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## Preliminary sensory and chemical profiling of Cypriot wines made from indigenous grape varieties Xynisteri, Maratheftiko and Giannoudhi and acceptability to Australian consumers

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### ABSTRACT

**Aim:** The aims of this study were to (1) generate sensory and chemical profiles of commercial Cypriot wines made from the white grape Xynisteri and the red grapes Maratheftiko and Giannoudhi and (2) assess the Australian consumers' response to these wines.

**Methods and Results:** A Rate-All-That-Apply (RATA) method was used for sensory profiling of the wines (n=56 panellists on Xynisteri and n=60 on Maratheftiko and Giannoudhi) and to guide chemical analysis of flavour compounds. Chemical analysis involved quantitative analysis of aroma compounds by gas chromatography mass spectrometry (GC-MS) and non-targeted profiling of phenolic compounds (non-volatile secondary metabolites) using liquid chromatography mass spectrometry (LC-MS). Australian wine consumer's hedonic responses towards wines made from Cypriot grape varieties were also investigated. Consumers completed a questionnaire exploring their demographics, wine consumption habits, environmental/sustainability opinions and neophobic tendencies prior to the tasting. The first tasting (n=111 consumers) consisted of six commercial Xynisteri, one Australian Pinot Gris and one Australian unwooded Chardonnay wines. The second (n=114) consisted of three Maratheftiko, one Giannoudhi and one Australian Shiraz wines.

**Conclusions:** Principal Component Analysis (PCA) of the RATA study identified the following sensory characteristics for Xynisteri wine: stone fruit, dried fruit, citrus, herbaceous, grassy, apple/pear, confectionary, vanilla, creamy, buttery, wood, and toasty. Maratheftiko wines were described as woody, dried fruit, chocolate, herbaceous, confectionary, jammy, sweet and full bodied. Giannoudhi wine was described as woody, dried fruit, chocolate and full bodied. Chemical analysis identified 15 phenolic compounds in the white wine samples and 17 in the red wine samples, as well as 21 volatile/aroma compounds in the white wine samples and 26 in the red wine samples. These chemical compounds were then correlated with sensory data from the RATA and consumer hedonic responses using Agglomerative Hierarchical Clustering (AHC) and PCA to determine consumer liking drivers for the wines. Three clusters of consumers were identified for the white and red wines. The overall consumer means for liking indicated that Cypriot wines were liked similarly to Australian wines.

**Significance and impact of the study:** Australia's changing climate is placing great pressure on the resources for sustainable viticulture. Many vineyards and wineries base their businesses on European grape varieties traditionally grown in regions with abundant water resources. It is therefore necessary for the Australian wine industry to investigate grape varieties that are indigenous to hot climates similar to Australia. The eastern Mediterranean island of Cyprus is one such place with indigenous grape varieties that grow well in a hot climate without irrigation. These popular Cypriot wines have the potential to be popular with Australian consumers, thus offering new grape varieties to the Australian market that are better suited to the changing climate.

### KEYWORDS

Rate-All-That-Apply (RATA), wine consumers, Gas Chromatography Mass Spectrometry (GC-MS), Liquid Chromatograph Mass Spectrometry (LC-MS), Partial Least Squares (PLS), Agglomerative Hierarchical Clustering (AHC), vineyard sustainability, Cypriot wines

## INTRODUCTION

The climate in Australia and the rest of the world is undergoing rapid change. Since the middle of the 20<sup>th</sup> century, Australian temperatures have on average risen by about 1°C with an increase in the frequency of heat waves and a decrease in the number of frosts and cold days (Webb, 2011). These changes have made observable impacts on viticulture. Trends to earlier harvest maturity were observed in numerous regions across the country (Webb *et al.*, 2013). These trends are partly due to warming climates, but also due to reduced water availability. Jarvis *et al.* (2019) report that unusually warm and dry spring conditions have been linked to earlier budburst, with a more rapid rate of growth and development for the remainder of the growing season, regardless of temperatures later in the season.

Further climate change and rainfall reduction is expected over the coming decades (Johnson *et al.*, 2018). For most locations the best estimate of mean warming over Australia by 2030 is 0.7–0.9°C in coastal areas and 1–1.2°C inland and annual precipitation is estimated to decrease by 2.5 to 5% in most regions of Australia. Objectives to assist the wine industry in mitigating and adapting to these changes in climate include establishing adaptation scenarios for major wine regions based on changes to phenology and temperature tolerance of major varieties and future water demand and availability (Webb *et al.*, 2007).

Cyprus is reported to have the oldest wine tradition in the Mediterranean with more than 5,500 years of wine production with a vineyard area of approximately 7,000 hectares (Chrysargyris *et al.*, 2018b). It has been described by Evans (2009) and Lelieveld *et al.* (2016) as the cradle of viticulture and that this area is gradually and steadily becoming hotter and drier due to climate change. Many indigenous varieties of grapes originating from the region have been hand selected for millennia for their resistance to heat and drought (Fraga *et al.*, 2016; Patakas *et al.*, 2005). During the summer period, grapevines cultivated in the Mediterranean are often subjected to a combination of environmental stresses including strong winds, high air temperatures (heat waves) and soil/atmospheric water deficits (Beis and Patakas, 2012; Chrysargyris *et al.*, 2018a). There are more than 10 indigenous Cypriot grape

varieties on the island, with many of them very well adapted to drought. They require less water and fertilisers when compared to introduced varieties and offer promising prospects for adaptation to climate change (Litskas *et al.*, 2017). This climate scenario of Cyprus is very similar to that of southern Australia and as such their indigenous varieties may also be a suitable strategy to mitigate climate change effects in Australian conditions. This study sought to analyse Cypriot wines made from indigenous grape varieties Xynisteri, Maratheftiko and Giannoudhi using chemical and sensory profiling. The white grape Xynisteri is the most widely planted white variety in Cyprus and is utilised for table wine, the sweet wine Commandaria and traditional sweets. Maratheftiko is considered a red floral variety capable of producing high quality wines and the rare Giannoudhi has been gaining popularity recently with the local market (Vrontis and Paliwoda, 2008). To date there is limited research on sensory and chemical profiling of wines made from Cypriot grape varieties. Research has mainly focused on investigating the chemical composition and metabolic fingerprints of sun dried Xynisteri grape musts (Constantinou *et al.*, 2017; Constantinou *et al.*, 2018a), the phenolic content and antioxidant capacity of Cypriot wines (Galanakis *et al.*, 2015) and the authenticity of Cypriot wines using isotopic markers (Kokkinofa *et al.*, 2006 and 2017).

There have been no consumer sensory studies on Cypriot wines to date. A consumer survey by Vrontis and Papolomou (2007) suggested that there has been a shift in Cypriot consumer preference, with 87.2% of the 600 consumers surveyed preferring to drink wine made from the local varieties. Wine flavour and aroma were found to be the main drivers for purchasing wine made from local varieties, rather than more popular European varieties. Similar results have been noted with Greek consumers and Greek wines. Krystallis and Chrysochou (2010) studied consumer loyalty determinants in Greek wine varieties and found that 87% of those surveyed purchased Xinomavro and 89% purchased Agiorgitiko at an average frequency of six bottles a month.

The aims of this study were to (1) generate sensory and chemical profiles of commercial Cypriot wines made from the white grape Xynisteri and the red grapes Maratheftiko and Giannoudhi and (2) assess the Australian

consumer's response to these wines that are very popular amongst wine consumers in Cyprus. This would enable the Australian wine industry to potentially introduce new grape varieties to the market that are both acceptable to consumers and better suited to the Australian climate.

## MATERIALS AND METHODS

### 1. Wines

The wines used for both studies included four Cypriot Xynisteri 2016, one Cypriot Xynisteri 2015, one Australian Pinot Gris and one Australian Chardonnay 2017. The red wines were two Cypriot Maratheftiko 2015, one Cypriot Maratheftiko 2013, one Cypriot Giannoudhi 2014 and one Australian Shiraz 2014. The Cypriot wines were chosen as they were common brands and were spread across a range of price points (5-20 Euros). Some older wines and oaked aged wines were also chosen to assist in consumer preference for younger or older wine styles. The Australian wines were used as a reference to the otherwise unknown Cypriot varieties. They were also common brands readily available at wine retailers for between \$20-\$25 AUD. More detailed information on the wines used in this study is provided in Table 1.

### 2. Sensory analysis

The Rate-All-That-Apply (RATA) technique described by Danner *et al.* (2018) was utilised for sensory profiling of the wines. RATA is a

rapid sensory profiling method with industry and research applications and aims to describe the sensory characteristics of wines, making it particularly relevant when resources and time are limited, and/or additional consumer responses i.e. hedonic ratings or willingness-to-pay are of interest (Ares *et al.*, 2014; Danner *et al.*, 2018). This method has demonstrated that using untrained consumers to evaluate commercial wine samples can result in very similar sample discrimination and sample configurations as descriptive analysis (DA) (Ares *et al.*, 2014).

RATA analysis of the white commercial wines occurred in November 2017 involving 57 tasters. The tasters were recruited from the School of Agriculture, Food and Wine staff members and post-graduate students who had previous experience in tasting and evaluating wines.

Nine wines were presented sequentially, monadic, blind and in a random order to the tasters to overcome serving order effects. Wines were served in International Standards Organisation (ISO) tasting glasses at 15°C. Tasters were required to select only the attributes that were applicable to the wine and additionally indicate the perceived intensity of these sensory attributes using a 7-point rating scale. Attributes included 3 colour, 22 aroma intensity, 3 taste, 22 flavour intensity, 6 mouthfeel intensity and 2 length of aftertaste questions (Supplementary Tables 1 and 2).

**TABLE 1.** Basic chemical, oak treatment and other information of wines used in sensory, consumer acceptance and chemical analysis.

| Code | Wine              | pH   | TA   | Alc % | Oak | Other     |
|------|-------------------|------|------|-------|-----|-----------|
| M1   | Maratheftiko 2015 | 3.43 | 5.86 | 14.8  | Yes |           |
| M2   | Maratheftiko 2013 | 3.62 | 5.45 | 13.2  | Yes |           |
| M3   | Maratheftiko 2015 | 3.44 | 5.88 | 14.5  | Yes |           |
| SH   | Shiraz 2014       | 3.57 | 6.13 | 14.5  | Yes |           |
| Yia  | Giannoudhi 2014   | 3.65 | 5.5  | 13.4  | Yes |           |
| CH   | Chardonnay 2017   | 3.33 | 7.35 | 12.9  | No  |           |
| PG   | Pinot Gris 2017   | 3.54 | 6.65 | 12.5  | No  |           |
| X1   | Xynisteri 2016    | 3.21 | 5.93 | 12.8  | No  |           |
| X2   | Xynisteri 2015    | 3.26 | 5.94 | 12.8  | Yes |           |
| X3   | Xynisteri 2016    | 3.22 | 5.52 | 13.7  | No  |           |
| X4   | Xynisteri 2016    | 3.35 | 5.44 | 12.8  | No  | 5% Muscat |
| X5   | Xynisteri 2016    | 3.16 | 4.72 | 12.6  | No  |           |
| X6   | Xynisteri 2016    | 3.42 | 5.02 | 12.6  | No  |           |

Ethics approval for the sensory analysis was given by the University of Adelaide, approval number: H-2017-204. The tasting took place in the wine sensory lab at the Wine Innovation Central (WIC) building at the University of Adelaide Waite Campus. Results were collected using Red Jade sensory software.

RATA analysis of the red commercial wines involving 60 tasters occurred in July 2018 using the same protocols as 2017. The red wines were served at a room temperature of 22°C.

### 3. Consumer acceptance trials

Participants completed a questionnaire utilising a 9-point hedonic scale prior to the tasting. The questions explored their demographics, wine consumption habits, environmental/sustainability opinions and neophobic tendencies. The questions were taken directly from previously published and validated questionnaires. The questions came from: The Fine Wine Instrument (Johnson and Bastian, 2015), Wine Neophobe Scale (Ristic *et al.*, 2016) and The Concern About Sustainability questionnaire (Grunert *et al.*, 2014).

The white commercial wines (n=111) were assessed in December 2017 and the red commercial wines (n=114) in July 2018. Consumers were recruited from social media and the University of Adelaide registered taster database. Pre-requisites for consumers in the trial were to be over 18 years of age and consume wine at least once every 2 weeks.

As with the RATA trial, wines were presented sequentially monadic, blind and in a random order. During the tasting, the consumers were required to answer five questions on a 9-point Likert scale relating to their perception of the wine quality, how much they liked the wine, how likely they would be to recommend the wine, how likely they were to buy the wine again and how much they would pay for the wine.

### 4. Chemical analysis

Wine samples were analysed by the Australian Wine Research Institute (AWRI) and Metabolomics Australia at the Waite Campus (AWRI-Metabolomics South Australia, 2019). As this was a preliminary study, only a small number of wines were able to be imported to Australia quickly and easily with an aim to gain an initial understanding of the attributes of these

wines and preliminary investigation of chemical compounds. Thus, only single measures were utilised in the chemical analysis.

#### 4.1 Non-volatile profiling of secondary metabolites by Liquid Chromatography-Mass Spectrometry (LC-MS/MS), non-targeted analysis

The non-targeted method was developed to detect as many phenolic compounds as possible and was not specifically optimised for one class of phenols.

The sample set consisted of 13 samples (5 red wine and 8 white wine samples). Prior to analyses wine samples were submitted to a standard clean-up procedure using Strata-X reversed phase SPE cartridges. After conditioning the cartridge (1 mL methanol and 1 mL Milli-Q water), 2 mL of each sample were diluted with 8 mL of Milli-Q water and loaded on the cartridge. The eluted fraction was discarded, while compounds of interest were retained on the cartridge phase. Cartridges were then washed with 1 mL of aqueous solution of methanol (2%) and dried at full vacuum for 5 minutes. Analytes were eluted using 1 mL of methanol. The eluted fractions were collected in test tubes and methanol evaporated. The dried extracts were resuspended prior to analysis using 25  $\mu$ L and 75  $\mu$ L of solvent B (2% formic acid, 2% Milli-Q water, 40% acetonitrile in methanol) and solvent A (2% formic acid, 0.5% methanol in Milli-Q water) respectively. Chemical Analysis Separation was performed on an Agilent 1200SL High-performance liquid chromatography (HPLC) coupled to a Bruker MicroTOFQ-II. Samples were acquired in the MS negative mode. HPLC conditions included: injection volume 1  $\mu$ L, flow rate 0.22 mL/min, column - Phenomenex Kinetex PFP 150mm x 2.1mm ID, oven temperature 30°C and DAD acquisition range 200-500 nm. MS conditions of the detector were: source temperature 200°C, capillary voltage 3500 V, end plate offset -500 V, nebuliser pressure 2.0 bar, dry gas flow rate 8.0 L/min, mass range 50-1650 m/z and acquisition rate 0.5 Hz.

A calibration solution of sodium formate (5 mM sodium hydroxide in 50% (v/v) 2-propanol) was introduced during LC-MS analysis via an inline post-column switching valve and sample loop. Using Bruker's Data Analysis (v4.0 SP4) software, mass spectra were calibrated in the

range 100-1650 m/z from the sodium formate clusters using an enhanced quadratic algorithm. Each file was exported in the mzXML generic file format for further processing using R (statistical programming environment) v3.3.2 and Bioconductor v2.14 under a Debian Linux 64-bit environment. Analyses were divided into two batches (acquired within the same sequence), for white wines and red wines respectively. For each batch a Master Mix (a pooled mix of the samples) was prepared and several analytical replicates of the mix were acquired along the samples sequence. This was done to monitor the instrument performances along the instrument sequence. Each batch was processed using an R based script that allowed the extraction of all the molecular features from the data matrix. The term molecular feature describes a two-dimensional bounded signal: a chromatographic peak (retention time) and a mass spectral peak (m/z).

#### **4.2 Quantitative analysis of fermentation products (aroma compounds) by Gas Chromatography/Mass Spectrometry (GC/MS)**

The wine samples were diluted by factor 10. This was done to ensure that the concentrations of the detected analytes were within the instrument linear range. 1 mL of each sample was transferred into individual 20 mL vials containing 9 mL of buffer solution (pH 3.39) and 2 g of salt.

The analysis was performed on an Agilent 7890A gas chromatograph equipped with a Gerstel MPS2 multi-purpose sampler and coupled to an Agilent 5975C VL mass selective detector. Instrument control was performed with Agilent ChemStation E.02.00. The gas chromatograph was fitted with an Agilent DB-624UI column (30m x 0.25mm x 1.4µm). Helium (Ultra High Purity) was used as the carrier gas in constant flow mode. The oven temperature was started at 40°C, then increased to 60°C at 20°C/min (held for 14 mins) and followed by a series of temperature ramps. First ramp to 70°C at 10°C/min, second ramp to 80°C at 10°C/min, third ramp to 160°C at 20°C/min, and final ramp to 260°C at 10°C/min and held for 2 mins. The total run time was 45.5 mins. The vial and its contents were heated to 40°C for 5 minutes with agitation. The SPME fibre (polyacrylate) was exposed to the headspace in the sample for 15 minutes and was then desorbed

in the injector (splitless mode) for 15 minutes. The injector temperature was set at 260°C. The mass spectrometer quadrupole temperature was set at 150°C, the source was set at 230°C and the transfer line was held at 260°C. Positive ion electron impact spectra at 70 eV were recorded in SIM and SCAN mode with solvent delay of 4 mins.

The raw data from Agilents' ChemStation software (v E.02.02.1431) were converted into MassHunter data files and processed using MassHunter Workstation Software for Quantitative Analysis (v B.04.00). The concentration of analytes in the samples are determined using stable isotope dilution analysis (SIDA) and are reported in µg/L. Aroma detection thresholds (DT) were determined from Wang *et al.* (2016), Waterhouse *et al.* (2016) and Gonzalez-Alvarez *et al.* (2011). Odour activity values (OAV) were calculated (concentration/DT).

#### **4.3 Spectral analysis**

The white wine samples underwent spectral analysis to determine Flavonoid Extractives, Total Hydroxycinnamates, Total Phenolics and Relative Brown colour. Procedures and conditions were based on standard techniques described by Cozzolino (2015).

#### **4.4 Modified Somers and tannin assays**

The red wine samples underwent modified Somers and tannin assays to determine Colour Density, Free Anthocyanins, Pigmented Tannin, Total Pigment, Percent of Pigmented Tannin and Total Phenolics. Procedures and conditions were based on standard techniques described by Mercurio *et al.* (2007).

#### **5. Statistical analysis**

Basic chemical data were processed with Microsoft Excel 2010. Chemical data are presented as mean values with standard deviation from replicate determinations. Sensory data and chemical data were analysed by one-way ANOVA (sample) using the statistical package XLSTAT (version 2018.7, Addinsoft SARL, Paris, France). The significantly different attribute means were subjected to Pearson's type Principal Component Analysis (PCA) using XLSTAT and partial least squares (PLS) regression using The Unscrambler (version 9.7, CAMO Software AS, Oslo, Norway) with

**TABLE 2.** Significant attributes identified by RATA in (a) white wine samples and in (b) red wine samples.

|                              | Attribute               | Code             | Minimum | Maximum | Mean | Standard deviation | p-value |
|------------------------------|-------------------------|------------------|---------|---------|------|--------------------|---------|
| (a)                          | Colour brown            | CB               | 0.71    | 1.66    | 1.02 | 0.30               | <.0001  |
|                              | Colour green            | CGr              | 0.88    | 2.04    | 1.48 | 0.34               | <.0001  |
|                              | Colour yellow           | CYe              | 2.95    | 4.56    | 3.67 | 0.56               | <.0001  |
|                              | Aroma apple pear        | AA/P             | 1.98    | 2.80    | 2.34 | 0.33               | 0.050   |
|                              | Aroma citrus            | ACit             | 2.23    | 3.09    | 2.72 | 0.31               | 0.022   |
|                              | Aroma dried fruit       | ADrF             | 0.86    | 1.68    | 1.16 | 0.27               | 0.0419  |
|                              | Aroma stone fruit       | AStF             | 2.45    | 3.50    | 3.02 | 0.39               | 0.009   |
|                              | Aroma confectionary     | ACon             | 1.07    | 1.99    | 1.45 | 0.33               | 0.005   |
|                              | Aroma tropical          | ATr              | 2.16    | 3.46    | 2.76 | 0.41               | 0.0003  |
|                              | Aroma floral            | AFI              | 1.46    | 2.75    | 2.19 | 0.50               | 0.0001  |
|                              | Aroma grass             | AGr              | 0.32    | 1.07    | 0.77 | 0.25               | 0.0097  |
|                              | Aroma herbal            | AHe              | 0.60    | 1.09    | 0.82 | 0.21               | 0.0457  |
|                              | Aroma butter            | ABu              | 0.86    | 1.57    | 1.14 | 0.28               | 0.0286  |
|                              | Aroma nutty             | ANu              | 0.78    | 1.89    | 1.19 | 0.41               | <.0001  |
|                              | Aroma savoury           | ASav             | 0.29    | 1.18    | 0.61 | 0.34               | <.0001  |
|                              | Aroma toast             | ATo              | 0.48    | 1.29    | 0.91 | 0.27               | 0.0069  |
|                              | Aroma wood              | AWo              | 0.38    | 1.29    | 0.77 | 0.32               | 0.0001  |
|                              | Aroma bread             | ABr              | 0.57    | 1.50    | 0.98 | 0.33               | 0.0007  |
|                              | Taste bitter            | TB               | 1.68    | 2.39    | 2.15 | 0.23               | 0.0062  |
|                              | Taste sweet             | TSw              | 2.11    | 2.88    | 2.37 | 0.26               | <.0001  |
|                              | Taste acid              | TA               | 3.65    | 4.45    | 3.99 | 0.23               | 0.0010  |
|                              | Flavour stone fruit     | FStF             | 2.52    | 3.32    | 2.89 | 0.30               | 0.0183  |
|                              | Flavour confectionery   | FCon             | 0.84    | 1.69    | 1.09 | 0.28               | 0.0009  |
|                              | Flavour tropical        | FTr              | 1.79    | 2.99    | 2.40 | 0.37               | 0.0011  |
|                              | Flavour floral          | FFI              | 1.25    | 2.39    | 1.79 | 0.44               | 0.0002  |
|                              | Flavour nutty           | FNu              | 0.83    | 1.77    | 1.18 | 0.29               | 0.0027  |
|                              | Flavour toast           | FTo              | 0.53    | 1.54    | 0.91 | 0.31               | 0.0003  |
|                              | Flavour wood            | FWo              | 0.45    | 1.19    | 0.72 | 0.26               | 0.0165  |
|                              | Flavour vanilla         | FVan             | 0.41    | 1.32    | 0.98 | 0.31               | 0.0023  |
|                              | Flavour bread           | FBr              | 0.48    | 1.39    | 0.94 | 0.30               | 0.0020  |
|                              | Mouth feel alcohol      | MFOH             | 3.21    | 3.89    | 3.62 | 0.22               | 0.0025  |
|                              | Mouth feel astringent   | MFA <sub>s</sub> | 1.89    | 2.55    | 2.26 | 0.22               | 0.0045  |
|                              | Mouth feel creamy       | MFCr             | 2.02    | 2.88    | 2.47 | 0.29               | 0.0045  |
|                              | After taste fruitlength | ATFL             | 3.68    | 4.25    | 3.94 | 0.22               | 0.0195  |
| After taste non-fruit length | ATNFL                   | 3.34             | 4.12    | 3.77    | 0.24 | 0.0201             |         |
| (b)                          | Colour red              | CR               | 3.53    | 4.93    | 4.39 | 0.57               | <.0001  |
|                              | Colour purple           | CP               | 1.38    | 4.92    | 2.75 | 1.72               | <.0001  |
|                              | Colour brown            | CB               | 0.98    | 3.15    | 2.16 | 1.03               | <.0001  |
|                              | Aroma dried fruit       | ADrF             | 2.08    | 3.15    | 2.67 | 0.45               | 0.0017  |
|                              | Aroma jammy             | AJ               | 2.37    | 3.22    | 2.69 | 0.34               | 0.0231  |
|                              | Aroma confectionery     | ACon             | 1.58    | 2.28    | 1.84 | 0.27               | 0.0541  |
|                              | Taste bitter            | TB               | 2.25    | 3.02    | 2.81 | 0.32               | 0.0025  |
|                              | Taste sweet             | TSw              | 2.15    | 2.80    | 2.49 | 0.24               | 0.0297  |
|                              | Flavour dried fruit     | FDrF             | 2.13    | 2.97    | 2.57 | 0.37               | 0.0051  |
|                              | Flavour jammy           | FJ               | 1.58    | 2.68    | 1.91 | 0.44               | 0.0001  |
|                              | Flavour chocolate       | FCh              | 1.05    | 1.80    | 1.51 | 0.31               | 0.0105  |
|                              | Flavour herbal          | FH               | 1.42    | 2.02    | 1.68 | 0.29               | 0.0175  |
|                              | Flavourwood             | FWo              | 2.13    | 2.95    | 2.58 | 0.33               | 0.0127  |
|                              | Mouth feel bitter       | MFB              | 3.98    | 4.47    | 4.31 | 0.21               | 0.0036  |
|                              | Mouth feel astringent   | MFA <sub>s</sub> | 4.15    | 5.15    | 4.69 | 0.38               | <.0001  |
|                              | Mouth feel smooth       | MFSm             | 3.05    | 3.90    | 3.37 | 0.35               | 0.0002  |
|                              | Mouth feel rough        | MFRo             | 2.98    | 3.95    | 3.57 | 0.39               | <.0001  |

chemical parameters (x-variables) and RATA data (y-variables). All variables were standardised before analysis and significance p-values where  $p < 0.05$ .

## RESULTS

### 1. Sensory analysis

Panellists utilising the RATA technique identified 35 statistically significant attributes for the white wines and 17 for the red wine samples that defined the properties of the Cypriot wines (Tables 2 and 3). Figures 1 and 2 display the scores and loadings from the PCA of sensory data, chemical analysis and wine samples.

The white wine samples in Figure 1 show the first two principal components, which accounted for 73.05% of the variation in the data. The first principal component (x-axis, 44.5%) separated samples that were floral, tropical, sweet, confectionary, apple, pear, herbaceous, stone fruit, citrus, vanilla and creamy from samples that were woody, bread, nutty, buttery, dried fruit, alcohol, bitter and astringent. The second principal component (y-axis, 28.5%) separated samples that were floral, tropical, sweet, confectionary, apple, pear, citrus, herbaceous, stone fruit, vanilla and creamy from samples that were woody, bread, nutty, buttery, dried fruit, alcohol, bitter and astringent. Wines were well distributed within the four quadrants. The upper right quadrant contained X2, which was perceived as toasty, wood, nutty, creamy and vanilla. The upper left quadrant contained X4, X6, PG, CH which were perceived as apple, pear, grass, herbaceous, confectionary, sweet, tropical, floral, stone fruit, citrus, grass and herbaceous. The lower left quadrant contained X1 which was perceived as green in colour. The lower right quadrant contained X3, X5 which were perceived as woody, bread, toast, nutty, buttery, dried fruit, alcohol, bitter and astringent.

The red wine samples in Figure 2 show the first two principal components, which accounted for 79.19% of the variation in the data. The first principal component (x-axis, 45.83%) separated samples that were jammy sweet, chocolate, confectionery and dried fruit from samples that were woody, bitter, astringent, rough and herbaceous. The second principal component (y-axis, 33.36%) separated samples that were sweet, jammy, confectionery, bitter, astringent and rough from those that were woody, chocolate, dried fruit, smooth and had fruit driven after

taste. Wines were well grouped in three quadrants with SH in the upper right quadrant perceived as jammy, sweet, smooth, dried fruit and chocolate. The lower right quadrant contained M1 and M3 which were perceived as confectionary, bitter, rough, astringent and herbaceous. The lower left quadrant contained M2 and Yia which were perceived as chocolate, dried fruit and wood.

### 2. Consumer acceptance

Agglomerative Hierarchical Clustering (AHC) was applied to the consumer data and revealed three clusters for the white and red wines.

The consumer means for liking before clustering revealed that the white wines were liked in the following order: PG, X4, CH, X3, X1, X2, X6, X5 driven by the attributes apple, pear, confectionery, sweet, floral, and tropical. Following clustering, the cohort in cluster 1 preferred X4, PG, X6, X2, X1, X5, X3 driven by the sensory attributes floral, tropical, sweet, confectionary, apple, pear, stone fruit, vanilla, creamy, woody, bread, nutty, buttery, dried fruit, alcohol, bitter and astringent. Cluster 2 preferred X2, PG, X1, CH, X3, X5 driven by the sensory attributes floral, stone fruit, vanilla, creamy, woody, bread, nutty, buttery, dried fruit, alcohol, bitter and astringent. Cluster 3 preferred CH, PG, X4, X5, X6 driven by the sensory attributes floral, tropical, sweet, confectionary, apple, pear, herbaceous, stone fruit, and citrus (Table 3).

The consumer means for liking before clustering revealed that the red wines were liked in the following order: SH, M3, M2, Yia, M1 driven by the attributes jammy, sweet, smooth and dried fruit. Following clustering, the cohort in cluster 1 were found to prefer M1, M3 driven by the sensory attributes sweet, jammy, confectionery and bitter. Cluster 2 preferred M2, SH, Yia driven by the attributes jammy, smooth, dried fruit, woody and chocolate. Cluster 3 liked all samples, but particularly M1, M3.

Analysis of the pre-tasting consumer questionnaire did not find any statistically significant relationships between the clusters and demographics, wine consumption habits, environmental/sustainability opinions, neophobic tendencies and wine acceptance. While the consumers in this trial were recruited from social media and the University of Adelaide volunteer taster database, it may be that the group were too homogenous to elicit any significant results.



**TABLE 3.** Sample, consumer means and clusters (C1, C2, C3) for (a) white wines and (b) red wines.

|     | Sample | Consumer mean | C1   | C2   | C3   |
|-----|--------|---------------|------|------|------|
| (a) | CH     | 5.78          | 4.82 | 6.31 | 6.56 |
|     | PG     | 6.43          | 6.53 | 6.71 | 6.03 |
|     | X1     | 5.76          | 5.97 | 6.31 | 4.97 |
|     | X2     | 5.75          | 6.02 | 6.78 | 4.44 |
|     | X3     | 5.78          | 5.60 | 5.93 | 5.88 |
|     | X4     | 5.90          | 6.60 | 4.87 | 5.94 |
|     | X5     | 5.52          | 5.37 | 5.28 | 5.94 |
|     | X6     | 5.68          | 6.35 | 4.87 | 5.56 |
| (b) | M1     | 5.80          | 5.79 | 4.25 | 7.05 |
|     | M2     | 6.00          | 4.46 | 7.09 | 6.65 |
|     | M3     | 6.20          | 5.59 | 5.50 | 7.19 |
|     | SH     | 6.50          | 5.46 | 7.09 | 6.88 |
|     | YIA    | 5.90          | 4.79 | 6.28 | 6.58 |

Overall however, the Cypriot wines were well liked by the Australian consumers in this study with the majority of mean liking scores greater than 5 on a 9-point hedonic scale.

### 3. Non-volatile profiling of secondary metabolites by Liquid Chromatography-Mass Spectrometry (LC-MS/MS), non-targeted analysis

As this was a preliminary study, it was decided to use non-targeted analysis of phenolic compounds. These normalised values were obtained by dividing the intensity value of each feature by the median intensity value across all features for that sample. The median value is the midpoint of all the feature intensities recorded separately for each sample. These values are reported as median normalised intensity values.

Analysis of the white samples identified 12 compounds and 3 unknown compounds (Table 4). Although not quantified, these phenolic compounds identified are consistent with the phenolic compounds identified in Xynisteri grape must by Constantinou *et al.* (2018a and b). PCA analysis in Figure 1 separated compounds caffeic acid, caffeic acid ethyl ester, coumaric acid A and epicatechin in the upper left quadrant correlating with PG, CH, X4, X6. The upper right quadrant contained ferulic acid and quercetin-3-O-glucuronide (correlating to X2). The lower left quadrant contained catechin, ethyl gallate and gallic acid which correlated with X1

and the lower right quadrant contained caftaric acid, epigallocatechin and coumaric acid B with X3, X5.

To date only phenolic classes have been identified in Maratheftiko and Giannoudhi wines (Galanakis *et al.*, 2015). This study has confirmed the identity of these classes and has also identified 15 preliminary compounds and 3 unknown compounds for Maratheftiko and Giannoudhi (Table 4). PCA analysis in Figure 2 separated compounds larcitrin, epigallocatechin and syringetin-3-O-glucoside in the upper right quadrant correlating to SH. The upper left quadrant contained compounds epicatechin, procyanidin B1, fisetin and quercetin. The lower left quadrant contained compounds catechin, gallic acid, quercetin-3-galactoside, quercetin-3-O-glucuronide, caftaric acid, and coumaric acid a, correlating to M1, M3. The lower right quadrant did not contain any phenolic compounds and correlated to M2, Yia.

### 4. Quantitative analysis of fermentation products (aroma compounds) by GC/MS

Analysis identified 21 volatile/aroma compounds in the white wine samples and 26 compounds in the red samples. Compounds, concentrations and OAV are presented in Tables 5 et 6.

PCA analysis of the white wines in Figure 1 separated the volatile compounds into the following quadrants. The upper right quadrant contained ethyl hexanoate (apple), 2-

**TABLE 4.** Phenolic compounds (median normalised intensity values) identified in (a) white wines and (b) red wines by LC-MS/MS.

| (a)                 | Class | Compound                  | CH     | PG     | X1     | X2    | X3     | X4    | X5    | X6     |
|---------------------|-------|---------------------------|--------|--------|--------|-------|--------|-------|-------|--------|
| Hydrolysable tannin |       | Gallic acid               | 4.59   | 8.05   | 84.35  | 17.63 | 17.41  | 54.09 | 36.89 | 46.50  |
|                     |       | Ethyl gallate             | 7.49   | 10.37  | 115.40 | 25.24 | 20.88  | 74.16 | 44.39 | 62.88  |
| Hydroxycinnamate    |       | Caftaric acid             | 7.05   | 30.65  | 80.02  | 62.18 | 82.32  | 42.89 | 85.38 | 46.58  |
|                     |       | Coutaric acid A           | 0.65   | 73.86  | 20.61  | 10.70 | 35.21  | 26.40 | 9.66  | 42.27  |
|                     |       | Coutaric acid B           | 0.57   | 12.66  | 27.44  | 12.77 | 38.55  | 20.10 | 12.33 | 35.47  |
|                     |       | Caffeic acid              | 120.29 | 129.25 | 55.07  | 70.09 | 22.37  | 20.66 | 73.66 | 22.55  |
|                     |       | Caffeic acid ethyl ester  | 61.45  | 65.34  | 48.10  | 55.54 | 11.11  | 16.91 | 50.27 | 15.39  |
|                     |       | Fertaric acid             | 0.59   | 1.32   | 1.01   | 3.55  | 0.56   | 0.29  | 1.47  | 1.50   |
| Flavan-3-ol         |       | (+)-Catechin              | 0.18   | 0.10   | 1.54   | 0.24  | 1.99   | 1.17  | 0.71  | 1.56   |
|                     |       | (-)-Epicatechin           | 14.79  | 28.56  | 15.15  | 10.68 | 6.85   | 7.68  | 7.59  | 14.41  |
|                     |       | Epigallocatechin          | 2.50   | 6.14   | 28.44  | 16.75 | 9.26   | 12.58 | 11.73 | 20.47  |
| Flavanol            |       | Quercetin-3-O-glucuronide | 0.00   | 1.01   | 0.26   | 2.58  | 0.54   | 1.50  | 0.82  | 20.14  |
| Unknowns            |       | C7 H12 O5                 | 34.50  | 102.21 | 41.84  | 37.50 | 42.24  | 25.45 | 27.08 | 23.85  |
|                     |       | C10 H11 NO4 S             | 6.70   | 4.81   | 46.94  | 28.92 | 177.76 | 6.42  | 1.97  | 126.89 |
|                     |       | C15 H28 N2 O4             | 35.94  | 40.49  | 0.81   | 2.86  | 8.70   | 2.22  | 15.91 | 2.84   |
| (b)                 | Class | Compound                  | M1     | M2     | SH     | M3    | Yia    |       |       |        |
| Hydrolysable tannin |       | Gallic acid               | 28.96  | 16.75  | 9.00   | 30.35 | 14.27  |       |       |        |
|                     |       | Ethyl gallate             | 9.43   | 4.35   | 5.31   | 12.92 | 5.16   |       |       |        |
| Hydroxycinnamate    |       | Caftaric acid             | 25.64  | 18.78  | 6.79   | 35.77 | 19.10  |       |       |        |
|                     |       | Coutaric acid A           | 43.52  | 41.55  | 10.83  | 71.48 | 38.92  |       |       |        |
| Flavan-3-ol         |       | (+)-Catechin              | 86.30  | 79.87  | 77.73  | 98.50 | 82.50  |       |       |        |
|                     |       | (-)-Epicatechin           | 44.27  | 32.04  | 51.19  | 42.91 | 33.45  |       |       |        |
|                     |       | Epigallocatechin          | 0.83   | 1.40   | 4.54   | 1.03  | 1.51   |       |       |        |
| Proanthocyanidin    |       | Procyanidin B1 (1)        | 77.46  | 63.86  | 46.14  | 87.01 | 60.51  |       |       |        |
|                     |       | Procyanidin B1 (2)        | 39.39  | 25.14  | 33.25  | 41.96 | 25.19  |       |       |        |
|                     |       | Quercetin-3-O-glucuronide | 48.83  | 35.75  | 1.61   | 54.99 | 36.34  |       |       |        |
|                     |       | Quercetin-3-O-galactoside | 43.47  | 3.96   | 0.03   | 21.19 | 9.63   |       |       |        |
| Flavanol            |       | Syringetin-3-O-glucoside  | 22.47  | 21.04  | 47.29  | 21.29 | 24.03  |       |       |        |
|                     |       | Quercetin                 | 48.24  | 25.28  | 100.20 | 64.13 | 29.01  |       |       |        |
|                     |       | Laricitrin                | 0.91   | 1.89   | 27.47  | 1.11  | 1.87   |       |       |        |
|                     |       | Fisetin                   | 15.73  | 1.31   | 9.44   | 16.98 | 1.92   |       |       |        |
| Unknowns            |       | C15 H10 O8                | 6.58   | 11.69  | 50.00  | 9.58  | 10.91  |       |       |        |
|                     |       | C16 H12 O7                | 6.65   | 6.16   | 40.55  | 9.46  | 7.30   |       |       |        |
|                     |       | C30 H26 O13               | 44.18  | 31.52  | 23.16  | 48.51 | 30.20  |       |       |        |

methylpropanol and 3-methylbutanol (solvent) which correlated with X2. The upper left quadrant contained 3-methylbutyl acetate (banana), 2-methylpropyl acetate (banana), ethyl octanoate (pear, pineapple), ethyl butanoate (lactate), ethyl decanoate (floral), 2-phenylethyl acetate (stone fruit, floral), decanoic acid (fat), hexyl acetate (pear, apple), hexanoic acid (leafy, woody), hexanol (fruity) and octanoic acid (butter) which correlated with X4, X6, CH, PG. The lower left quadrant contained ethyl propanoate (fruity) which correlated with X1.

The lower right quadrant contained 2-phenylethanol (honey), ethyl-3-methylbutanoate (fruity), butanoic acid (cheese), ethyl-2-methylpropanoate (sweet), ethyl acetate (acetone), acetic acid (vinegar), ethyl-2-methylbutanoate (strawberry), 3-methylbutanoic acid & 2-methylbutanol (solvent), 2-methylbutyl acetate (fruity), 3-methylbutyl acetate (banana), 2-methylbutanoic acid (cheese) and butanol (malty) which correlated with X3, X5.

PCA analysis of the red wines in Figure 2 separated the volatile compounds in the

**TABLE 5.** Volatile compounds identified in white wine samples.

| Family         | Compounds                | CH     | PG     | XI     | X2     | X3     | X4     | X5     | X6     | DT    | CH OAV | PG OAV | X1 OAV | X2 OAV | X3 OAV | X4 OAV | X5 OAV | X6 OAV |
|----------------|--------------------------|--------|--------|--------|--------|--------|--------|--------|--------|-------|--------|--------|--------|--------|--------|--------|--------|--------|
| Acids          | Acetic acid              | 82443  | 91940  | 247462 | 199352 | 206846 | 346071 | 23877  | 238876 | 20000 | 4,12   | 4,59   | 12,37  | 9,96   | 10,34  | 17,3   | 11,94  | 11,94  |
|                | Butanoic acid            | 1697   | <LOQ   | 961    | 1262   | 1387   | 1326   | 1040   | 2205   | 200   | 8,49   | nd     | 4,81   | 6,31   | 6,94   | 6,63   | 5,2    | 11,03  |
|                | Hexanoic acid            | 6071   | <LOQ   | <LOQ   | <LOQ   | <LOQ   | <LOQ   | <LOQ   | 6138   | 420   | 14,45  | nd     | nd     | nd     | nd     | nd     | nd     | 30,69  |
|                | Octanoic acid            | 11988  | 6979   | 4211   | 7038   | 7112   | 6473   | 6311   | 10899  | 500   | 23,98  | 13,96  | 8,42   | 14,08  | 3,01   | 2,17   | 2,56   | 27,99  |
|                | Decanoic acid            | 4464   | 2836   | 681    | 937    | 1505   | 1086   | 1282   | 1378   | 1000  | 4,46   | 2,84   | 0,68   | 0,94   | 1,51   | 1,09   | 1,28   | 1,38   |
|                | 2-methylpropanol         | 13027  | 19865  | 13740  | 34016  | 20065  | 18302  | 15741  | 13993  | 40000 | 0,33   | 0,5    | 0,34   | 0,85   | 0,5    | 0,46   | 0,39   | 0,35   |
| Alcohols       | 3-methylbutanol          | 120594 | 150944 | 140360 | 209280 | 173834 | 154846 | 144071 | 150217 | 30000 | 4,02   | 5,03   | 4,68   | 6,98   | 5,8    | 5,16   | 4,8    | 5,01   |
|                | Hexanol                  | 1801   | 2007   | 677    | 748    | 848    | 1326   | 524    | 1190   | 8000  | 0,23   | 0,25   | 0,08   | 0,09   | 0,11   | 0,17   | 0,07   | 0,15   |
| Acetate esters | 2-phenylethanol          | 14199  | 11929  | 35317  | 44604  | 35273  | 37277  | 25686  | 31378  | 14000 | 1,01   | 0,85   | 2,52   | 3,19   | 2,52   | 2,66   | 1,83   | 2,24   |
|                | 2-methylpropyl acetate   | 28,8   | 64,1   | <LOQ   | <LOQ   | <LOQ   | <LOQ   | <LOQ   | <LOQ   | 1600  | 0,02   | 0,04   | nd     | nd     | nd     | nd     | nd     | nd     |
|                | 3-methylbutyl acetate    | 2445   | 4109   | 220    | 186    | 470    | 377    | 385    | 1096   | 30    | 81,05  | 136,97 | 7,33   | 6,2    | 15,67  | 12,57  | 12,83  | 36,53  |
|                | Hexyl acetate            | 353    | 401    | 9,94   | 4,06   | 20,5   | 37,8   | 12,3   | 88,2   | 1500  | 0,24   | 0,27   | 0,01   | 0,001  | 0,01   | 0,03   | 0,01   | 0,06   |
|                | 2-phenylethyl acetate    | 221    | 215    | 61,2   | 53,5   | 139    | 96,6   | 68,1   | 191    | 250   | 0,88   | 0,86   | 0,24   | 0,21   | 0,56   | 0,39   | 0,27   | 0,76   |
|                | Ethyl acetate            | 25752  | 32238  | 32467  | 34440  | 38138  | 5539   | 51792  | 36331  | 15000 | 1,72   | 2,15   | 2,16   | 0,23   | 2,54   | 3,69   | 3,45   | 2,42   |
|                | Ethyl propanoate         | 153    | 119    | 150    | 126    | 144    | 162    | 130    | 153    | 1800  | 0,09   | 0,07   | 0,08   | 0,07   | 0,08   | 0,09   | 0,07   | 0,09   |
|                | Ethyl-2-methylpropanoate | 28,4   | 39,5   | 118    | 281    | 211    | 204    | 130    | 92,7   | 15    | 1,89   | 2,63   | 7,87   | 18,73  | 14,07  | 13,6   | 8,67   | 6,18   |
|                | Ethyl butanoate          | 562    | 347    | 259    | 345    | 421    | 349    | 349    | 586    | 20    | 28,1   | 17,35  | 12,95  | 17,25  | 21,05  | 17,45  | 17,45  | 29,3   |
|                | Ethyl-3-methylbutanoate  | <LOQ   | <LOQ   | 43,3   | 65,6   | 61,7   | 41,8   | 44,4   | 31,8   | 3     | nd     | nd     | 14,43  | 21,87  | 20,57  | 13,93  | 14,8   | 10,6   |
| Ethyl esters   | Ethyl hexanoate          | 1426   | 867    | 661    | 1060   | 1130   | 975    | 1014   | 1526   | 14    | 101,86 | 61,93  | 47,21  | 75,71  | 80,71  | 69,64  | 72,43  | 109    |
|                | Ethyl octanoate          | 1747   | 1101   | 827    | 1074   | 1257   | 1128   | 1131   | 1555   | 600   | 2,91   | 1,84   | 1,38   | 1,79   | 2,1    | 1,88   | 1,89   | 2,59   |
|                | Ethyl decanoate          | 669    | 466    | 145    | 114    | 259    | 206    | 243    | 235    | 200   | 3,35   | 2,33   | 0,73   | 0,57   | 1,3    | 1,03   | 1,22   | 1,18   |
|                |                          |        |        |        |        |        |        |        |        |       |        |        |        |        |        |        |        |        |

All values reported in µg/L based on single measurements. DT (Detection Threshold), OAV (Odour Activity Value = concentration/DT).

**TABLE 6.** Volatile compounds identified in red wine samples.

| Family          | Compounds                | M1     | M2      | SH      | M3      | Yia     | DT    | M1 OAV | M2 OAV | SH OAV | M3 OAV | Yia OAV |
|-----------------|--------------------------|--------|---------|---------|---------|---------|-------|--------|--------|--------|--------|---------|
| Acids           | Acetic acid              | 903075 | 1411587 | 1253019 | 2842986 | 3892968 | 20000 | 45,15  | 70,58  | 62,65  | 142,15 | 194,65  |
|                 | Propanoic acid           | <LOQ   | 2908    | 5198    | 9561    | 6872    | 8000  | nd     | 0,36   | 0,65   | 1,12   | 0,86    |
|                 | Butanoic acid            | 2611   | 2036    | 2981    | 7189    | 4860    | 200   | 13,1   | 10,18  | 14,91  | 35,95  | 24,3    |
|                 | 3-methylbutanoic acid    | <LOQ   | <LOQ    | <LOQ    | 3040    | 4815    | 30    | nd     | nd     | nd     | 101,33 | 160,5   |
|                 | Hexanoic acid            | 6249   | 5831    | 6967    | 16397   | 12961   | 420   | 14,88  | 13,88  | 16,59  | 39,04  | 30,86   |
|                 | Octanoic acid            | 4170   | 3455    | 5088    | 11975   | 8651    | 500   | 8,34   | 6,91   | 12,11  | 28,51  | 20,6    |
|                 | Decanoic acid            | 415    | 296     | 1124    | 2016    | 1288    | 1000  | 0,42   | 0,3    | 1,12   | 2,02   | 1,29    |
| Alcohols        | 2-methylpropanol         | 101109 | 117621  | 129219  | 287726  | 265075  | 40000 | 2,53   | 2,94   | 3,23   | 7,12   | 6,63    |
|                 | Butanol                  | 3420   | 4131    | 7422    | 9504    | 9713    | 590   | 5,8    | 7      | 12,58  | 16,11  | 16,46   |
|                 | 3-methylbutanol          | 472190 | 566188  | 636626  | 1383105 | 1393035 | 30000 | 15,74  | 18,87  | 21,22  | 46,1   | 46,43   |
|                 | 2-methylbutanol          | 161333 | 195019  | 215352  | 462861  | 472136  | 1200  | 134,44 | 162,52 | 179,46 | 385,7  | 393,45  |
|                 | Hexanol                  | 2333   | 2252    | 8848    | 12285   | 6114    | 8000  | 0,29   | 0,28   | 1,11   | 1,54   | 0,76    |
| Acetate esters  | 2-phenylethanol          | 100558 | 107505  | 117513  | 248898  | 267200  | 14000 | 7,18   | 7,68   | 8,39   | 17,78  | 19,09   |
|                 | 3-methylbutyl acetate    | 570    | 859     | 613     | 1858    | 1998    | 30    | 19     | 28,63  | 20,43  | 61,93  | 66,6    |
|                 | 2-methylbutyl acetate    | 87     | 159     | 109     | 274     | 422     | 10    | 8,7    | 15,9   | 10,9   | 27,4   | 42,2    |
|                 | Hexyl acetate            | 4,32   | <LOQ    | 7,84    | 26      | 10,9    | 1500  | 0,001  | nd     | 0,01   | 0,02   | 0,01    |
|                 | 2-phenylethyl acetate    | 70,1   | 106     | 58,8    | 244     | 275     | 250   | 0,28   | 0,42   | 0,24   | 0,98   | 1,1     |
| Ethyl esters    | Ethyl acetate            | 132458 | 213643  | 214802  | 446547  | 540668  | 15000 | 8,83   | 14,24  | 14,32  | 29,7   | 36,04   |
|                 | Ethyl propanoate         | 389    | 431     | 967     | 1244    | 1170    | 1800  | 0,22   | 0,24   | 0,54   | 0,69   | 0,65    |
|                 | Ethyl-2-methylpropanoate | 478    | 628     | 825     | 1229    | 1579    | 15    | 31,87  | 41,87  | 55     | 81,93  | 105,27  |
|                 | Ethyl butanoate          | 586    | 433     | 738     | 1814    | 1052    | 20    | 29,3   | 21,65  | 36,9   | 90,7   | 52,6    |
|                 | Ethyl-2-methylbutanoate  | 80     | 103     | 161     | 177     | 268     | 1     | 80     | 103    | 161    | 177    | 268     |
|                 | Ethyl-3-methylbutanoate  | 132    | 218     | 300     | 355     | 520     | 3     | 44     | 72,67  | 100    | 3,55   | 173,33  |
|                 | Ethyl hexanoate          | 1141   | 796     | 1294    | 2894    | 1815    | 14    | 81,5   | 56,86  | 92,43  | 206,71 | 129,64  |
| Ethyl octanoate | Ethyl octanoate          | 925    | 762     | 1206    | 2739    | 1430    | 600   | 1,54   | 1,27   | 2,01   | 4,57   | 2,38    |
|                 | Ethyl decanoate          | 97,5   | 73,2    | 350     | 463     | 186     | 200   | 0,49   | 0,37   | 1,75   | 2,32   | 0,93    |

All values reported in µg/L based on single measurements. DT (Detection Threshold), OAV (Odour Activity Value = concentration/DT).

following ways. The upper left quadrant contained ethyl decanoate (pear), hexanol (fruity), decanoic acid (fatty), hexyl acetate (cherry) and ethyl octanoate (pear). The upper right quadrant contained ethyl propanoate (fruity), propanoic acid (pungent) and butanol (solvent) which correlated with SH. The lower left quadrant contained ethyl hexanoate (strawberry), butanoic acid (cheese), hexanoic acid (woody/leafy), octanoic acid (butter) and ethyl butanoate (strawberry) which correlated with M1, M3. The lower right quadrant contained ethyl-2-methylbutanoate (strawberry), 3-methylbutanol & 2-methylbutanol (solvent), ethyl-2-methylpropanoate (sweet), ethyl-3-methylbutanoate (fruity), 2-methylbutyl acetate (fruity), 2-phenylethyl acetate (plum), 3-methylbutyl acetate (banana), 3-methylbutanoic acid (cheese), 2-methylpropanol (solvent), ethyl acetate (fruity), acetic acid (vinegar), 2-phenylethanol (rose, honey), 2-methylpropyl acetate (banana, cherry), 2-methylpropanoic acid (cheese) and 2-methylbutanoic acid (fruity) which correlated with M2, Yia.

### 5. Spectral analysis and modified Sommers and tannin assays

There have been limited studies on the phenolic content of Cypriot wines, however, our results in Table 7 for total phenolics mirror the work done by Galanakis *et al.* (2015). The only measure

that stands out is the total phenolics for X1 at 423.35 mg/L which is very high for a white wine, levels are generally around 200 mg/L (Waterhouse *et al.*, 2016). This is however consistent with the high levels of phenolic compounds such as ethyl gallate, gallic acid and epigallocatechin identified for this wine in the non-volatile profiling of secondary metabolites by LC-MS/MS, non-targeted analysis.

### 6. Relating wine composition and sensory data by PLS regression

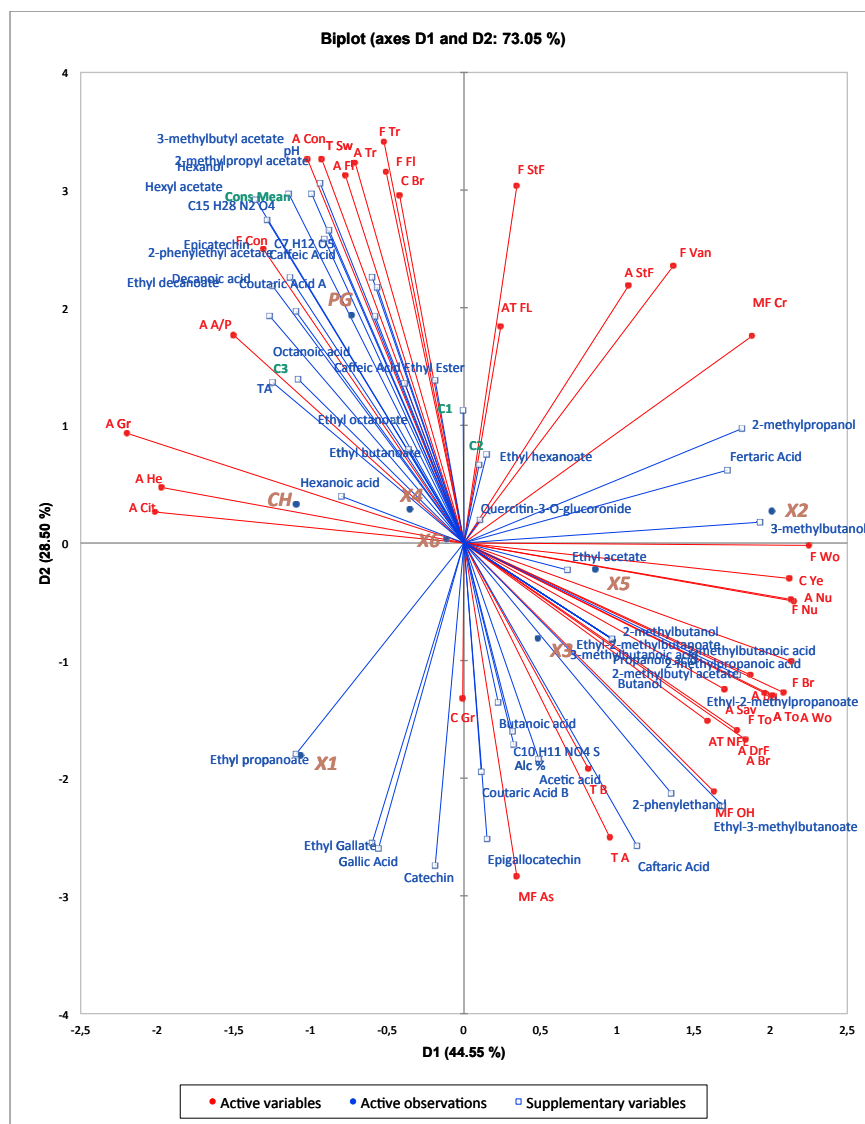
Volatile composition, basic chemical parameters and sensory data determined for eight white and five red wines were analysed through PLS regression to explore their underlying relationship. This PLS approach has been used successfully to evaluate mixed sensory and chemical data sets in Sauvignon Blanc wines (Benkwitz *et al.*, 2012). The first two principal components explained 60% of the variation in white wine composition (x-variables) and 62% of the variation in sensory properties (y-variables). In the red wine samples, the first two principal components explained 79% of the variation in wine composition (x-variables) and 58% of the variation in sensory properties (y-variables).

White wines (Figure 3a and 3b) were separated on the left side of the plot (PG and CH) based on characteristics such as stone fruit, sweet,

**TABLE 7.** Phenolic and anthocyanin composition of (a) white wine samples and (b) red wine samples.

| (a) | Wine code | Total phenolics<br>mg/L (GAE per a.u. @280 nm) | Flavonoid extractives<br>mg/L | Total hydroxycinnamates<br>mg/L                |
|-----|-----------|--|-------------------------------|--|
|     | CH        | 86.5   | 35.75                         | 34   |
|     | PG        | 68   | 0.25                          | 46   |
|     | X1        | 423.3  | 365                           | 39   |
|     | X2        | 86   | 33.75                         | 35   |
|     | X3        | 53.75  | 11.5                          | 28   |
|     | X4        | 30.1   | 80                            | 32   |
|     | X5        | 84.5   | 30.25                         | 34   |
|     | X6        | 124.3  | 68                            | 37   |
| (b) | Wine code | Free anthocyanins<br>mg/L                      | Total tannins<br>mg/L         | Total phenolics<br>mg/L (GAE per a.u. @280 nm) |
|     | M1        | 136  | 3220                          | 2075   |
|     | M2        | 154  | 2360                          | 1775   |
|     | SH        | 127  | 2030                          | 1625   |
|     | M3        | 186  | 2430                          | 1825   |
|     | Yia       | 147  | 2510                          | 1825   |

All values reported in mg/L based on single measurements.

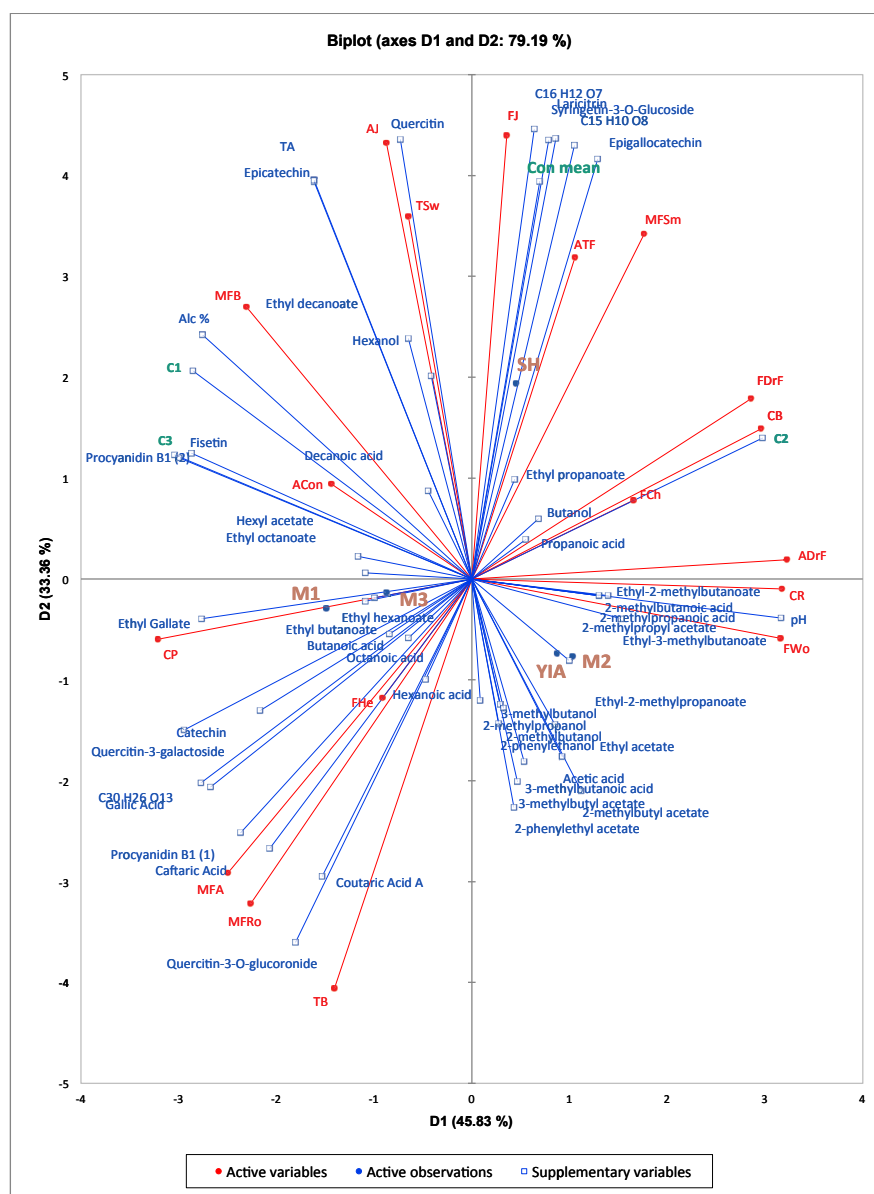


**FIGURE 1.** PCA biplot of white wine samples generated from correlation with chemical compounds and sensory attributes.

Sensory attributes (red), Chemical compounds (blue), Wines (orange), Consumer mean and Clusters (green). Colour Brown (CB), Colour Green (CGr), Colour Yellow (CYe), Aroma Apple Pear (AA/P), Aroma Citrus (ACit), Aroma Dried Fruit (ADrF), Aroma Stone Fruit (AStF), Aroma Confectionary (ACon), Aroma Tropical (ATr), Aroma Floral (AFI), Aroma Grass (AGr), Aroma Herbal (AHe), Aroma Butter (Abu), Aroma Nutty (ANu), Aroma Savoury (ASav), Aroma Toast (ATo), Aroma Wood (AWo), Aroma Bread (ABr), Taste Bitter (TB), Taste Sweet (TSw), Taste Acid (TA), Flavour Stone Fruit (FStF), Flavour Confectionary (FCon), Flavour Tropical (FTr), Flavour Floral (FFI), Flavour Nutty (FNu), Flavour Toast (FTo), Flavour Wood (FWo), Flavour Vanilla (FVan), Flavour Bread (FBr), Mouth Feel Alcohol (MFOH), Mouth Feel Astringent (MFAs), Mouth Feel Creamy (MFCr), After Taste Fruit Length (ATFL), After Taste Non-Fruit Length (ATNFL).

confectionery, tropical, floral, herbaceous, citrus, apple and pear. These characteristics correlated with fruity aroma compounds such as hexanol, hexyl acetate, 3-methylbutyl acetate and 2-methylpropyl acetate. Wines on the right side of the plot (X1, X2, X3, X5, X5, X6) had more astringent, bitter, savoury, bread, wood, toasty, alcohol characteristics. In particular X2, X3, X5 in the upper right quadrant exhibited more

developed, secondary characteristics associated with oak intervention and ageing. These characteristics correlated with compounds such as 2-phenylethanol, ethyl-3-methylbutanoate, ethyl-2-methylpropanoate, 3-methylbutanol and 2-methylpropanol. X1, X4, X6 in the lower right quadrant were associated with bitterness, astringency and green characteristics, which correlated to compounds such as ethyl acetate,



**FIGURE 2.** PCA biplot of red wine samples generated from correlation with chemical compounds and sensory attributes.

Sensory attributes (red), Chemical compounds (blue), Wines (orange), Consumer mean and Clusters (green). Colour Red (CR), Colour Purple (CP), Colour Brown (CB), Aroma Dried Fruit (ADrF), Aroma Jammy (AJ), Aroma Confectionery (ACon), Taste Bitter (TB), Taste Sweet (TSw), Flavour Dried Fruit (FDrF), Flavour Jammy (FJ), Flavour Chocolate (FCh), Flavour Herbal (FHe), Flavour Wood (FWo), Mouth Feel Bitter (MFB), Mouth Feel Astringent (MFA), Mouth Feel Smooth (MFSm), Mouth Feel Rough (MFRo).

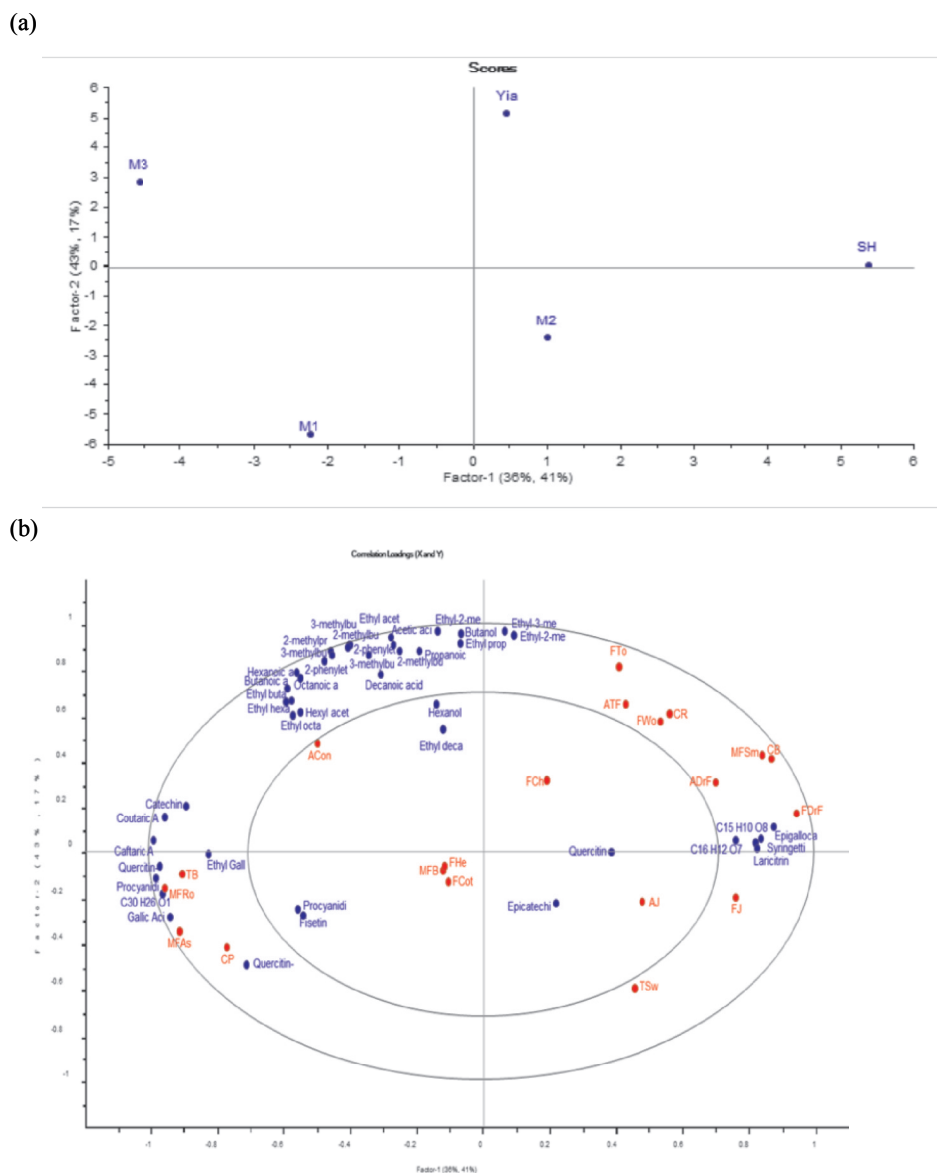
ethyl propanoate, butanoic acid, acetic acid, catechin, epigallocatechin and coumaric acid.

Red wines (Figure 4a and 4b) were separated on the left side of the plot (M1, M3) based on characteristics such as bitterness, astringency, herbal and confectionary, while wines on the right side of the plot (M2, Yia, SH) were separated based on characteristics such as toast, woody, dried fruit, jammy, sweet and fruity after taste. M3 in the upper left quadrant correlated to

compounds such as hexyl acetate, ethyl octanoate, ethyl hexanoate, butanoic acid, hexanoic acid and octanoic acid. Sample Yia, which was close to the centre line in the upper right quadrant, correlated with propanoic acid, butanol, ethyl-2-methylbutanoate, ethyl-2-methylpropanoate, ethyl-3-methylbutanoate, acetic acid and ethyl propanoate. SH was associated with compounds such as epigallocatechin, lactic acid, quercetin and syringetin-3-O-glucoside. M2 in the lower right







**FIGURE 4. (a)** PLS Regression plots of standardised volatile aroma compounds in red wines. **(b)** Correlation loadings between chemical (blue) and sensory (red) data 50% (inner), 100% (outer) explained variance limits.

Chemical compounds (Blue), Sensory attributes (Red). Colour Red (CR), Colour Purple (CP), Colour Brown (CB), Aroma Dried Fruit (ADrF), Aroma Jammy (AJ), Aroma Confectionery (ACon), Taste Bitter (TB), Taste Sweet (TSw), Flavour Dried Fruit (FDrF), Flavour Jammy (FJ), Flavour Chocolate (FCh), Flavour Herbal (FHe), Flavour Wood (FWo), Mouth Feel Bitter (MFB), Mouth Feel Astringent (MFAs), Mouth Feel Smooth (MFSm), Mouth Feel Rough (MFRo).

sensory active compounds in red wine (predominately Tempranillo and Grenache) that influence wine experts and consumers perception of quality. They found that there was a difference between consumers and experts in terms of relating sensory compounds and wine quality. Their consumers linked high quality with oak ageing and leather-like compounds, while the wine experts linked high quality with red fruity aromas (Sáenz-Navajas *et al.*, 2015). A study by Johnson *et al.* (2013) involving wine

experts concur with Sáenz-Navajas *et al.* (2015), with wine experts preferring berry fruit, spice, red fruit, dark fruit and oak characteristics to developed and savoury characteristics in Shiraz wines. Likewise, Niimi *et al.* (2018) had difficulties predicting wine quality from sensory profiling wines. Winemakers were consistently able to sort Cabernet-Sauvignon wines based on quality but found that Chardonnay wines were poorly discriminated in both sensory profiles and quality.

When relating wines made from the indigenous Cypriot varieties to other varieties, the following characteristics have been explored in terms of being positive or negative: for white wine King *et al.* (2010) explored Sauvignon Blanc wines made with different yeast strains. They found that flavours such as bruised apple, cooked, estery and floral aromas were not well liked while the box hedge/cat urine aromas were liked by both consumers and winemakers. Ali *et al.* (2011) studied the sensory attributes of Riesling and Mueller Thurgau. Their 'superior' wines were found to contain high levels of amino acids (proline and arginine), organic acids (malic and tartaric) and phenolic compounds (quercetin, catechin and epicatechin). Poor quality wines contained higher levels of lactic, acetic, and succinic acids, as well as amino acids (threonine and alanine) and phenolic compounds (caffeic acid, gallic acid and vanillic acid). Riesling was found to have higher levels of catechin, epicatechin, caftarate and coumarate. González-Álvarez *et al.* (2011) explored the sensory and chemical profile of wines made from the Spanish white variety Godello. They found that the sensory descriptors with the highest intensity were fruity (apple, citrus), floral aromas and herbaceous notes. The chemical compounds attributed to these compounds were ethyl esters, acetates, fatty acids and terpenes. Danish researchers Liu *et al.* (2015) analysed sensory and chemical composition of Solaris wines and found that 3-methyl-1-butanol, 3-methylbutyl acetate, ethyl acetate and ethyl hexanoate are important amongst the 79 compounds identified. Acetates and ethyl esters of fatty acids were correlated with floral and fruity aromas. The positive sensory attributes were described as floral and fruity (peach/apricot, Muscat, melon, banana and strawberry) while the negative attributes were described as chemical, wood and rooibos/smoke.

Many of these positive attributes have also been identified from our analysis of Xynisteri which was described sensorially as citrus, herbaceous, bitter, astringent, creamy, alcohol, dried fruit, bread, savoury, toast, wood, nutty, apple, pear, grass, herbaceous with a full length of fruit and non-fruit flavours in the after taste. Some of these attributes such as toast, wood, creamy and nutty however, are related to the wine making process and the use of oak barrels and are not grape variety attributes. Chemical analysis supported sensory analysis with aroma compounds of ethyl propanoate (fruity), 2-

phenylethanol (honey), ethyl-3-methylbutanoate (fruity), ethyl acetate (acetone), ethyl-2-methylpropanoate (sweet), 3-methylbutanol & 2-methylbutanol (solvent), hexanoic acid (leafy, woody), ethyl octanoate (pear, pineapple), hexanoic acid (leafy, woody) and ethyl butanoate (lactate) identified in wines. Phenolic compounds of catechin, caftaric acid, epigallocatechin, coumaric acid B, epigallocatechin, ethyl gallate and gallic acid and have been associated with quality in Riesling wines (González-Álvarez *et al.*, 2011).

Shiraz is the most widely planted and consumed red variety in Australia; it was therefore chosen to assist in benchmarking the red Cypriot varieties (Australian Bureau of Statistics, 2015). Shiraz sensory quality has been described by Li *et al.* (2017) as having aromas of red fruit, dark fruit, and confectionary, as well as flavours of jam, and high intensity along with five palate attributes: sweetness, palate fullness, astringency, surface coarseness, and hotness. These characteristics have been linked to ethyl acetate, ethyl 2-methylpropanoate, 2-methylpropyl acetate, ethyl butanoate, ethyl 3-methylbutanoate, ethyl hexanoate, ethyl lactate, ethyl octanoate, 2-methyl-1-butanol, 3-methyl-1-butanol and 2-phenylethanol (Li *et al.*, 2017).

When comparing the phenolic content of Cypriot varieties to Greek varieties Agiorgitiko, Xinomavro and Mandilaria, the Cypriot varieties have an equivalent total phenolic content to Agiorgitiko and less phenolics than Xinomavro and Mandilaria and have been shown to be less astringent than these two varieties (Kallithraka *et al.*, 2011). The same can be said for total tannins, Maratheftiko and Giannoudhi exhibit equal or less total tannins than Greek varieties Araklinos, Bakouri, Fidia, Karvounaris, Kotselina, Limniona, Mavrotragano, Nerostafilo, Papadiko and Thrapsa (Kallithraka *et al.*, 2015).

Koussissi *et al.* (2007) employed a sensory profiling of aroma in Greek wines using a rank rating technique. They investigated Agiorgitiko, Xinomavro, Syrah and Cabernet Sauvignon and found that Agiorgitiko wines differentiated from the other wines by aroma characteristics of floral, vanilla, caramelised (confectionery), fruity and berry. Xinomavro has been linked to high astringency and bitter/sour taste (Koussissi *et al.*, 2003). Cypriot red wines, Maratheftiko and Giannoudhi therefore compare favourably with common European varieties and less

common Greek varieties being described sensorially as dried fruit, jammy, confectionery, bitter, sweet, chocolate, herbaceous, woody, astringent and rough with full length of fruit flavours in the after taste. The Cypriot wines were also assessed to have aroma compounds that contributed to the above attributes, that is: strawberry, sweet, fruity, banana, cherry, pear, woody/leafy, and butter. As with the Xynisteri wines, the attributes of buttery and wood are due to the use of barrels in the wine making process and are not direct varietal attributes.

It is also worth noting that due to the small number of wine samples available for this preliminary study, it is difficult to make in depth comparisons with the more common European varieties. However, when we consider these quality parameters above and the consumer data generated in this study, we can speculate that the wines made from Cypriot varieties are comparable to common Australian wines and potentially similar to other quality European wines made from varying grape varieties.

These studies have provided us with useful information which will be followed up with further in-depth studies to investigate specific phenolic compounds by LC-MS/MS (targeted, quantitative analysis) as well as analysis of thiols and terpenes with repeated measures, along with further quantitative analysis of specific aroma compounds by GC/MS with repeated measures. Further RATA studies of Cypriot wines may involve research wines made from different locations and standardised wine making techniques to eliminate any wine making influence on the sensory analysis.

We believe that these studies have given wine producers in Australia and Cyprus further insight into a few of the popular Cypriot grape varieties and how Australian consumers might respond to these wines in the market place. Considering the similar climates of Australia and Cyprus, it is also predicted that these Cypriot grape varieties will be a source for environmentally sustainable wines which require less resources and aid in the future adaptation of the wine industry to a changing climate.

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