The Impact of Vineyard Origin and Winery on the Elemental Profile of Red Wines

Agilent ICP-MS with Mass Profiler Professional Chemometric Software

Application Note

Food Testing and Agriculture

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Introduction

There is an increasing demand among consumers to know that the food they purchase is both safe and authentic. The adulteration or substitution of high value food with lower grade ingredients is of concern. Mislabeling of an inexpensive product as a valued brand, which is then sold on as a top grade product, can also be highly profitable, especially for foodstuffs that are associated with a certain geographical origin. This is often the case with wine, cheese, ham, olive oil, and honey. Together with DNA fingerprinting and testing for various organic markers, multielement profiling has been proposed as a method to establish the authenticity of foods.

Various factors affect the elemental composition of food and beverages. The metal profile of wine, for example, depends on the composition of the soil where the vine is grown, viticultural practices (for example, application of agrochemicals and irrigation), and winemaking processes, including storage and aging [1].

Pattern recognition of trace elements by atomic spectroscopy has been used to determine if wine can be identified as coming from a specific region [2]. ICP-MS has been used for elemental fingerprinting of wines for decades [3-7]. In this study, ICP-MS was used to investigate the combined effects of vineyard origin and winery processing on 65 red wine samples. Agilent Mass Profiler Professional (MPP) integrated chemometric software was used to model the geographical origin of the wines, characterized by the concentrations of 63 elements. By including wines that originated from the same vineyard but were processed in different wineries, and *vice versa*, the vineyard effect can be separated from the winery effect. This adds to an understanding of how much the elemental profile of wine changes during the production process.

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Experimental

Chemicals and standards

All calibration solutions and dilutions were carried out using ultrapure water (18 MΩcm^{-1}, EMD Millipore) and ethanol (200 proof, Gold Shield Distributors, Hayward, CA). The internal standard mix was diluted 1:10 in 1% HNO_3 before use. SPEX CertiPrep multi-element calibration standards (1, 2A, 3, and 4) and internal standard mix (10 mg/L in 1% HNO_3) were from SPEX, Metuchen, NJ. Ultrapure nitric acid was from Fisher Scientific and the environmental spike mix from Agilent (p/n 5183-4687).

Samples and sample preparation

Sixty-five commercial red wine samples from five different vineyards in Northern California were processed in five different commercial wineries, as indicated in Figure 1. All wines were made from monovarietal grapevine cultivars, including *Vitis vinifera* cv. Cabernet Sauvignon, cv. Merlot, and cv. Pinot Noir. Every winery fermented the grapes from each individual vineyard in a separate fermentation vessel. Samples were taken directly from the stainless steel tanks after fermentation was finished, but before any additional postfermentation treatments took place. Wines were sampled in metal-free 50 mL plastic tubes (VWR, Radnor, PA), and stored at 4 °C until analysis. Before analysis, all wine samples were diluted 1:3 in 5% HNO_3 to decrease the ethanol levels to around 4%, and each sample was analyzed in duplicate.

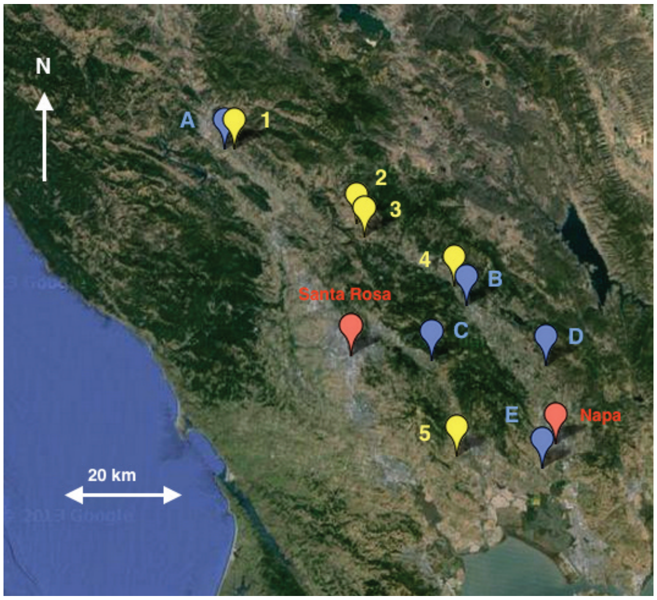

Figure 1. Map of California showing the five vineyards (1-5) and five wineries (A-E) used to source and process the 65 red wine samples.

Instrumentation

An Agilent 7700x ICP-MS with Octopole Reaction System (ORS^3) collision/reaction cell was used for the study. An internal standard (ISTD) solution containing ^{6}Li, Sc, Ge, Y, In, Tb, and Bi was diluted to 1 µg/L in 1% nitric acid. The ISTD solution was mixed online with the sample, using a mixing tee before the nebulizer. The ICP-MS was calibrated and tuned daily, using an Agilent tuning mix (Li, Y, Ce, Tl, Co, p/n 5188-6564) and Agilent Detector P/A Calibration solutions (Zn, Be, Cd, As, Ni, Pb, Mg, Th, Ca, Co, Sr, V, Cr, Mn, ^{6}Li, Sc, In, Lu, Bi, Y, Yb, Mo, Sb, Sn, Ge, Ru, Pd, Ti, and Ir, p/n 5188-6524). An Agilent environmental spike mix standard was used to spike the wine sample. Table 1 presents the instrument operating parameters.

Table 1. Agilent 7700x ICP-MS operating conditions.

	No gas	**He mode**	**High energy (HE) He**
RF power (W)	1,550		
Nebulizer type	MicroMist		
Carrier gas flow (L/min)	1.05		
Sampling depth (mm)	10		
Spray chamber temp. (°C)	2		
Cell gas flow (mL/min)	NA	4.3	10

Calibration

A six-point calibration between 0 and 500 µg/L was carried out for all 63 monitored elements using calibration solutions that were matched for the acid and carbon content of the wine samples (5% HNO_3 and 4% ethanol). Table 2 lists all 46 elements that were detected, and their limits of detection (LOD). Higher concentration elements (> 500 µg/L; B, Na, Mg, Si, P, K, Ca, Mn, Cu, Rb, Sr, and Ba) were analyzed following a 1:1,000 dilution in 5% HNO_3 (calibration solutions matrix matched with 5% HNO_3). Spiked samples were analyzed throughout the run to ensure the validity of the analytical method and continuous calibration blank and continuous calibration verification runs were performed every 10th sample.

Table 2. List of elements detected and LOD (ppb), from Hopfer *et al.* 2015 [1].

Element	*m/z*	Mode[a]	LOD[b]
Li	7	No gas	2.25E-01
Be	9	No gas	1.40E-02
B	11	No gas	1.12E-01
Na	23	No gas	1.79E+00
Mg	24	He	5.76E-01
Al	27	He	4.83E-01
P	31	He	5.45E+00
K	39	He	1.16E+00
Ca	43	He	8.79E-01
Ti	47	He	5.43E-01
V	51	He	2.40E-02
Cr	52	He	7.70E-02
Mn	55	He	2.30E-02
Co	59	He	9.00E-03
Ni	60	He	3.47E-01
Cu	63	He	2.30E-02
Zn	66	He	1.50E-02
Ga	69	He	5.00E-03
As	75	He	7.00E-03
Se	78	HEHe	5.20E-02
Rb	85	He	6.88E-03
Sr	88	He	1.00E-02
Mo	95	He	8.30E-02
Rh	103	He	1.00E-03
Cd	111	He	9.00E-03
Sn	118	He	2.10E-02
Sb	121	He	4.00E-03
Cs	133	He	4.60E-03
Ba	137	He	1.30E-02
La	139	He	6.00E-04
Ce	140	He	2.00E-03
Pr	141	He	3.00E-04
Nd	142	He	7.00E-04
Sm	147	He	2.00E-03
Eu	153	He	4.60E-06
Gd	157	He	1.00E-03
Dy	163	He	1.11E-03
Ho	165	He	6.00E-04
Er	166	He	2.00E-03
Tm	169	He	7.30E-03
Yb	172	He	1.00E-03
W	182	He	1.90E-02
Re	185	He	5.00E-04
Tl	205	He	1.40E-02
Pb	208	He	5.00E-03
U	238	He	6.60E-06

[a] He = helium; HEHe = high energy helium

[b] LOD, n = 10 calibration blank measurements, 99% confidence interval

Data analysis using Mass Profiler Professional software

Agilent ICP-MS MassHunter software (G7201B, version B.01.03) was used to acquire and analyze the data. Postanalysis, elemental concentrations for all wines were imported into the Agilent integrated MPP chemometric software to perform statistical analysis of the large and complex data sets (65 wine samples × 46 detected elements × three replicates). MPP also provided data visualization tools for a convenient way to investigate and determine relationships in multidimensional data sets, in this case, 65 wines from five different vineyard and winery combinations and 46 elemental concentrations. Analyses of variance (ANOVA) on $\log_2$ scale concentration data were done in MPP to assess winery and vineyard effects, as well as winery-by-vineyard interactions. Statistical significance was set at 5%. Canonical variate analysis (CVA) was chosen as a classification technique to study how individual wineries, vineyards, and the winery-vineyard combinations differed from each other using multivariate ANOVA models.

Results and Discussion

Elemental profiling

Of the 63 monitored elements, 46 were detected and included in the subsequent data analysis. Recoveries were between 93% (for Ba) and 103% (for Ca), measured with spiked samples analyzed throughout the sequence. None of the detected elements showed significant differences associated with the different wine cultivars. However, significant differences in elemental content were found between the five different wineries (33 significantly different elements), the five different vineyards (26 significantly different elements), and the 15 different winery-vineyard combinations (17 significantly different elements: Be, Na, P, Ti, Zn, As, Rb, Cd, Sb, Cs, La, Pr, Dy, Er, Tm, Yb, and Tl). These findings show that both grape growing and winemaking have an effect on the elemental composition of wine. More elements differed significantly in the wines across the different processing wineries compared to the different vineyard origins. This could be interpreted that winemaking has a larger impact on the elemental content of wines than vineyard location. Full details of the data for the vineyard and winery effects can be found in Hopfer *et al.* 2015 [1].

Winery and vineyard interaction

Based on the 17 elements mentioned above, a CVA graphical representation of the samples was obtained using MPP software (Figure 2). There is a clear cluster of wines from the A and B wineries. Grapes grown in vineyard 1 (A1, C1, and D1) are grouped, irrespective of the winery where the wine was produced. A similar pattern can be observed for grapes grown in vineyard 5. Vineyards 2 and 3 are geographically close to each other, where soil conditions are likely to be similar. This is reflected in the score plot (Figure 2) with A2 and B2 wines positioned on the right-hand side of the graph and A3 and B3 wines on the left. Only two samples were prepared using grapes grown at vineyard 4 (B4 and E4) and the results suggest that the contribution from the winery obscures the effect of the vineyard on the elemental composition.

Looking at the score boxplots and the total structure coefficients in Figures 3A and 3B, it is possible to study which elements contribute to the separation of the wine samples shown in Figure 2. Figure 3A shows that along the horizontal first dimension, CV1, wines are either separated by a strong vineyard effect, as for vineyards 1 and 5 that appear on opposite sides of the CV1 scale, or a combined winery-vineyard effect, as for wineries A and B and vineyards 2 and 4. Elements responsible for the horizontal separation of the wines are shown in Figure 3B. Wines on the right-hand side show higher levels of Be, Rb, Cs, Tl, and some rare earth elements, while wines on the left-hand side are correlated to Na, P, Ti, Zn, As, and, to a smaller degree, Cd and Sb.

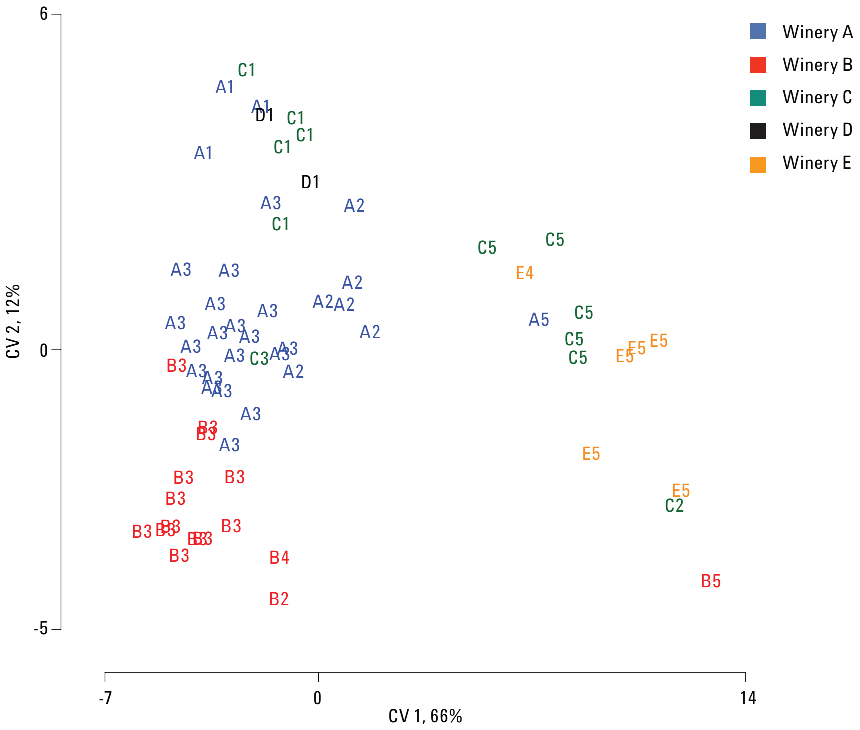

Figure 2. Canonical variate analysis for the first two dimensions using the winery-by-vineyard interaction term as classifier. The individual wine samples are shown color-coded by winery. The numbers 1-5 indicate the five different vineyards. Reprinted from: Hopfer, H.; Nelson, J.; Collins, T. S.; Heymann, H.; Ebeler, S. E. The combined impact of vineyard origin and processing winery on the elemental profile of red wines. *Food Chemistry* **2015**, *172*, pp. 11, with permission from Elsevier.

Along the vertical second dimension (CV2), wines processed in winery B are grouped at the bottom of the plot (Figure 3C). Figure 3D shows that wines processed in winery B had higher levels of all elements, except for P and Ti.

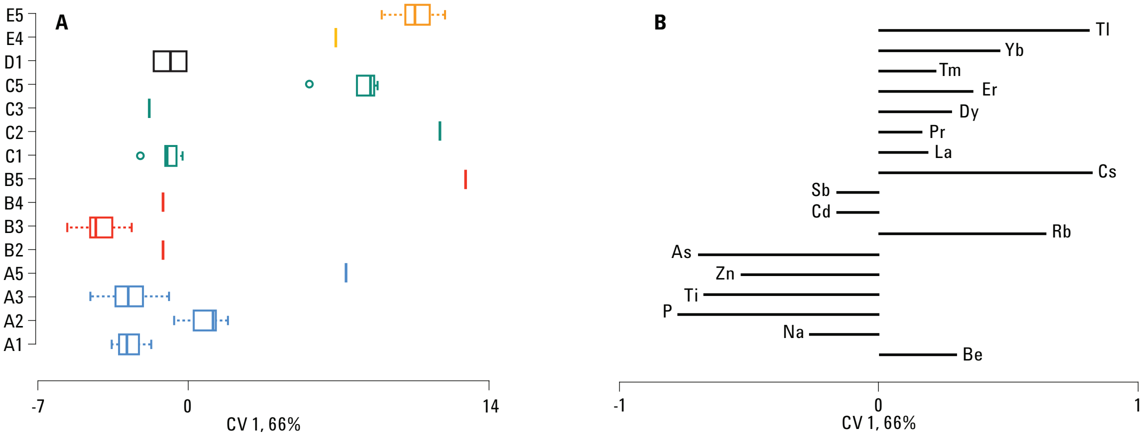

Figure 3a. Canonical variate analysis for the first two dimensions using the winery-by-vineyard interaction term as classifier. CV 1 box plot (A) and structure coefficient plot (B). Reprinted from: Hopfer, H.; Nelson, J.; Collins, T. S.; Heymann, H.; Ebeler, S. E. The combined impact of vineyard origin and processing winery on the elemental profile of red wines. *Food Chemistry* **2015**, *172*, pp. 11, with permission from Elsevier.

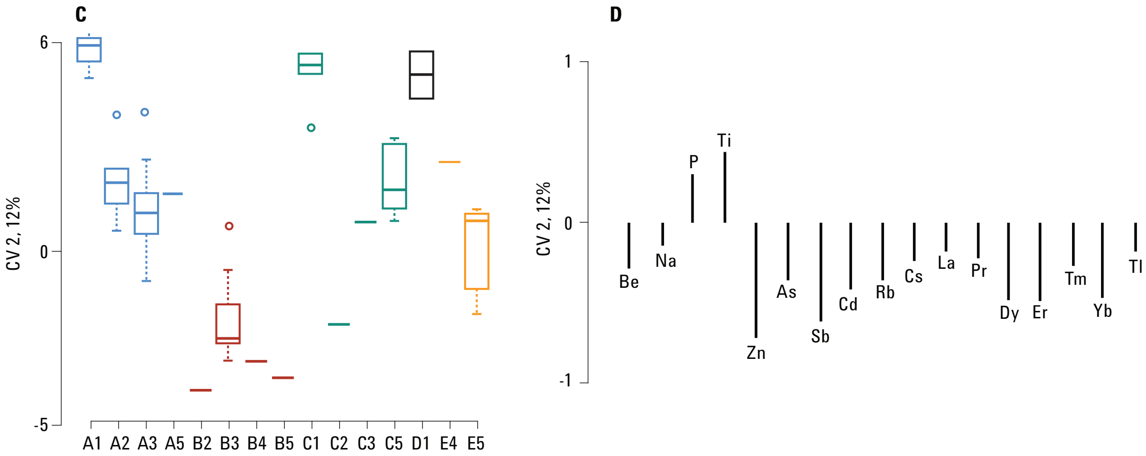

Figure 3b. CV 2 box plot (C) and structure coefficient plot (D). Elements represented by a long line have a larger contribution to the observed separation in the CVA. Reprinted from: Hopfer, H.; Nelson, J.; Collins, T. S.; Heymann, H.; Ebeler, S. E. The combined impact of vineyard origin and processing winery on the elemental profile of red wines. *Food Chemistry* **2015**, *172*, pp. 11, with permission from Elsevier.

Conclusions

Elemental profiling is increasingly used to characterize the geographical origin of foods and beverages, including wine. To better understand the combined effects of vineyard origin and winery processing, the elemental content of 65 red wines was studied using an Agilent 7700x ICP-MS, combined with Agilent Mass Profiler Professional chemometric software. The study demonstrated that elemental profiles could not distinguish the different grape types (vine cultivars). However, elemental fingerprinting was able to classify the wine samples according to vineyard origin, processing winery, and the combination of both factors. Seventeen elements showed a significant winery-by-vineyard interaction, that is, the elemental concentrations of these 17 elements were affected by both grape growing and winemaking to a different degree among the 15 winery-vineyard combinations. Studying these combined effects provides further insight into the determination of the geographical origin of red wines using multi-elemental fingerprints.

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Printed in the USA
September 9, 2015
5991-6111EN

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