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REGIONALITY IN AUSTRALIAN PINOT NOIR WINES: A STUDY USING NMR AND ICP-MS WITH COMMERCIAL WINES

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Abstract

Aim: Wine quality and character are defined in part by the *terroir* in which the grapes are grown. Metabolomic techniques, such as nuclear magnetic resonance (NMR) spectroscopy and inductively coupled plasma mass spectrometry (ICP-MS), are used to characterise wines and to detect wine fraud in other countries but have not been extensively trialled in Australia. This study aimed to investigate the use of ICP-MS and NMR to characterise a selection of Pinot noir wines.

Methods and Results: Duplicate bottles of commercial Pinot noir wines from seven viticultural regions (six in Australian and one in New Zealand) were collected during 2013/4, either as donations from the wineries or via commercial sources. These regions represented a range of viticultural climates and vintages (2010-2013). These wines were analysed using NMR and ICP-MS by the Institut Heidger (Osann-Monzel, Germany) using their proprietary methods. Multivariate data analysis was then undertaken, trialling principal component analysis (PCA), multifactorial analysis, and analysis of coinertia. Interestingly, the results showed that the wines from varying *terroirs* could be best distinguished using PCA of their mineral content, and this statistical separation of the wines was clearest by geological region. Metabolomic analysis of the wines using NMR did not reveal any correlations with climate in terms of daytime temperatures. NMR metabolites did not prove useful for distinguishing wines by region, but interestingly there was a better separation based on Australian states, presumably reflecting the marked differences in climates. An analysis of coinertia suggested that the two datasets were not redundant.

Conclusions: ICP-MS appears to have promise in determining regionality in Australian and New Zealand wines, perhaps reflecting the extremes in geology often found in these two nations. Although the regional characteristics relating to contributions by *terroir* were frequently overwhelmed by strong local mineral contributions to the wines – possibly resulting from varying soil types, previous mining activity, and viticultural methods such as irrigation – these differences showed promise in providing distinctive 'fingerprints' for individual wines. NMR may also be useful for analysing and refining metabolite composition during winemaking and viticulture.

Significance and Impact of the Study: This was the first such study in Australia using both NMR and ICP-MS. The study provided valuable data for future 'fingerprinting' commercially bottled wines, as a precaution against wine 'forgery.'

Keywords: NMR, IPCMS, PCA, Pinot Noir wine, terroir, metabolomics

Introduction

The environment in which grapes are grown has an impact on the eventual characteristics of the wine. The organic metabolites in a wine are derived from the grapes, as influenced by the climate and the vineyard methods, then modified by the winemaking techniques including yeasts, fermentation, and subsequent filtering and ageing. In particular, Roullier-Gall *et al.* (2014) drew attention to the environmental effects on the metabolic pattern (or 'metabolome') of a wine, noting that "*Wine can be described as a molecular kaleidoscope integrating signatures from grapes, yeasts, the winemaking and the environment*" (Roullier-Gall *et al.,* 2014).

In this study, the chemical profiles of a series of commercially bottled Pinot noir wines from Australia and New Zealand were analysed and compared against environmental and geographic factors that form part of what is widely referred to as '*terroir*'. The methods utilised to analyse the 'metabolome' – the metabolic profile – were nuclear magnetic resonance (NMR) and colour spectroscopy, while the inorganic minerals present were determined by inductively coupled plasma mass spectrometry (ICP-MS).

This study (which will be published as Duley *et al.* (2021), and a summary of which is reported here) aimed to determine whether bottled Pinot noir wines from different regions in Australia can be distinguished by chemical analysis, using NMR, ICP-MS, and spectroscopy. We proposed that principal component analysis (PCA) could categorise the wines by region, and possibly vintage, based on ICPM-MS and/or NMR data.

Materials and Methods

The methods for this study are described in detail in the forthcoming paper Duley *et al.* (2021), which discusses this research in further detail. Consequently, only a brief summary is provided here. Commercially bottled vintage wines (2010-13 vintages), labelled 'Pinot noir', were donated or purchased from a number of regions across Australia. Central Otago, New Zealand, has a cooler climate compared to Australia and was used as an out-group. Wine samples are labelled with an abbreviation of the region name (AH: Adelaide Hills, CO: Central Otago (New Zealand), MP: Mornington Peninsula, MV: McLaren Vale, SF: Southern Fleurieu, TAS: Tasmania, and YV: Yarra Valley) plus an arbitrarily assigned number. The geographic separation of most areas was very large by European standards, with the selection including both cool and warm climates.

NMR and ICP-MS were undertaken at the Institut Heidger (Osann-Monzel, Germany), according to their validated proprietary methods. PCA was undertaken, following the example of previous authors (Coetzee *et al.,* 2014; Son *et al.,* 2009) using the 'PCA' function of the 'FactoMineR' package (Le *et al.,* 2008). Data were normalised using the scale argument of the 'FactoMineR' functions. Factors that were invariant or only had one measurement were removed from the analysis.

To evaluate the contributions of the NMR and ICP-MS data, a multifactorial analysis (MFA) and an analysis of coinertia were undertaken. MFA analysed the described observations in several "blocks" or sets of variables, seeking the common structures present in all or some of these sets (Husson *et al.,* 2017). This was undertaken using the 'MFA' function of the 'FactoMineR' (Le *et al.,* 2008) and 'factoextra' packages (Kassambara and Mundt, 2017), and the analysis of coinertia was undertaken using the 'dudi.pca' and 'coinertia' functions of the 'ade4' package. Analysis of coinertia explores the covariance between two datasets, by finding a structure or co-structure to examine the relationship between two set of variables where the two datasets are linked by the same individuals (Dolédec and Chessel, 1994).

Results and Discussion

ICP-MS

An initial PCA showed that several variables were highly correlated, including most of the rare earth elements, which all correlated with each other, and thorium, which correlated with bismuth, tungsten, germanium, and vanadium. In addition, three outlier vineyards (AH02, AH06, YV04) were found to have a disproportionate influence on the model, and so were removed from subsequent analysis. The wines from these 'outlier' vineyards can be hypothesised to indicate the presence of particular mineral deposits, and thus they may represent extreme values that may typify some geological aspects of outcrops or washouts in some areas. Consequently, the analysis was rerun without these wines (see Figure 1a). In this analysis, the remaining wines were more clearly separated, and this allowed the relationships between all wines to be more clearly observed.

The wines were categorised into groups using confidence ellipses (see Figures 1b and 1c) to show 'clustering'. Figure 1b shows the wines grouped by region. While some regions overlapped considerably, several others were easily distinguishable. The South Australian regions of Southern Fleurieu and McLaren Vale overlapped, but the two together formed a relatively distinct group, though there was overlap with some other regions, notably the Adelaide Hills region in South Australia. This appears logical as the Southern Fleurieu, McLaren Vale, and Adelaide Hills regions are contiguous. Some regions were more difficult to distinguish, although Tasmania, the Adelaide Hills, Yarra Valley, and Mornington Peninsula, which are widely separated, were all quite distinct from each other. However, the McLaren Vale overlapped with the widely separated Yarra Valley, and Central Otago (New Zealand). Interestingly, Central Otago also overlapped with diverse Australian regions of Tasmania, the Adelaide Hills, McLaren Vale, and Yarra Valley. This suggests that the mineral contents of soils of the NZ Central Otago region are not radically different to those of the Australian winegrowing regions studied, despite the different geological histories of Australia and New Zealand. Further research is needed to determine if this is true for other regions of New Zealand. It should be noted, however, that the mineral content of wine is influenced by vineyard and winery processes, not solely the mineral content of the corresponding viticultural soils (Martin *et al.,* 2012).

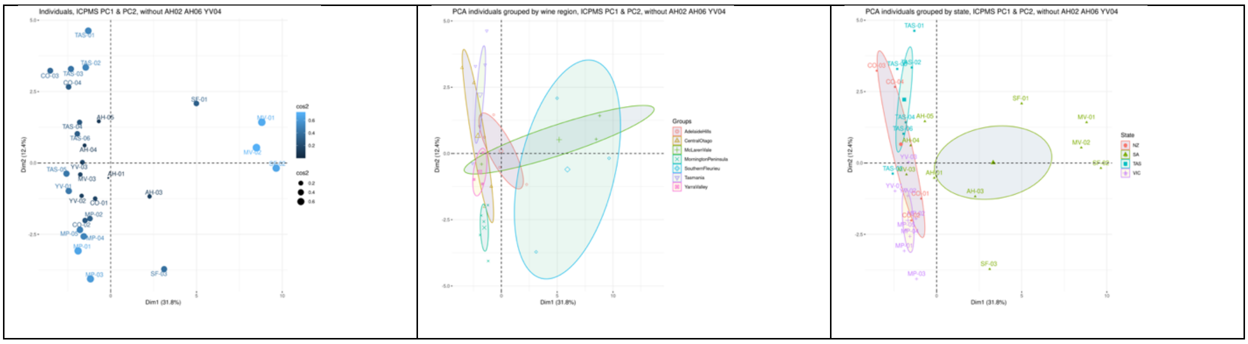

Figure 1: PCA of ICP-MS data. a) PCA without extreme individuals, providing a clearer picture of their distribution within the PCA space; b) Individuals grouped by wine region using confidence ellipses, without extreme individuals; c) Individuals grouped by state using confidence ellipses, without extreme individuals (NZ: New Zealand; SA: South Australia; TAS: Tasmania, VIC: Victoria).

NMR Data

An initial analysis of wine organic metabolites by NMR showed that CO-04 had a disproportionate influence on the analysis, contributing 20% to the first plane (Figure 2a). When the analysis was rerun excluding CO-04, valine, alanine, leucine, lactic acid, catechin, and succinic acid were all important variables in the PC1-PC2 plane.

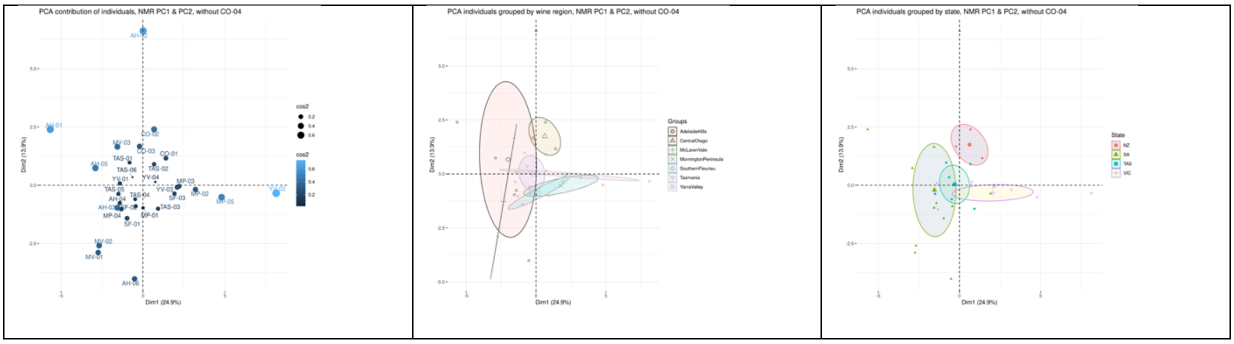

Figure 2: PCA of NMR data. a) PCA without extreme individual, providing a clearer picture of their distribution within the PCA space; b) Individuals grouped by wine region using confidence ellipses, without CO-04; c) Individuals grouped by state using confidence ellipses, without CO-04 (NZ: New Zealand; SA: South Australia; TAS: Tasmania, VIC: Victoria).

NMR metabolites did not prove particularly useful in this study for distinguishing wines by region, with a lot of overlap between the groups (Figure 2b). Interestingly, there was a better separation based on Australian states (Figure 2c), particularly in PC1 & PC2 (Figure 2c) and PC3 & PC4. This presumably reflected the differences in climates, which is quite marked between the three states studied. Central Otago, in New Zealand, was well separated in Figure 2c, again presumably influenced by the climate, as it is the coldest, driest part of NZ. South Australia, probably the hottest, was somewhat separated. As with ICP-MS, it was not possible to confidently distinguish between vintages, based on NMR data. A larger sample set is probably required.

Multifactorial Analysis

ICP-MS made the highest contribution to PC1, and NMR to PC2 and PC3 (see Duley *et al.*, 2021). Although PC1 and PC2 together only accounted for 38% of total variability, they together represented the first two main factors of MFA. PC1 summarised the characteristics of South Australian wines, typified by high levels of rare earth metals yttrium, holmium, and dysprosium; and alkaline metals sodium and strontium. The metabolite shikimic acid. which is an essential precursor for synthesis of aromatic (aryl) metabolites including flavonoids, was also important. PC2 summarises the characters of wines that are determined by NMR, particularly the energy metabolites lactate, succinate, and tartrate; the plant growth hormone trigonelline; and the amino acids proline, histidine, and leucine.

Conclusions

The study mostly upheld our hypothesis, and it was concluded that ICP-MS in combination with PCA was a useful analytical technique for identifying regions of origin of the bottled Pinot noir wines from Australia and New Zealand. Combining the results of ICP-MS with NMR did not provide any additional discrimination. Given the large areas, and therefore the heterogeneity, of many of the regions included in the study, it is unsurprising that the regions were not as distinctive as has been shown in other countries. For example, studies in Burgundy have confirmed the distinctiveness of village level appellations (Roullier-Gall *et al.,* 2014). In comparison, Tasmania is an island with a landmass of over 68,000 square km. A comparison of more discrete subregions may offer more clear-cut results. It must be noted as well that variable ageing conditions for these bottles may have had an impact on the diversity of metabolites, in particular as seen by NMR.

Eventually, the distinctiveness of Australian wine regions and subregions may be able to be discriminated based on NMR metabolomes reflecting climatic differences, and ICP-MS profiles based on distinctive soil compositions. In other countries, similar analyses have also been used to determine wine fraud, and similar work could be undertaken in Australia. This will probably depend upon the establishment of open databases of wine composition linked to regions and/or specific vineyards.

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