.937 ± 0.018	1.09300 ± 0.00005	2.370 ± 0.003	0.71034 ± 0.00026
Long-term average	δ	0.18 ‰	-0.10 ‰					-0.06 ‰
	Ratio	4.0445	12.1757		16.933	1.0933	2.370	0.71029
Reproducibility (2σ)	δ	0.6	0.13 ‰					0.07
	Ratio	0.0021	0.0015		0.006	0.0020	0.0008	0.00005
RSD (RM, %)		0.26	0.006		0.018	0.09	0.017	0.0004
N		46	135		150			61
QC sample								
Average		23.85 ‰	12.42		19.371	1.2320	2.467	0.710284
Reproducibility (2σ)		4.54	0.28		0.14	0.0010	0.04	0.00005
N		32	26		24			23
95% CI		0.98 ‰	0.06		0.030	0.0003	0.0086	0.000013
RSD (QC, %)		9.5	0.24		0.36	0.041	0.81	0.0035
Duplicates								
R^2		0.932			0.977	0.864	0.822	0.999
Slope		0.937			1.006	0.895	0.968	0.989
Intercept		1.847			-0.127	0.128	0.083	0.008
N		40	30		29	29	29	27
Comparison of sample variance to analytical reproducibility								
Pooled SD†		6.8	0.3	2.5	0.8	0.0340	0.05	0.00290
QC SD ‡		2.27	0.14	0.08	0.01	0.0006	0.02	0.00003
$F_{ALL/QC}$§		8.9	5.7	980	30.4	3200	6.9	9300

† All samples in survey, n= 386.; ‡SD taken from QC batch control samples (n = 23-33, depending on analyte) for all parameters except $\delta^{18}O$ where SD on duplicate determinations (n = 157).); §F_{CRIT} = 2.34; α=0.01; $DF_{ALL,QC}$ = 385,22.

Table S2. Mean isotope ratios for Australia and overseas wines with ANOVA results comparing all and red wines only.

All wines							Red wines only			
Parameter	Mean[†]	SD	Range	F_{ANOVA}[‡]		SE_{DIFF}	Mean[§]	SD	F_{ANOVA}[‡]	
$\delta^{11}B_{NIST\ 951}$ (‰)	29.2	6.3	8.9–47.2	310	***	14.2	29.7	6.5	170	***
	15.0	8.42	-9.2–36				15.9	8.0		
$\delta^{7}LiI_{RMM-016}$ (‰)	21.3	25.2	-67–84	0.1	ns	-	20.7	23.4	0.8	ns
	22.1 ‰	24.2	-58–82.8				22.2	24.2		
$\delta^{18}O_{V-SMOW}$ (‰)	6.22	2.43	0.28–11.38	136	***	3.46	6.46	2.58	80	***
	2.79	2.60	-2.65–9.9				2.95	2.57		
$R\ ^{206}Pb/^{204}Pb$	18.0	0.8	15.7–20.1	4.7	*	0. 2	17.6	0.6	1.3	ns
	17.8	0.7	16.0–19.6				17.7	0.6		
$R\ ^{206}Pb/^{207}Pb$	1.172	0.036	1.081–1.279	3.7	+	0.0078	1.157	0.025	2.9	+
	1.164	0.029	1.113–1.257				1.163	0.022		
$R\ ^{208}Pb/^{207}Pb$	2.44	0.05	2.30–2.54	6.48	*	0.02	2.42	0.05	0.7	ns
	2.42	0.06	2.31–2.53				2.42	0.05		
$R\ ^{87}Sr/^{86}Sr$	0.71189	0.00300	0.70544–0.72460	67	***	0.00253	0.71201	0.00273	54	***
	0.70917	0.00207	0.70473–0.71598				0.70914	0.00213		

[†] Australian wines, n=292 (top value), overseas wines, n=94 (bottom value). [‡]Compares Australia to overseas; significance indicated by *** P <0.001, ** P <0.01, * P <0.05, + <0.1;
[§] All Australian red wines, n=168 (top value), overseas red wines, n=58 (bottom value).

Table S3. Mean and SD of $R^{87}Sr/^{86}Sr$ for commercial wines from selected overseas countries

Country	N	Mean	SD	Range	Literature values for comparison	References
Argentina	6	0.70838	0.00165	0.70704–0.71106	Cordoba, 0.7104 (n=24); Mendoza, 0.7072 (n=37); San Juan, 0.7081 (n=39)	Di Paola-Naranjo et al. (2011)
Chile	8	0.70606	0.00092	0.70473–0.70746	0.70471 (n=1)	Barbaste, Robinson, et al. (2002)
France	13	0.70929	0.00119	0.70657–0.71117	Bordeaux, 0.70800–0.71050 (n=46) Rhône, 0.70764–0.70862 (n=18)	(Epova, Berail, et al. 2019) Lurton et al. (1999)
Italy	12	0.70930	0.00128	0.70746–0.71284	Chianti, 0.70830–0.70900 (n=44) Central Italy, 0.70750–0.71150 (n=28) Cesanese, 0.70900–0.71000 (n=20)	Braschi et al. (2018) Marchionni et al. (2013) (Marchionni et al. 2016)
New Zealand	18	0.70864	0.00105	0.70692–0.71103	n/a	-
Spain	12	0.71098	0.00268	0.70859–0.71598	n/a	-
USA	8	0.70833	0.00215	0.70580–0.71230	California, 0.706882 (n=1)	Barbaste, Robinson, et al. (2002)

Argentina (Mendoza Province, Mendoza: Uco Valle, Mendoza: Maipú, Aconcagua: Casablanca Valley)
Chile (Aconcagua: Casablanca Valley, Central: Maipo Valley, Central: Rapel Valley, Central: Maule Valley)
France (Bordeaux, Burgundy, Rhone, Touraine, Languedoc)
Italy (Marche, Piemonte, Puglia, Sicilia, Toscana, Umbria, Veneto)
New Zealand (Central Otago, Hawke's Bay, Marlborough, Nelson, Waipara, Wairarapa)
Spain (Andalucía, Castilla y León, Catalunya, Galicia, La Rioja, Mallorca, Valencia)
USA (California, Oregon, Washington state)

Table S4. ANOVA results for element concentrations separated as Australia vs overseas wines

Element	$N_{<LOD}$	Average[†]	SD	F_{ANOVA}[‡]	
As	61	6.1	4.2	11	**
	9	8.1	5.3		
B (mg/L)	0	5.313	2.503	0.14	ns
	0	5.245	2.631		
Ba	0	154.5	93.8	15	***
	0	125.7	96.0		
Co	0	4. 2	2.3	10	***
	0	3.2	1.5		
Cr	30	10.8	5.0	2	***
	8	14.2	7.4		
Li	5	6.4	4.9	60	***
	0	24.5	52.8		
Mn	0	1140	565	3.0	+
	0	1230	515		
Ni	32	12.6	7.2	56	***
	0	20.3	11.0		
Pb	0	4.6	3.5	22	***
	0	8.2	8.1		
Rb	0	1430	910	0.6	ns
	0	1360	930		
Sn	128	5.947	3.271	11	***
	52	5.13	4.447		
Sr	0	704	403.4	22	***
	0	548.4	338.5		
	1	858	386.9	10	**
Zn	0	734.4	364		

[†] Average concentration for Australia (top value) or overseas (bottom value) in µg L^{-1} unless stated. [‡]F calculated on $\log_{10}$ transformed data; significance indicated by *** P <0.001, ** P <0.01, * P <0.05, [+] <0.1

Table S5. ANOVA results for Australian wines only classified by state, zone or region

Parameter	$N_{<LOD}$	F_{ANOVA}†					
		by state (Y=5)		by zone (Y=16)		by region (Y=34)	
$\delta^{11}B$ (‰)	not applicable	4.9	***	7.1	***	4.6	***
$R^{7}Li/^{6}Li$	not applicable	4.4	**	6.7	***	4.4	***
$\delta^{18}O_{V\text{-}SMOW}$ (‰)	not applicable	30.6	***	23.5	***	17.5	***
$R\ ^{206}Pb/^{204}Pb$	not applicable	9.2	***	4.5	***	3.5	***
$R\ ^{206}Pb/^{207}Pb$	not applicable	8.3	***	3.6	***	3	***
$R\ ^{208}Pb/^{207}Pb$	not applicable	11.2	***	6.6	***	5.1	***
$R\ ^{87}Sr/^{86}Sr$	not applicable	6.3	***	17.1	***	15.3	***
As	61	6.7	***	9.6	***	6.4	***
B (mg/L)	0	24.3	***	14.6	***	8.3	***
Ba	0	11.6	***	6.6	***	5.5	***
Be	162	2.3	ns	2.6	**	1.9	*
Cd	126	1.9	ns	2.1	*	2.1	**
Co	0	8.6	***	6.3	***	5.2	***
Cr	31	1.7	ns	2.5	***	1.9	**
Li	5	18	***	10.6	***	8.1	***
Mn	0	10.3	***	6.9	***	4.2	***
Ni	32	5.3	***	4.2	***	4.4	***
Pb	0	2	ns	2.8	***	2	**
Rb	0	20.9	***	12.7	***	8.8	***
Sn	128	4.2	**	2.4	**	2.2	**
Sr	0	14.4	***	16.1	***	8.2	***
Zn	1	2.4	ns	3.2	***	3.2	***

significance indicated by *** P <0.001, ** P <0.01, * P <0.05, ns P >0.05

Table S6. ANOVA results for Australian wines only classified by ACE designation (n=194, m=14)

Parameter	$N_{<LOD}$	F_{ANOVA}[†]	
$\delta^{11}B_{NIST\ 951}$ (‰)	not applicable	7.6	***
$R^{7}Li/^{6}Li$	not applicable	3	***
$\delta^{18}O_{V\text{-}SMOW}$ (‰)	not applicable	25	***
$R^{206}Pb/^{204}Pb$	not applicable	5.3	***
$R^{206}Pb/^{207}Pb$	not applicable	4.5	***
$R^{208}Pb/^{207}Pb$	not applicable	7.4	***
$R^{87}Sr/^{86}Sr$	not applicable	16.8	***
As	61	10.5	***
B_ppm	0	12.5	***
Ba	0	9.3	***
Be	162	1.9	*
Cd	126	1.8	*
Co	0	10.6	***
Cr	31	1.7	ns
Li	5	12.5	***
Mn	0	6.1	***
Ni	32	4.3	***
Pb	0	3.3	***
Rb	0	10.4	***
Sn	128	1.7	***
Sr	0	11.8	***
Zn	1	5	***

significance indicated by *** P <0.001, ** P <0.01, * P <0.05 ns, P >0.05

Table S7. Isotope ratios of three vertical series of single-vineyard wines over 12 years

Vertical series	Parameter	$\delta^{11}B$ ‰ NIST951	$^{7}Li/^{6}Li$	$\delta^{18}O$ ‰ VSMOW	$^{206}Pb/^{204}Pb$	$^{206}Pb/^{207}Pb$	$^{208}Pb/^{207}Pb$	$^{87}Sr/^{86}Sr$
Chardonnay	2008	30.9	12.25	5.3	16.6	1.19	2.37	0.70986
Margaret	2009	32.4	12.51	5.4	17.4	1.19	2.35	0.71037
River, WA	2010	35.4	12.27	4.3	16.9	1.18	2.40	0.71042
	2011	33.5	12.25	4.2	17.3	1.17	2.34	0.71040
	2012	29.8	12.23	5.7	17.7	1.20	2.37	0.71086
	2013	31.0	12.02	6.1	17.4	1.20	2.35	0.71028
	2014	28.5	11.98	4.9	17.5	1.19	2.35	0.71001
	2015	29.2	11.94	6.5	17.6	1.20	2.33	0.71047
	2016	31.1	11.92	4.7	17.6	1.19	2.35	0.71068
	Average	31.3	12.15	5.2	17.3	1.19	2.35	0.71037
	SD	2.2	0.20	0.8	0.4	0.01	0.02	0.00030
Riesling	2005	36.2	12.12	8.9	17.5	1.16	2.34	0.71038
Clare	2007	36.7	12.19	9.6	17.1	1.14	2.34	0.71237
Valley, SA	2009	36.2	12.47	7.3	17.5	1.16	2.31	0.71145
	2010	35.9	12.37	8.0	17.5	1.15	2.32	0.71173
	2012	37.2	12.38	7.6	16.9	1.15	2.33	0.71154
	2013	32.4	12.34	8.8	17.2	1.12	2.37	0.71296
	2014	33.1	12.36	9.8	17.6	1.13	2.31	0.71225
	2015	36.8	12.34	10.1	17.6	1.12	2.30	0.71265
	2016	32.5	12.36	8.1	17.2	1.14	2.31	0.71277
	Average	35.2	12.33	8.7	17.4	1.14	2.33	0.71201
	SD	2.0	0.11	1.0	0.2	0.02	0.02	0.00082
Semillon	2006	15.7	12.17	6.5	17.7	1.12	2.36	0.71069
Hunter	2007	16.1	12.21	7.5	17.4	1.11	2.37	0.71067
Valley,	2008	9.2	12.14	-0.7	17.1	1.13	2.32	0.71065
NSW	2009	16.3	12.29	6.3	17.4	1.11	2.31	0.71059
	2011	15.3	12.29	4.7	16.8	1.14	2.32	0.71044
	2012	9.3	12.37	0.7	17.5	1.14	2.33	0.71090
	2013	18.7	12.36	4.6	17.4	1.11	2.30	0.71093
	2014	9.2	12.12	4.7	17.1	1.13	2.33	0.71085
	2015	-4.9†	12.31	2.9	17.4	1.13	2.31	0.71100
	2016	-7.1†	12.48	1.4	17.5	1.12	2.39	0.71058
	Average	13.7	12.27	3.9	17.3	1.12	2.33	0.71073
	SD	3.9	0.11	2.7	0.3	0.01	0.03	0.00018
	Pooled SD	3.6	0.21	1.8	0.5	0.03	0.04	0.00140

†value omitted from average and SD calculations

Table S8. Absolute isotope ratios and element concentration for different grape cultivars grown in two separate vineyard blocks

Block	Cultivars		$\delta^{11}B$ ‰	$R^{7}Li/^{6}Li$	$\delta^{18}O$ ‰	$R^{206}Pb/Pb204$	$R^{206}Pb/^{207}Pb$	$R^{208}Pb/^{207}Pb$	$R^{87}Sr/^{86}Sr$	Element concentration (mg/L)							
										B	Ca	K	Mg	Na	P	S	Si
Nuriootpa	Chardonnay		31.7	12.40	7.78	17.677	1.153	2.388	0.71209	5	92	1723	104	50	233	252	4
	Riesling		37.4	12.26	7.97	17.649	1.159	2.341	0.71211	5	82	1015	87	73	161	383	3
	Semillon		25.2	12.42	6.87	17.943	1.163	2.391	0.71206	5	58	1251	72	91	189	279	3
	Cabernet		16.4	12.57	8.38	17.489	1.147	2.333	0.71214	4	90	1596	99	17	206	284	6
	Merlot		33.6	12.46	7.35	17.530	1.138	2.308	0.71216	8	74	1208	127	39	224	306	3
	Pinot Noir		19.1	12.48	8.53	17.888	1.155	2.307	0.71210	4	82	1161	86	20	141	240	4
	Shiraz		36.6	12.24	8.54	17.658	1.159	2.328	0.71216	6	85	1774	95	154	224	269	10
	Block average	All	28.6	12.40	7.92	17.690	1.153	2.342	0.71211	5	80	1390	96	63	197	288	5
		White	31.4	12.36	7.54	17.756	1.158	2.373	0.71208	5	77	133	88	71	194	304	3
		Red	26.4	12.44	8.20	17.641	1.150	2.319	0.71214	6	83	1435	102	58	199	275	6
	Block SD	All	8.4	0.12	0.63	0.169	0.009	0.035	0.00004	1	11	302	17	48	35	47	3
		White	6.1	0.09	0.59	0.162	0.005	0.028	0.00002	0	17	360	16	21	37	69	0
		Red	10.1	0.14	0.57	0.180	0.009	0.013	0.00003	2	7	298	18	65	40	28	3
Monash	Chardonnay		16.2	12.61	6.21	16.574	1.111	2.306	0.71028	5	73	1652	106	30	240	204	4
	Riesling		17.6	12.58	8.47	17.122	1.143	2.355	0.71030	5	94	930	111	36	217	426	5
	Semillon		13.8	12.58	8.64	16.883	1.150	2.355	0.71038	5	88	1040	104	33	207	344	5
	Cabernet		12.3	12.48	10.20	17.137	1.122	2.396	0.71040	6	84	1496	106	17	272	229	6
	Merlot		15.4	12.59	9.70	16.910	1.116	2.390	0.71044	7	86	1819	96	19	333	197	4
	Pinot Noir		11.9	12.71	6.68	17.096	1.132	2.323	0.71023	5	66	1743	85	14	211	158	4
	Shiraz		15.6	12.50	10.64	16.789	1.119	2.328	0.71054	9	75	2090	121	37	281	179	9
	Block average	All	14.7	12.58	8.65	16.930	1.127	2.350	0.71036	6	81	1539	104	27	252	248	5
		White	15.9	12.59	7.77	16.860	1.135	2.338	0.71032	5	85	1207	107	33	221	325	5
		Red	13.8	12.57	9.31	16.983	1.122	2.359	0.71040	7	78	1787	102	22	274	191	6
	Block SD	All	2.1	0.08	1.70	0.207	0.015	0.034	0.00011	2	10	420	11	10	46	99	2
		White	1.9	0.02	1.36	0.275	0.021	0.028	0.00005	0	11	389	3	3	17	112	0
		Red	2.0	0.11	1.79	0.163	0.007	0.039	0.00013	2	9	245	15	10	50	30	2
Pooled SD			6.1	0.10	1.28	0.189	0.012	0.034	0.00008	1	11	366	15	35	41	77	2

Table S9. Isotope ratios of B, Li, Pb and Sr before and after treatment with various protein-reducing bentonite clays

Bentonite	Variety	$\delta^{11}B$†	$^{7}Li/^{6}$ Li	$^{206}Pb/Pb204$	$^{206}Pb/^{207}Pb$	$^{208}Pb/^{207}Pb$	$^{87}Sr/^{86}Sr$
	Fiano	20.10	12.552	16.996	1.153	2.425	0.710013
Control	Pinot Grigio	26.61	12.719	17.365	1.141	2.448	0.713437
	Sauvignon Blanc	20.89	12.669	17.696	1.157	2.447	0.714080
	Fiano	17.00	12.494	18.783	1.203	2.489	0.709999
AG	Pinot Grigio	26.80	12.742	18.686	1.186	2.470	0.713269
	Sauvignon Blanc	20.64	12.718	18.889	1.201	2.475	0.713870
	Fiano	18.53	12.612	18.804	1.199	2.493	0.709864
EB	Pinot Grigio	26.18	12.452	18.485	1.196	2.481	0.711586
	Sauvignon Blanc	22.03	12.636	18.760	1.205	2.508	0.712104
	Fiano	17.30	12.563	20.011	1.262	2.517	0.709790
MA	Pinot Grigio	28.87	12.198	19.878	1.260	2.537	0.710773
	Sauvignon Blanc	24.18	12.316	19.728	1.267	2.549	0.711150
	Fiano	18. 20	12.593	18.573	1.199	2.473	0.709958
MG	Pinot Grigio	26.04	12.594	18.503	1.179	2.470	0.712730
	Sauvignon Blanc	19.15	12.815	18.598	1.196	2.496	0.713294
	Fiano	19.35	12.364	19.866	1.257	2.524	0.709786
VB	Pinot Grigio	27.33	12.368	19.822	1.260	2.524	0.710576
	Sauvignon Blanc	21.00	12.411	19.947	1.275	2.568	0.710940
	Fiano	26.86	12.635	19.885	1.262	2.566	0.709760
VC	Pinot Grigio	28.99	12.445	19.697	1.262	2.539	0.710651
	Sauvignon Blanc	21.31	12.492	19.770	1.262	2.530	0.711062

† expressed in ‰ relative to NIST SRM 951

Table S10. Metal ion concentrations before and after treatment with various protein-reducing bentonite clays

Bentonite	Variety	Al	B	Ca	Fe	K	Li†	Mg	Mn	Na	P	Pb†	S	Se	Si	Sr	Zn
Control	Fiano	<0.5	5.6	55.3	1.5	583	3.8	81	0.6	16.6	251	0.4	215	0.6	12.3	2.6	<0.5
	Pinot Grigio	<0.5	2.1	56.0	<1	429	2.9	55	0.6	25.5	262	1.5	126	0.6	4.2	<0.5	0.6
	Sauv. Blanc	<0.5	3.8	41.6	<1	408	2.2	64	<0.5	26.5	82	1.7	196	0.7	2.8	<0.5	0.8
AG	Fiano	1.2	5.8	63.0	2.2	575	4.4	85	0.9	20.9	249	10.1	216	0.6	15.4	2.6	<0.5
	Pinot Grigio	1.1	2.3	70.6	<1	455	3.2	63	0.9	31.7	284	15.1	142	0.7	7.8	<0.5	0.6
	Sauv. Blanc	1.1	3.8	49.6	1.5	387	2.1	64	0.7	29.5	80	17.3	201	0.7	5.9	<0.5	0.8
EB	Fiano	1.5	5.8	66.0	1.6	572	4.7	83	0.8	22.6	252	5.4	220	0.6	15.3	2.8	<0.5
	Pinot Grigio	1.5	2.3	77.4	<1	469	3.9	65	0.8	33.5	292	8.4	146	0.7	8.3	<0.5	0.6
	Sauv. Blanc	1.2	3.8	53.8	<1	394	2.8	67	0.5	31.5	80	8.4	207	0.7	5.6	<0.5	0.8
MA	Fiano	0.9	5.6	61.4	2.8	569	7.3	82	0.7	33.4	248	18.8	216	0.6	14.1	2.9	<0.5
	Pinot Grigio	0.6	2.2	68.2	1.3	446	7.6	61	0.7	42.8	274	25.7	143	0.6	6.1	0.5	0.6
	Sauv. Blanc	0.7	3.8	48.1	1.6	392	5.1	62	<0.5	42.4	81	26.1	205	0.7	4.6	0.6	0.8
MG	Fiano	1.4	5.7	62.0	1.9	576	3.2	82	0.7	35.5	251	4.5	221	0.6	15.4	2.7	<0.5
	Pinot Grigio	1.2	2.3	69.8	<1	465	2.7	64	0.8	46.4	292	6.1	148	0.6	7.9	<0.5	0.7
	Sauv. Blanc	1.2	3.8	49.3	<1	401	2.0	66	<0.5	44.7	81	6.2	211	0.7	6.0	<0.5	0.8
VB	Fiano	0.8	5.4	59.6	3.1	544	7.7	76	0.7	31.2	236	23.2	205	0.5	13.3	2.8	<0.5
	Pinot Grigio	0.7	2.3	74.6	1.8	476	6.4	66	0.8	44.8	290	27.4	146	0.7	6.5	0.6	0.7
	Sauv. Blanc	0.7	3.8	49.2	2.2	399	5.5	63	0.6	42.2	80	28.7	207	0.7	4.5	0.6	0.8
VC	Fiano	0.9	5.6	58.6	2.6	561	7.1	78	0.7	35.2	246	20.4	208	0.6	14.1	2.7	<0.5
	Pinot Grigio	0.8	2.3	71.5	1.4	471	5.7	63	0.7	46.6	293	26.5	149	0.6	6.6	0.6	0.7
	Sauv. Blanc	0.8	3.8	48.8	1.5	402	5.0	64	<0.5	45.3	82	27.3	210	0.6	5.0	0.6	0.8

All concentrations in mg/L unless indicated by †where it is expressed in µg/L