Unravelling regional typicality of Australian premium Shiraz through an untargeted metabolomics approach

Sijing Li[1], Leigh Schmidtke[1], Andrew Hall[1], Wes Pearson[1,2], Leigh Francis[2], Urska Vrhovsek[3], Silvia Carlin[3], John Blackman[1]

1. National Wine and Grape Industry Centre, Charles Sturt University, School of Agricultural and Wine Science, Locked Bag 588, Wagga Wagga, NSW 2678, Australia
2. The Australian Wine Research Institute, PO Box 197, Glen Osmond, SA 5064, Australia
3. Fondazione Edmund Mach, Research and Innovation Centre, Department of Food Quality and Nutrition, San Michele all'Adige (TN), Italy

Background and Aims

Geographical indications (GIs) are legally delineated areas used for describing the place of origin of agricultural products. GI usually affords a strong reputation for consistently producing quality goods in particular categories. In the wine context, consumers usually have preferences for wines produced from certain grape varieties originated in specific GIs.

Shiraz is one of the most iconic varieties of Australia, accounting for 29.5% of total vine plantings and 46% of total red grape crush in 2018 (Table 1). Although many regions produce Shiraz wines, experts and consumers both agree that variations exist to distinguish Shiraz wines of different GIs. This investigation is part of a larger project that aims to identify similarities and differences between Australian GIs renowned for premium Shiraz.

Table 1. Snapshot of Shiraz plantings and crush in Australia in 2018 [1].

Plantings

Source: ABS National Vineyard Survey 2015	Shiraz	All varieties
Area planted in Australia (hectares)	39,893	135,133
Percentage of total area	29.5%	
Percentage of red varieties	46.0%	

Crush

Source: National Vintage Survey 2018	Shiraz	All varieties
Tonnes crushed in 2018	429,106	1,794,182
Percentage of total crush	23.9%	
Percentage of red crush	46.0%	

Figure 2. Growing season degree-days (left) and topography (right) of Australian wine regions selected for the current study [2]. In the topography graph, the vertical lines represent the range of elevation while the median elevations are given in number.

Results and Discussion

Sample preparation and analysis

22 commercial wines produced in 2015 and 2016 vintages were selected from 6 different regions (Figure 2), using the Pivot© Profile sensory analysis method. Wines were extracted through LiChrolute-EN SPE cartridges in triplicate (3 different bottles), using an established protocol [3]. A quality assurance (QA) was created by pooling 100 mL of each wine and 1 QA sample was extracted for every 10 samples. The extracts were analysed with a GC-Q-TOF-MS installed with a 60 m DB-WAXetr column and operated in scan mode.

Raw mass spectrometry data was imported into R and processed with packages "xcms" and "CAMERA" [4]. This approach extracted 'features' (i.e. an ion of exact mass that elutes within a defined time window). Initially, more than 10,000 features were reported. This was narrowed down to 5005 by selecting features that were present in at least 80% of all samples belonging to the same region. Peak areas of the features were normalised against those of internal standards. An initial principle component analysis (PCA) was performed (Figure 3), which demonstrated robustness of the current approach, since all QAs tightly positioned near the centre of the score plot, with all samples distributed on PC 1 and 2. In addition, sample triplicates were tightly clustered.

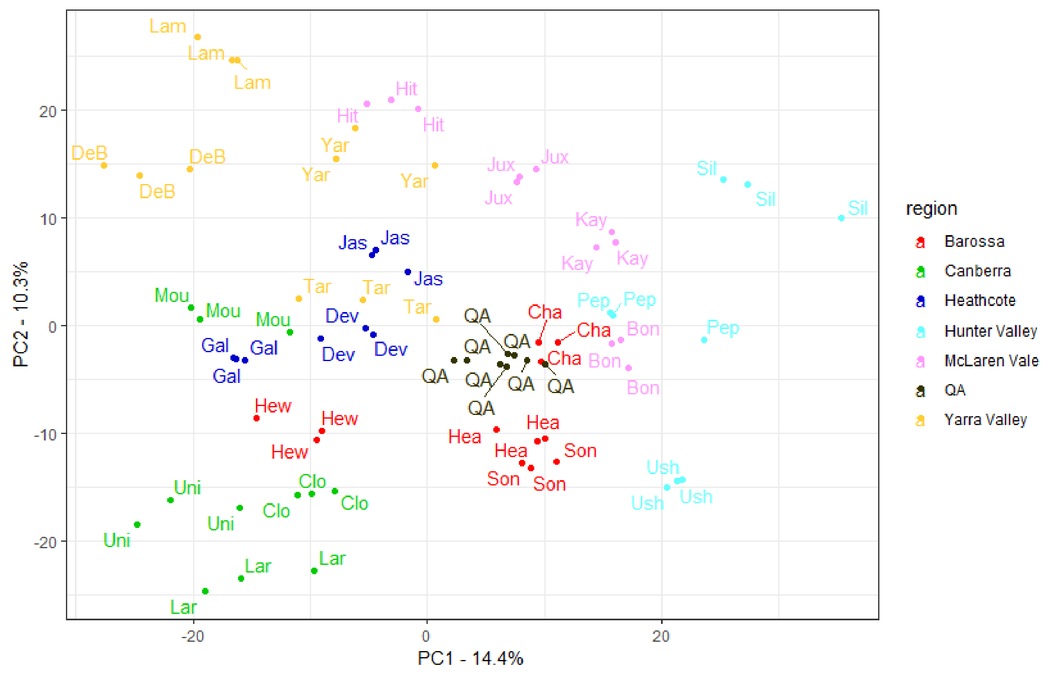

Figure 3. PCA based on 5005 features extracted from all samples, the 3-letter codes are abbreviations for wine names.

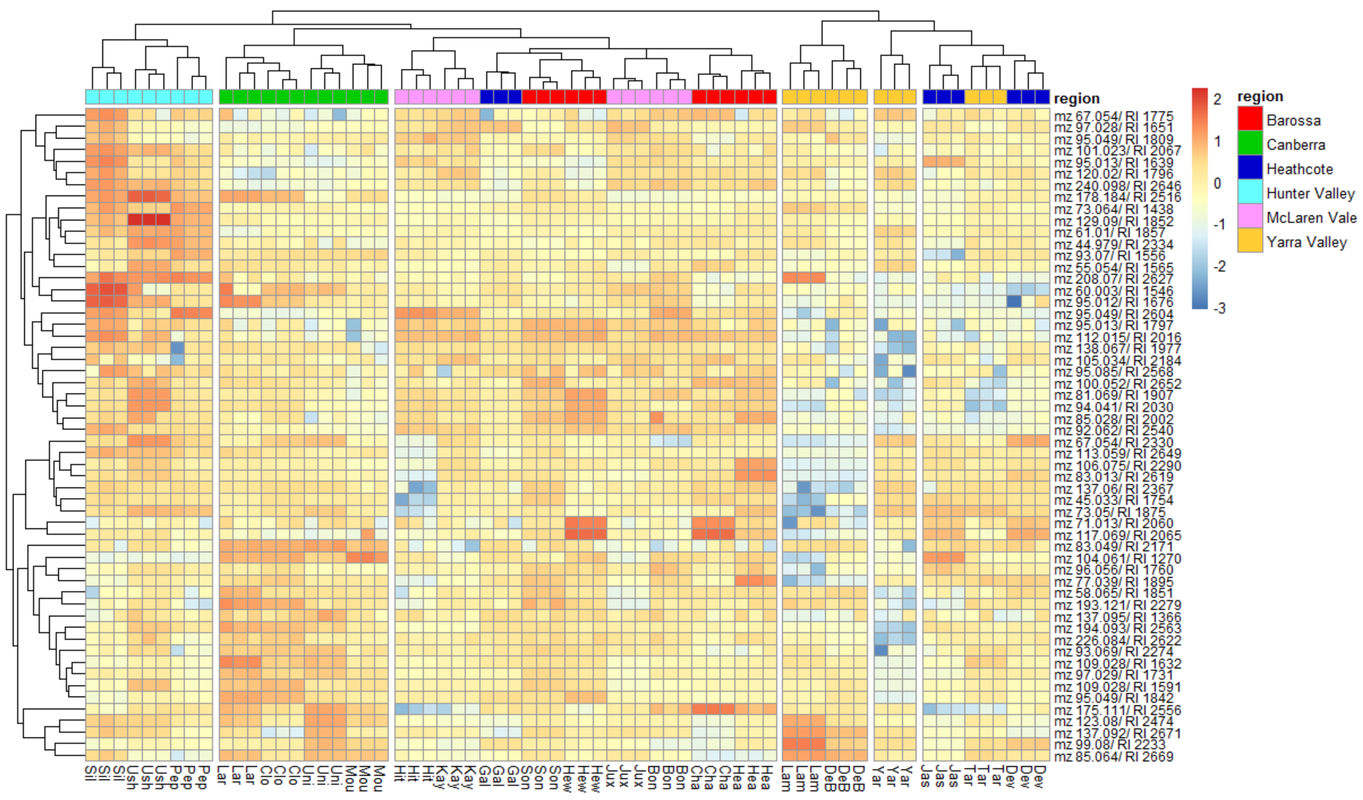

Figure 4. Cluster analysis based on important features.

Cluster analysis

Important features were then selected based on ANOVA analysis (post hoc Bonferroni adjustment, $p < 0.01$), and subsequently grouped based on proximity in retention time and peak width. The most intense feature from each group was chosen for a second PCA analysis. The first 4 PCs accounted for 55% of total variance. Features with the highest loadings (56 in total) were chosen for cluster analysis. Both samples and features were clustered by complete-linkage analysis, using Euclidean distance between elements. Results of cluster analysis were divided into 6 blocks by cutree analysis to represent 6 regions (Figure 4).

- Hunter Valley and Canberra District wines were clearly separated based on this untargeted metabolomics approach.
- The largest block consisted of all wines from Barossa Valley and McLaren Vale. The relative proximity of location is probably a key reason for this finding.
- Yarra Valley wines were clustered closely together, but were divided into different blocks. Within region variability in addition to varying practices in the vineyard and winery may have contributed to this result.
- The largest variation was observed between wines from Heathcote and warrants examination in depth.

References: [1] Wine Australia: https://www.wineaustralia.com/market-insights/variety-snapshots; [2] Hall and Jones, Aust. J. Grape Wine Res. 2010, 16(3), 389 – 404; [3] Schmidtke et al., J. Agric. Food Chem. 2013, 61, 11957−11967; [4] R packages available at https://bioconductor.org/

Future directions:

The untargeted metabolomics approach presents an opportunity to discover markers that can effectively distinguish wines of different GI. The features used in cluster analysis need to be subjected to MS/MS analysis for structure elucidation. Furthermore, the exact topography and climate data is currently being compiled for each individual vineyard involved in this study. Sensory evaluations were also performed on these wines. Collating and correlating data from all these aspects will help unravel the complex factors that define the typicality of various Australian GIs.

Acknowledgement:
The authors thank Wine Australia and Charles Sturt University for providing funding for the current project.