

Profiling the trace metal composition of wine as a function of storage temperature and packaging type

Cite this: *J. Anal. At. Spectrom.*, 2013, **28**, 1288

Helene Hopfer,^{*a} Jenny Nelson,^b Alyson E. Mitchell,^{cd} Hildegard Heymann^a and Susan E. Ebeler^{ad}

The effect of wine packaging and storage temperature on the elemental profile of a commercial Cabernet Sauvignon wine was studied using inductively coupled plasma-mass spectrometry (ICP-MS). In the method, 14 trace elements were monitored in a concentration range between 0.1 and 500 $\mu\text{g L}^{-1}$, with five of these elements showing significant concentration differences among the samples as a result of the different wine packaging and storage temperatures. Among others, increased tin levels were found in screw capped samples, probably originating from the tin liner used inside the screw caps.

Received 24th March 2013

Accepted 25th April 2013

DOI: 10.1039/c3ja50098e

www.rsc.org/jaas

Introduction

Metals present in wines are the result of both endogenous and exogenous sources, deriving from either the grape itself, or being introduced during various steps of the grape growing and winemaking process.

Ideally, the endogenous metal composition of wine could be used for authenticity and determination of provenance, however, due to potential contamination, this task is rather complicated, and, consequently, only with careful experimental design accounting for all possible exogenous impact factors, can a true endogenous metal profile be obtained.

From a legal point of view, knowledge about the elemental profile of wines is important, as some elements have maximum legal limits, with most countries following the International Organisation of Vine and Wine (OIV) recommendations, which limit the amounts of As, B, Br, Cd, Pb and Zn in wine. Other countries have more stringent regulations in place, for example, in Croatia 16 different metals are monitored, including Cr, Sn, B and Br.¹

While most research in the area of elemental wine profiling is focused on the determination of fingerprints of various winegrowing regions using the trace metal content,^{2,3} the contribution of exogenous metal sources is often much larger. Application of various agrochemicals, or even a coastal location of the vineyard, were shown to increase the K, Ca, Cu, Cd, Mn, Pb and Na content of the finished wine.⁴

Common application areas of ICP-MS for wines are fingerprinting of trace elements for the determination of origin and

provenance. In a study on Canadian wines, Taylor *et al.*³ determined 34 trace elements in 95 wines from single-vineyard plots, together with corresponding soil samples from two Canadian wine regions. Using multivariate statistics, the authors report 100% discrimination accuracy between the two regions using only 10 elements. However, the soil and the wine trace element composition did not correlate well, indicating a severe impact of the winemaking process, taking place after the grape growing, thus complicating the fingerprinting of wines for provenance reasons.

It is known that during winemaking, grapes are often in long contact with various materials (stainless steel, glass, wood, *etc.*) and are treated with products such as fining agents, which were identified as a source for increased concentrations in Al, Cd, Cr, Cu, Fe, Zn and Ca,⁴ so clearly, certain steps during winemaking influence the trace element pattern of wine. One aspect of winemaking was studied by Jakubowski and co-workers:⁵ they showed that the use of bentonite, a commonly used fining agent, dramatically increased the concentration of rare earth elements by an order of magnitude and more, when they compared the elemental profiles of unfinished and finished wines.

In another study the impact of clarification, filtration and short-term storage on the rare earth elements was evaluated in white wine by Rossano *et al.*⁶ Similarly to Jakubowski, in all filtered and/or clarified wines (using membrane filters, cellulose sheets or bentonite or combinations thereof) higher levels of rare earth elements (REEs) were detected, on average about one order of magnitude higher in concentration than in the unfiltered and non-clarified wine, where REEs were found in the lower nanogram per litre range. Additionally, the authors report that storing the wine in either a glass bottle, an oak barrel or a stainless steel tank resulted in different REE patterns. However, the authors did not give any further information about the storage containers used (*e.g.* new or used barrel and tank, type

^aDept. of Viticulture and Enology, University of California, Davis, USA. E-mail: hhopfer@ucdavis.edu; Fax: +1 530 752 0382; Tel: +1 530 752 9356

^bAgilent Technologies, Santa Clara, USA

^cDept. of Food Science and Technology, University of California, Davis, USA

^dFood Safety and Measurement Facility, University of California, Davis, USA

of bottle closure, cleaning regime, *etc.*), and so these results can only serve as an indication for a potential contamination with REE due to storage vessels.

These results together with the discrepancy between soil and wine elemental compositions found by Taylor *et al.*³ lead to the question of other factors that may have an impact on the trace element profile of wine as well. In this study we report the effect of wine packaging materials and storage temperatures on the trace elemental profile of red wine.

Materials and methods

Materials

The samples used were part of a larger storage study about the sensory and chemical changes due to storage temperature and wine packaging.⁷ In short, the same commercially available Cabernet Sauvignon from the Californian Central Coast, vintage 2009, was used for 12 different treatments, which included all combinations of three storage temperatures (10 °C, 20 °C, 40 °C) and four packaging types (0.75 L green glass bottle with natural cork closure (29 mm × 49 mm, AC-1 grade, ACI Cork, Fairfield, CA, USA), 0.75 L green glass bottle with screw cap closures filled with 2 filling heights (aluminium Stelvin cap 30 mm × 60 mm, Federfin Tech S.R.L., Tromello, Italy, with 28.6 mm × 2 mm tin-polyvinylidene chloride (PVDC) liner, Oenoseal, Chazay D'Azergues, France), and a bag-in-box (3 L DuraShield 34 ES, Scholle Packaging, Nohlake, IL, USA). Samples were stored upright during the entire storage period.

Multi-element calibration standards (2A and 3) were purchased from SPEX CertiPrep (Metuchen, NJ, USA), ultrapure concentrated nitric acid was obtained from Fisher Scientific (Pittsburgh, PA, USA), ultrapure water (18 MΩ cm, EMD Millipore Beillerica, MA, USA) and 200 proof ethanol (GoldShield, Hayward, CA, USA) were used for the matrix matching of the calibration curves.

Instrumentation

A quadrupole-ICP-MS 7700x from Agilent Technologies was used for all trace element measurements (RF power 1550 W, RF matching 1.8 V, sample depth 10 mm, carrier gas flow of 1.03 L min⁻¹). The quartz double-pass spray chamber was cooled to 2 °C with a Peltier system, and a borosilicate micromist nebulizer (nebulizer pump 0.1 rps) was used. A total of 100 sweeps per replicate with three replicate measurement of each vial were realized. Helium was used as a collision cell gas with a flow rate of 4.3 mL min⁻¹, with the exception of As⁷⁵ and Se⁷⁸, which were quantified using the high-energy helium mode, where helium had a flow rate of 10 mL min⁻¹. The quantified elements were ⁵¹V, ⁵²Cr, ⁵⁵Mn, ⁵⁶Fe, ⁵⁷Fe, ⁵⁸Ni, ⁵⁹Co, ⁶⁰Ni, ⁶⁶Zn, ⁷⁵As, ⁷⁸Se, ¹¹¹Cd, ¹¹⁷Sn, ¹¹⁸Sn, ¹¹⁹Sn, ¹²⁰Sn, ¹³³Cs, ²⁰⁵Tl and ²⁰⁸Pb.

Sample preparation

Wine samples were diluted 1 : 3 with 1% nitric acid to decrease the ethanol levels to around 4%, as various studies have shown that ethanol as an organic solvent has an effect on the plasma stability; a dilution of the ethanol to a level of 5% is a good

compromise for most elements in terms of sensitivity and selectivity.^{6,8–11} Wines were sampled directly out of the bottle, and special care was taken not to shake the bottles, as the wine closure would have been in contact with the wine during that step. All calibrated elements (⁵¹V, ⁵²Cr, ⁵⁵Mn, ⁵⁶Fe, ⁵⁷Fe, ⁵⁸Ni, ⁵⁹Co, ⁶⁰Ni, ⁶⁶Zn, ⁷⁵As, ⁷⁸Se, ¹¹¹Cd, ¹¹⁷Sn, ¹¹⁸Sn, ¹¹⁹Sn, ¹²⁰Sn, ¹³³Cs, ²⁰⁵Tl and ²⁰⁸Pb) were quantified using two five-point multi-element calibration curves in the concentration range from 0.1 to 500 µg L⁻¹ in matrix-matched solutions (1% HNO₃ and 4% ethanol). Wines were measured in triplicate and spiked wine samples containing 0.5, 1 or 10 µg L⁻¹ tin were measured as quality control samples throughout the sample queue. An internal standard solution diluted to 1 µg L⁻¹ in 1% nitric acid (Claritas PPT Grade Internal Standard Mix 1, SPEX CertiPrep, Metuchen, NJ, USA) was mixed using a mixing tee with the sample before the nebulizer and contained ⁶Li, ⁴⁵Sc, ⁷²Ge, ⁸⁹Y, ¹¹⁵In, ¹⁵⁹Tb and ²⁰⁹Bi. Seven sample blanks were run to determine limits of detection and quantitation (LOD, LOQ) as suggested by the International Union of Pure and Applied Chemistry (IUPAC).¹²

Data analysis

All obtained data were statistically evaluated with RStudio version 0.97.248 (Boston, MA). All isotopes were tested for significance in a model with the sample and replicate as fixed effects, using an α level of 5%. Elements that differed significantly were then used in the graphical representation of the sample differences using Principal Component Analysis (correlation matrix).

Results and discussion

Using the 19 monitored masses for a total of 14 different elements, five elements differed significantly among the samples due to the packaging type and storage temperature. Table 1 shows the significant concentration differences of the samples. All elements were below their legal limits, if applicable. Significantly higher V levels were found in high fill screw capped bottles, while all other packaging types showed lower levels of V. Additionally, the high fill screw cap samples also showed a temperature effect for the V concentration, with decreasing V levels as storage temperature increased. We speculate that these differences are a result of the high fill level in combination with the screw cap closure, and the immediate contact of the wine with the tin-PVDC liner inside the screw cap. This may have resulted in reactions between the wine and the closure, taking place during the storage period at higher storage temperatures. Almeida and coworkers reported increased V levels as a result of the winemaking process, where the grape material was in contact with winery equipment (*e.g.* stainless steel containers).¹³

For Cr, all bottle samples showed significantly higher levels than the bag-in-box samples, and these differences were constant over the three storage temperatures. Cr is a common stainless steel alloy element, and all bottle samples were transported and stored for 2 days in a stainless steel keg before

Table 1 Concentrations of the significantly different elements in the measured wine samples in $\mu\text{g L}^{-1}$ ($n = 3$), together with limits of detection (LOD), limits of quantification (LOQ) and detection limits (DL). Columns sharing the same letter are not significantly different ($P \leq 0.05$)

	$^{\circ}\text{C}$	V^{51}	Cr^{52}	Cu^{63}	Sn^{118}	Pb^{208}
Limits	—	—	100 ^a	1,000 ^a	10,000 ^b	150 ^a
Bag-in-box	10	14.8 c	14.1 b	21.1 h	0.1 hi	4.3 d
	20	15.4 c	14.7 b	22.0 h	0.0 i	4.5 d
	40	13.9 c	14.1 b	11.0 i	0.0 i	4.6 d
Natural cork	10	15.5 c	22.0 a	84.1 b	0.8 f	5.4 bcd
	20	15.6 c	22.3 a	59.8 d	0.6 fg	5.2 cd
	40	13.1 c	18.4 ab	28.5 gh	0.4 gh	4.5 d
Low fill screw cap	10	15.5 c	22.7 a	41.5 f	6.0 e	5.1 d
	20	15.7 c	23.0 a	50.5 e	8.7 c	5.5 bcd
	40	15.3 c	22.7 a	33.8 fg	12.3 b	5 d
High fill screw cap	10	34.7 a	22.9 a	152.7 a	6.3 e	8.8 a
	20	22.1 b	22.1 a	68.7 c	8.3 d	6.5 b
	40	20.4 b	22.5 a	50.2 e	16.0 a	6.4 bc
LOD ^c		0.001	0.007	0.044	0.018	0.001
LOQ ^c		0.003	0.023	0.14	0.057	0.003
DL ^d		0.004	0.036	0.027	0.027	0.006

^a Legal limits by the OIV.¹ ^b Legal limits by the Croatian government.¹ ^c LOD and LOQ were determined *via* the standard deviation (sd) of 7 blank runs (LOD = $3.14 \times \text{sd}$, LOQ = $10 \times \text{sd}$). ^d Detection limits (DL) were reported by the Agilent's ICP-MS MassHunter Workstation software (version A.01.02, Agilent, Santa Clara, CA, USA).

they were bottled, which could explain the observed differences, similarly to the observation of Almeida and Vasconcelos¹³ and Kristl *et al.*¹⁴

A significant temperature effect was found for the Cu levels in all samples. In all samples Cu levels decreased with increasing storage temperature, indicating chemical reactions consuming Cu, for example, copper is known to react with thiol groups of proteins, forming precipitates that are not analysed because they settle at the bottom of the bottle.¹⁵ Due to the sampling directly out of the liquid part of the bottle we did not sample any precipitates from the bottom of the bottle. The decrease in copper levels was observed independent of the packaging type. According to previous studies, copper measured in wine can be explained from copper accumulation in the soil from copper sulphate treatments in the vineyard to reduce fungal pressure, the use of copper for wine fining purposes or the use of copper-containing winery equipment during the winemaking process.¹³

Significant differences in lead levels due to the different packaging types and storage temperatures were observed, with highest levels of Pb in the high fill screw cap samples stored at 10 $^{\circ}\text{C}$. We believe that these differences are a result of the different packaging types, with an additional temperature-driven effect of metal complex formation with other wine components (*e.g.* polyphenols) as observed for all other elements, in accordance to the observations by Kristl *et al.*¹⁴ and Almeida and Vasconcelos.¹⁶

Lastly, elevated tin levels were measured in the screw cap samples only, a strong indicator that tin leached out of the tin-PVDC liner into the stored wine. This effect was pronounced at the highest storage temperature of 40 $^{\circ}\text{C}$, due to the volumetric

expansion of the wine when being warmed up, thus, being in contact with the screw cap liner during the storage period. We are not sure how the tin leached into the wine at the lower storage temperatures, as the wine was not in contact with the liner, but assume that short bottle movements were enough to splash wine onto the liner, leaching tin out. Additionally, the acidic environment of wine (pH of the samples was between 3.70 and 3.74, determined after the storage period⁷) is most likely facilitating the leaching of elements into the wine.

Using the significantly different elements, a graphical representation of the sample similarities and dissimilarities was obtained in a Principal Component Analysis (PCA), shown in Fig. 1. Within the first two principal components (PCs) over 90% of the total variance was explained, with 69.4% in the first dimension and an additional 21.3% in the second dimension. Samples are separated by packaging type along PC1, with the bag-in-box samples (bib) located on the left hand side of the PCA biplot, next to the natural cork closure bottles (naco). The two screw cap closures (high and low fill screw cap) are positioned in the middle and on the right hand side of the biplot. Along PC2 storage temperature separates the samples, with samples stored at the highest storage temperature of 40 $^{\circ}\text{C}$ at the top of each packaging group, the 10 $^{\circ}\text{C}$ samples at the bottom and the 20 $^{\circ}\text{C}$ samples in between. Interestingly, a higher variability due to the storage temperature was observed for the bottle samples (natural cork and both screw cap closures), indicating temperature-driven changes in the elemental pattern dependent on the packaging type. This could be the result of the lower concentration of all significant

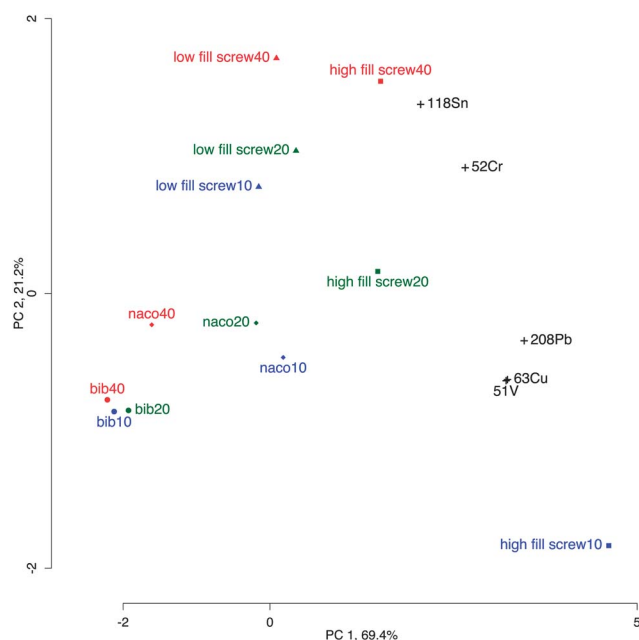


Fig. 1 PCA biplot for the statistically significant elements determined in the wine samples with ICP-MS. Samples are coded with different symbols and colors for the different packaging types and storage temperatures, respectively (bag-in-box (●), natural cork closure (◆), low fill screw cap closure (▲), high fill screw cap (■), 10 $^{\circ}\text{C}$ in blue, 20 $^{\circ}\text{C}$ in green, 40 $^{\circ}\text{C}$ in red).

elements in the bag-in-box samples, compared to the bottle samples.

Both screw cap samples (low and high fill) showed the highest levels of tin and chromium (Fig. 1 and Table 1), while highest levels in copper, vanadium and lead were found in the high fill screw cap sample stored at 10 °C. All bag-in-box samples had significantly lower levels of Cr, thus being negatively correlated in the PCA biplot to Cr.

Conclusions

In this study we could show that exogenous factors have a clear impact on the elemental profile of wine. Besides previously reported contamination of wine with fining agents, clarification and filtration devices, we showed with our results that also the type of wine packaging impacts the elemental profile of wine.

For the future, a closer look at the different steps in wine making and wine storage should enable us to better understand which elements are most influenced by the wine making processes. From a toxicological point of view, the observed levels of elements were all well below available official regulations.

Acknowledgements

The Food Safety and Measurement Facility is supported by loans and gifts from Agilent Technologies, Inc., Gerstel U.S., Inc., and Constellation Brands U.S. Jerry Lohr provided post-doctoral financial support. We are grateful for the help of Christopher A. Jenkins, Charles 'Chik' Brenneman, Scott C. Frost and Paul Green for help with the analysis and bottling.

Notes and references

- 1 B. Tariba, *Biol. Trace Elem. Res.*, 2011, **144**, 143.
- 2 M. J. Baxter, H. M. Crews, M. J. Dennis, I. Goodall and D. Anderson, *Food Chem.*, 1997, **60**, 443.
- 3 V. F. Taylor, H. P. Longerich and J. D. Greenough, *J. Agric. Food Chem.*, 2003, **51**, 856.
- 4 P. Pohl, *TrAC, Trends Anal. Chem.*, 2007, **26**, 941.
- 5 N. Jakubowski, E. Brandt, D. Stuewer, H. R. Eschnauer and S. Görtges, *Anal. Bioanal. Chem.*, 1999, **364**, 424.
- 6 E. C. Rossano, Z. Szilágyi and A. Malorni, *J. Agric. Food Chem.*, 2007, **55**, 311.
- 7 H. Hopfer, P. A. Buffon, S. E. Ebeler and H. Heymann, *J. Agric. Food Chem.*, 2013, **61**, 3320.
- 8 A. W. Boorn and R. F. Browner, *Anal. Chem.*, 1982, **54**, 1402.
- 9 R. F. J. Dams, J. Goossens and L. Moens, *Microchim. Acta*, 1995, **119**, 277.
- 10 J. Goossens, T. Smaele, L. Moens and R. Dams, *Anal. Bioanal. Chem.*, 1993, **347**, 119.
- 11 J. Goossens, L. Moens and R. Dams, *Anal. Chim. Acta*, 1994, **293**, 171.
- 12 V. Thomsen, D. Schatzlein and D. Mercurio, *Spectroscopy*, 2003, **18**, 112.
- 13 C. M. R. Almeida and M. T. S. D. Vasconcelos, *J. Agric. Food Chem.*, 2003, **51**, 4788.
- 14 J. Kristl, M. Veber and M. Slekovec, *Anal. Bioanal. Chem.*, 2002, **373**, 200.
- 15 M. Ugliano, M. Kwiatkowski, S. Vidal, D. Capone, T. Siebert, J.-B. Dieval, O. Aagaard and E. J. Waters, *J. Agric. Food Chem.*, 2011, **59**, 2564.
- 16 C. M. R. Almeida and M. T. S. D. Vasconcelos, *J. Agric. Food Chem.*, 2003, **51**, 3012.