

Multi-element Analysis of South African Wines by ICP–MS and Their Classification According to Geographical Origin

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Wines from three important wine-producing regions, Stellenbosch, Robertson, and Swartland, in the Western Cape Province of South Africa, were analyzed by ICP–MS and the elemental composition used in multivariate statistical analysis to classify the wines according to geographical origin. The method is based on the assumption that the provenance soil is an important contributor to the trace element composition of a wine. A total of 40 elements were determined in 40 wines. Of these, 20 elements: Li, B, Mg, Al, Si, Cl, Sc, Mn, Ni, Ga, Se, Rb, Sr, Nb, Cs, Ba, La, W, Tl, and U showed differences in their means across the three areas. In a stepwise discriminant analysis procedure, functions based on linear combinations of the log-transformed element concentrations of Al, Mn, Rb, Ba, W, and Tl were generated to correctly classify wines from each region. In an alternative approach, a pairwise discriminant analysis procedure, not previously used in wine provenance studies, was tested. In this procedure, the classification was done in three steps, with each step classifying a wine as coming from a certain region or not. The combination of elements characterizing wines from a particular region was different in each region. The discriminant functions were based on the following elements: Al, Mn, Rb, Ba, and W for Stellenbosch; Se, Rb, Cs, and Tl for Robertson; and Al, Mn, Rb, Sr, Ba, and Tl for Swartland. After this procedure, the classification of the wines into one of the groups was 100% successful.

KEYWORDS: Wine analysis; fingerprinting; multivariate statistical analysis; provenance; multi-element analysis; ICP–MS

INTRODUCTION

Fingerprinting techniques based on chemical composition and multivariate statistical analysis (1, 2) of analytical data can be used for identification and classification of a specific agricultural product type according to geographical origin. The method assumes that the chemical composition of an agricultural product such as wine (3–10), coffee (11), tea (12), olive oil, and fruit juice (13, 14) will reflect the composition of the provenance soil, at least for certain elements. The key to the successful application of this technique is the selection of suitable elements that would reflect the link with soil geochemistry and thus have discriminating potential for that particular product. Only a limited number of elements qualify for this purpose. Reliable information on the elemental composition, mostly at trace level, is required to apply this method with any degree of success. One of the most versatile techniques for this purpose

is ICP–MS, which can be used to determine the multi-element composition (3–9) of samples or the isotopic ratios (11, 15–17) of elements that show variation in their isotopic composition in different geographical regions. Elements with isotopes of radiogenic origin, such as Sr and Pb, fall into this category.

In this study, the trace element composition of South African wines from three major wine-producing regions in the Western Cape Province was compared with the aim of identifying and classifying the wines according to origin. Studies in different wine-producing countries, for example Canada (4), Spain (8), Portugal (5), Germany (3, 7), France (18, 19), and Switzerland (20), have shown that fingerprinting techniques have potential in obtaining information about the origin of wines. The technique is by no means simple, because many factors may cause the trace element composition of a wine to differ from that related to the elemental composition of the soil. These factors include agricultural practices such as the use of fertilizers and pesticides (5) and climatic factors such as heavy rains during the growth season. Pollution (16) and the quality of the irrigation water must be considered. The vinification process itself may contribute to the trace element composition of a wine. Examples

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Table 1. Optimized Operating Conditions and Acquisition Parameters for the Perkin–Elmer Sciex ELAN 5000 ICP-MS Instrument Used for the Analysis of Diluted Wine Samples

parameter	semiquantitative	quantitative
RF power (W)	1000	1000
plasma gas flow rate (L/min)	12	12
nebulizer gas flow rate (L/min)	0.80–1.0	0.80–1.0
auxiliary gas flow rate (L/min)	1.2	1.2
sample uptake rate (mL/min)	1.0	1.0
dwelt time (ms)	50	50
sweeps/reading	5	50
readings/replicate	1	1
number of replicates	3	3
mass–charge ratios monitored	6–238 excluding 28–38, 40–41, and 80 in peak hop mode using Total Quant II software	Cr-51, Mn-55, Rb-85, Sr-88, Mo-98, Cs-133, Ba-138, La-139, Ce-140, Nd-146, Tl-205, Pb-208, U-238

of elements that were found to be minimally changed during the vinification process include Li, B, Mg, Ca, Mn, Zn, Rb, Sr, Cs, and Pb (21), and those that could be affected were Al, Cd, Co, Cr, Cu, Fe, Mn, Pb, V, and Zn (22). No consensus is reached on this effect, and the elements included in the two lists may differ from study to study. Differential uptake of trace elements in the skin and flesh of the grape (23) can cause complications. White and red wine from the same region may therefore differ in trace element composition because for red wine the skin contact is longer after crushing than in the process for making white wine. More elements may therefore leach out into the red grape juice than in the white grape juice.

All of these factors may influence the correlation between wine and soil composition and limit the usefulness of the fingerprinting procedure. Some of these factors have been investigated, but the results reported in the literature are still inconclusive and in many cases contradictory. Ideally then, the elements selected for identification should not be affected by the vinification process, agricultural practices, and environmental conditions and should correlate with soil composition.

The specific aims of this study were the determination of the elemental composition of wines from Robertson, Stellenbosch, and Swartland, three main wine-producing areas in the Western Cape Province of South Africa, and the use of these data to uniquely classify wines from these areas according to a multivariate statistical procedure. A set of indicator elements suitable for discriminant analysis and specific for South African wines was to be determined. The feasibility of including red and white wines in the same data set for provenance determination was evaluated.

MATERIALS AND METHODS

Instrumentation. Multi-element determinations were carried out with a Perkin–Elmer Sciex Elan 5000 quadrupole-based ICP–MS instrument equipped with a cross-flow nebulizer, a Scott-type spray chamber, nickel sampling cone and skimmer, and a Gilson peristaltic sample delivery system. The optimized operating conditions for analysis of the diluted wine samples are listed in **Table 1**. Optimization was done for maximum sensitivity while maintaining the oxide ratio as low as possible. An oxide ratio for $I_{\text{ThO}}/I_{\text{Th}} < 0.05$ was attainable on the Elan 5000 system at a typical sensitivity of 5000 cps μg In.

Samples. A sample set of 40 wines was obtained from three major wine-producing regions in South Africa: Stellenbosch, Robertson, and Swartland. Each wine could be traced to a specific vineyard where the grapes were grown to produce the wine. Both red and white wines were included. **Table 2** lists the origin of the selected wines. The white cultivars were about equally divided among chardonnay, sauvignon blanc, and chenin blanc. The red cultivars consisted of cabernet sauvignon, shiraz, pinotage, and merlot. None were fermented or aged in wood. All wines were from the 2004 vintage. They were sampled

Table 2. Sampling Details of 40 Wine Samples

region	cellar	number of samples		
		total	red	white
Stellenbosch	Overgaauw, Simonsig, Jordan, Eikendal, Rust en Vrede	14	5	9
Robertson	Clairvaux, Rooiberg, Bonnievale, Roodezand	10	4	6
Swartland	Swartland, Perdeberg, Riebeeck, Porterville	16	8	8

before blending and final bottling to preserve the trace to the vineyard of origin.

Sample Preparation. Dilution. Wine samples were diluted 1:1 with 0.14 M HNO_3 . The dilution reduces the ethanol concentration to between 5 and 6%, which is sufficiently low to diminish matrix effects and plasma instability commonly associated with introducing matrixes containing organics into the plasma. After a 1:1 dilution, concentrations of most elements of interest, except the rare earth elements, remain comfortably above the detection limits of the instrument.

Microwave Digestion. Wine samples were also analyzed after microwave digestion (24–26) using a Milestone MLS1200 microwave digestion system. A total of 1.5 mL of wine sample, 150 μL of 65% HNO_3 , and 1.5 mL of 30% H_2O_2 were added into the PTFE digestion cells and microwaved for 33 min by increasing the power to 600 W in a stepwise fashion. The residues were dissolved in 12 mL of 0.14 M HNO_3 . The sample dilution factor was effectively 8. PTFE cells were cleaned by microwaving 5 mL of 65% HNO_3 for 10 min at 400 W. The H_2O_2 (Vel 6171) used in the digestion process was found to contain substantial amounts of Sn. Sn could therefore not be determined in digested samples.

Blanks, Standards, and Internal Standards. A standard solution containing 50 $\mu\text{g}/\text{L}$ of Be, Co, Rh, In, Pb, and Th in 6% ethanol/0.14 M HNO_3 was used to determine the sensitivity factors for all elements across the entire mass range for the measurement of diluted samples made in semiquantitative mode. In the case of measuring digested samples, ethanol was omitted in the calibration solution. High-purity ethanol (Panreac Quimica, Spain) was used for preparing matrix-matched standards. The internal standard In was added to the level of 50 $\mu\text{g}/\text{L}$ to all samples, standards, and blanks. External standards for quantitative analysis were prepared in 6% ethanol/0.14 M HNO_3 for diluted samples and in 0.14 M HNO_3 for digested samples. Blanks for the measurement of diluted and digested samples were a 6% ethanol/0.14 M HNO_3 solution containing 50 $\mu\text{g}/\text{L}$ of the internal standard and a 0.14 M HNO_3 procedure blank submitted to the microwave treatment and including the internal standard, respectively. Standards and the internal standard were prepared by appropriate dilution from 1000 mg/L Merck ICP standard stock solutions. HNO_3 was purified by distillation in a sub-boiling quartz distillation system. High-purity 18 M Ω cm water was obtained from a Millipore system and was used in the preparation of all solutions.

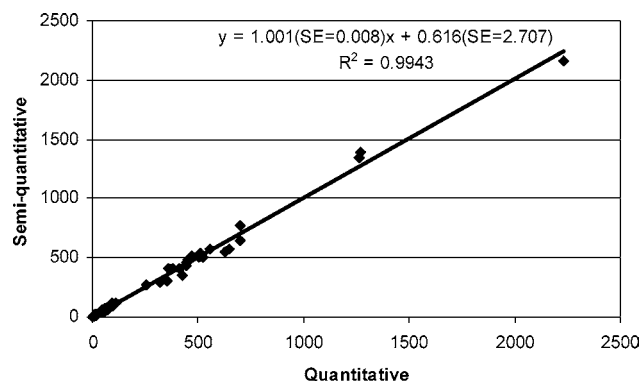


Figure 1. Comparison between quantitative and semiquantitative data obtained by ICP–MS for the analysis of 13 elements (Table 1) in 8 white wines.

Correction of Matrix Effects by Internal Standards. The effect of an ethanol matrix on calibration lines can be severe, and it is therefore essential to use matrix-matched standards for the analysis of diluted wine samples (25). In the current study, a 6% ethanol matrix for standards and blanks was used to approximate the 1:1 diluted wine matrix. To further correct for matrix effects, In was used as an internal standard.

Comparison of Digested and Diluted Samples. To establish whether the destruction of the organic matrix, in particular the ethanol in the wine samples, would bring any advantages, microwave-digested wine samples were measured using the semiquantitative mode of analysis and the results were compared with those obtained for the diluted samples. The results for diluted and digested samples compare adequately for most elements, but the LODs for most elements in the digested samples were between 2 and 10 times higher than those for the diluted samples. This could be attributed to the somewhat higher backgrounds in the blanks for the digested samples caused by impurities in the H_2O_2 and concentrated HNO_3 used during digestion and by possible contamination of the PTFE liners. It was concluded that the 1:1 dilution of the wine samples was to be preferred to the digestion procedure because of the following advantages: lower detection limits, less risk of contamination, less risk of the loss of volatile analyte elements, less time-consuming, and simple sample preparation. The 1:1 dilution approach was therefore used in the rest of the study.

Comparison of Semiquantitative and Quantitative ICP–MS Results. Many reports (27–30) can be found in the literature comparing the semiquantitative and quantitative mode in ICP–MS for multi-element analysis. The conclusions are generally that the results for most elements are comparable. In one study (27) pertaining to the analysis of diluted wine samples, semiquantitative ICP–MS results were compared to that obtained from neutron activation analysis (NAA). The average differences were generally <10%. Relative uncertainties were generally <30 and <15% for the heavier elements As, Rb, Sr, Mo, Cd, Sb, Cs, Ba, La, Ce, Tl, Pb, Th, and U. It can therefore be concluded that the semiquantitative mode of analysis with ICP–MS where only 5 elements (Be, Ge, Rh, In, and Bi) are used for calibration of the whole mass range is a reliable option for the estimation of the multi-element composition of wines.

To confirm that these conclusions were in fact valid for the analytical procedures and instrumentation used in the current study, results obtained using the semiquantitative procedure were compared with those obtained using the quantitative procedure for the elements listed in Table 1. Figure 1 shows the plot of the mean concentration values obtained by the quantitative mode on the *x* axis and the semiquantitative mode on the *y* axis. The trendline obtained by least-squares regression, $y = 1.001x + 0.615$ [standard error (SE) of the slope = 0.008, and SE of the intercept = 2.707], with a regression coefficient of 0.9943, shows a slope of 1.001, very close to the ideal line.

These results are similar to previously reported comparative tests (30) and support the use of multi-element concentration data obtained by the semiquantitative mode of ICP–MS analysis for wine provenance studies.

RESULTS AND DISCUSSION

Selection of Elements for Multivariate Analysis. The results for the analysis of the wine samples are summarized in Table 3 for the 40 elements (Li, B, Na, Mg, Al, Si, Cl, Ca, Sc, Ti, V, Cr, Mn, Fe, Ni, Cu, Zn, Ga, As, Se, Rb, Sr, Y, Zr, Nb, Mo, Ru, Cd, Sn, Sb, Te, Cs, Ba, La, Ce, Nd, W, Tl, Pb, and U).

The concentration ranges of most elements overlap within the three regions as can be inferred when comparing the variation in analyte concentration for each element in each region. Many binary and ternary scatterplots involving different combinations of elements, were examined. Typically, several combinations of elements could differentiate adequately between any two of the regions but not all three simultaneously. Scatterplots were therefore limited in their ability to display differences when the three groups were to be differentiated. Because the sample size (number of wines) was relatively small relative to the number of variables (element concentrations), a reduction in the number of variables was necessary to perform useful multivariate statistical analyses. An ANOVA test indicated that the group means of the following elements show significant differences at the 95% confidence level: Li, B, Mg, Al, Si, Cl, Sc, Mn, Ni, Ga, Se, Rb, Sr, Nb, Cs, Ba, La, W, Tl, and U. A few elements (Mg, Cl, Si, Nb, La, and U) were removed from this group in cases where the analytical uncertainty was large because of for example high polyatomic background interference or where concentration levels were close to the LOD. The remaining elements were used in the stepwise and pairwise discriminant analysis procedures.

Multivariate Statistical Analysis. The discriminant analysis was done by using the statistical package SPSS. Discriminant functions, that is, linear combinations of the independent variables (elemental concentrations), were derived that best differentiate among the dependent variables (the three regions Stellenbosch, Robertson, and Swartland). Element concentrations were \log_e -transformed for discriminant analysis to reduce the effect of outliers on skewing the data distribution and to bring the concentrations of elements with high and low abundance to within the same range. In this way, the robustness of the model is improved. The following canonical discriminant functions were derived by stepwise discriminant analysis:

$$f_1 = -0.416 \ln(\text{Al}) + 1.034 \ln(\text{Mn}) - 1.144 \ln(\text{Rb}) + 0.659 \ln(\text{Ba}) + 0.541 \ln(\text{W}) - 0.301 \ln(\text{Tl}) \quad (1)$$

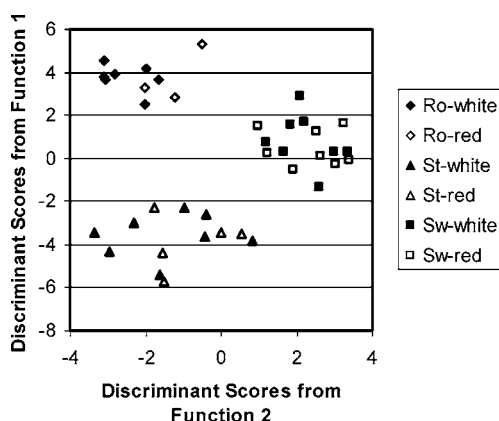
$$f_2 = -0.678 \ln(\text{Al}) + 0.326 \ln(\text{Mn}) - 0.597 \ln(\text{Rb}) + 0.509 \ln(\text{Ba}) - 0.012 \ln(\text{W}) + 0.996 \ln(\text{Tl}) \quad (2)$$

These functions enabled 100.0% of the wines to be correctly classified. A cross-validation procedure was applied to evaluate the robustness of the classification model. This means that each wine was in turn omitted from the estimation of model constants, and then its membership was determined from the resulting model. The cross-validation was again 100% correct. A graphical presentation of the two discriminant functions applied to the data set is given in Figure 2. It is clear from the plot in Figure 2 that the red and white wines are completely mixed in the three well-separated clusters, and it was therefore not deemed necessary to perform separate discriminant analyses for red and white wines (the sample sizes per wine type being too small for a meaningful discriminant analysis to be performed).

Pairwise Discriminant Analysis. Analysis of variance tests for the differences between the mean values of the three regions indicated that the regions were characterized by different element combinations. It seemed therefore logical to use a three-step

Table 3. Average Elemental Concentrations ($\mu\text{g/L}$) and Standard Deviations for 40 Elements in Red and White Wines from Stellenbosch, Robertson, and Swartland Wine Regions

element	LOD	Stellenbosch			Robertson			Swartland		
		white	red	all	white	red	all	white	red	all
Li	0.23	2.02 ± 0.98	1.04 ± 0.56	1.65 ± 0.97	16.5 ± 12	10.2 ± 7	13.9 ± 12	3.00 ± 1.9	4.10 ± 2.1	3.55 ± 2.1
B ^a	0.003	1.95 ± 0.36	3.49 ± 0.88	2.55 ± 0.95	2.70 ± 1.0	3.99 ± 0.88	3.38 ± 1.1	3.10 ± 0.90	3.90 ± 1.3	3.50 ± 1.2
Na ^a	0.002	14.6 ± 3.0	14.7 ± 4.0	14.6 ± 3.2	12.3 ± 6.8	16.6 ± 4.1	15.3 ± 5.7	15.2 ± 3.3	13.8 ± 7.2	14.0 ± 5.4
Mg ^a	0.009	126 ± 68	228 ± 68	163 ± 82	174 ± 91	300 ± 52	237 ± 87	205 ± 33	276 ± 70	241 ± 64
Al	1.1	453 ± 140	449 ± 131	451 ± 132	241 ± 200	215 ± 69	229 ± 144	129 ± 87	198 ± 123	163 ± 109
Si ^a	0.038	6.97 ± 1.7	9.89 ± 1.0	8.01 ± 2.0	9.98 ± 7.9	11.5 ± 2.1	11.3 ± 6.1	8.84 ± 1.1	12.0 ± 3.0	10.4 ± 2.7
Cl ^a	0.428	5.69 ± 2.3	17.0 ± 4.6	9.76 ± 6.4	15.8 ± 9.0	31.8 ± 8.0	22.7 ± 10.5	15.6 ± 7.5	19.4 ± 10.0	17.5 ± 8.9
Ca ^a	0.004	41.6 ± 4.0	61.3 ± 7.5	50.4 ± 10.4	45.9 ± 24	63.0 ± 12	49.7 ± 14	47.7 ± 9.7	57.1 ± 7.3	52.4 ± 9.6
Sc	0.77	8.20 ± 1.7	11.2 ± 0.9	9.28 ± 2.0	10.1 ± 6.5	13.5 ± 3.0	11.4 ± 2.9	11.4 ± 1.3	14.9 ± 4.1	13.1 ± 3.4
Ti	0.49	21.3 ± 11	19.3 ± 5.4	20.8 ± 8.7	26 ± 19	28 ± 9.4	25 ± 6.8	22.2 ± 4.4	23.1 ± 8.6	22.6 ± 6.6
V	0.06	1.60 ± 0.99	0.93 ± 0.32	1.36 ± 0.82	2.59 ± 2.4	0.61 ± 0.19	2.09 ± 2.2	1.06 ± 0.31	0.63 ± 0.32	0.85 ± 0.37
Cr	7.8	48.7 ± 22	17.4 ± 4.2	37 ± 24	48.3 ± 34	18.5 ± 19	42 ± 32	41.9 ± 8.9	30.6 ± 15	36 ± 14
Mn	0.14	664 ± 308	1493 ± 525	995 ± 555	1149 ± 720	1946 ± 990	1468 ± 759	1389 ± 466	2271 ± 646	1830 ± 710
Fe	2.0	904 ± 950	2274 ± 667	1393 ± 950	972 ± 500	1601 ± 1332	1021 ± 952	266 ± 183	1235 ± 740	751 ± 722
Ni	0.23	21.0 ± 11	17.9 ± 3.2	19.8 ± 8.8	23.1 ± 6.4	37.6 ± 9.9	29 ± 9.4	21.3 ± 11	40.8 ± 20	31 ± 19
Cu	0.27	204 ± 123	270 ± 121	229 ± 122	178 ± 146	246 ± 261	201 ± 185	600 ± 1221	509 ± 592	554 ± 929
Zn	2.7	1435 ± 581	899 ± 615	1229 ± 626	1210 ± 558	1403 ± 1327	1287 ± 807	2314 ± 1191	1563 ± 886	1938 ± 1086
Ga	0.07	2.83 ± 1.1	4.05 ± 1.5	3.26 ± 1.3	3.33 ± 1.4	6.97 ± 2.6	4.84 ± 2.4	4.95 ± 1.6	8.61 ± 2.8	6.78 ± 2.9
As	0.45	15.0 ± 9.7	5.74 ± 1.8	11.1 ± 8.6	15.3 ± 15	6.16 ± 3.0	11.8 ± 13	6.12 ± 1.9	7.06 ± 3.4	6.59 ± 2.7
Se	0.95	5.65 ± 3.5	6.58 ± 1.9	5.99 ± 2.9	3.07 ± 2.8	6.73 ± 4.6	4.68 ± 3.7	6.12 ± 3.2	11.7 ± 6.3	8.90 ± 5.6
Rb	0.04	2285 ± 934	4590 ± 1098	3182 ± 1508	449 ± 185	655 ± 185	532 ± 177	1360 ± 707	2664 ± 862	2012 ± 1017
Sr	0.08	400 ± 78	659 ± 227	510 ± 194	563 ± 239	1392 ± 303	905 ± 452	627 ± 143	1023 ± 322	825 ± 316
Y	0.02	0.48 ± 0.35	0.36 ± 0.43	0.44 ± 0.36	0.17 ± 0.18	0.14 ± 0.12	0.16 ± 0.15	0.25 ± 0.14	0.26 ± 0.20	0.26 ± 0.17
Zr	0.02	1.15 ± 1.1	1.11 ± 0.07	0.78 ± 1.0	1.44 ± 1.8	0.09 ± 0.04	0.94 ± 1.6	0.28 ± 0.5	0.10 ± 0.09	0.19 ± 0.36
Nb	0.01	0.06 ± 0.04	0.02 ± 0.01	0.05 ± 0.04	0.13 ± 0.29	0.01 ± 0.01	0.14 ± 0.25	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01
Mo	0.03	1.70 ± 0.68	0.90 ± 0.25	1.42 ± 0.65	1.96 ± 1.2	1.73 ± 1.2	1.89 ± 1.1	1.19 ± 0.45	1.49 ± 0.60	1.34 ± 0.54
Ru	0.01	0.06 ± 0.04	0.05 ± 0.02	0.06 ± 0.03	0.06 ± 0.07	0.14 ± 0.18	0.09 ± 0.12	0.07 ± 0.00	0.09 ± 0.1	0.08 ± 0.08
Cd	0.02	0.25 ± 0.18	0.06 ± 0.02	0.16 ± 0.16	0.22 ± 0.13	0.03 ± 0.04	0.15 ± 0.13	0.78 ± 1.3	0.13 ± 0.09	0.45 ± 0.96
Sn	0.05	1.82 ± 0.97	0.78 ± 0.18	1.45 ± 0.89	1.12 ± 0.58	0.92 ± 0.49	0.98 ± 0.47	0.95 ± 0.66	0.84 ± 0.44	0.90 ± 0.55
Sb	0.02	0.79 ± 0.44	0.37 ± 0.25	0.64 ± 0.41	2.17 ± 2.2	0.17 ± 0.07	1.27 ± 2.0	0.52 ± 0.24	0.44 ± 0.28	0.48 ± 0.25
Te	0.01	0.08 ± 0.07	0.07 ± 0.05	0.08 ± 0.06	0.09 ± 0.08	0.04 ± 0.07	0.06 ± 0.07	0.08 ± 0.1	0.08 ± 0.1	0.08 ± 0.09
Cs	0.02	10.4 ± 10	16.3 ± 6.0	13.4 ± 8.7	1.43 ± 0.73	1.21 ± 0.27	1.31 ± 0.62	2.59 ± 1.67	6.99 ± 5.2	4.79 ± 4.4
Ba	0.08	85.9 ± 35	105 ± 52	93 ± 41	101 ± 30	200 ± 55	143 ± 60	192 ± 87	338 ± 235	265 ± 188
La	0.01	0.24 ± 0.20	0.08 ± 0.07	0.19 ± 0.17	0.06 ± 0.17	0.02 ± 0.02	0.04 ± 0.10	0.05 ± 0.03	0.06 ± 0.06	0.05 ± 0.05
Ce	0.01	0.40 ± 0.34	0.15 ± 0.08	0.31 ± 0.29	0.16 ± 0.2	0.07 ± 0.06	0.13 ± 0.19	0.15 ± 0.06	0.14 ± 0.1	0.14 ± 0.08
Nd	0.01	0.24 ± 0.19	0.08 ± 0.07	0.18 ± 0.17	0.08 ± 0.10	0.03 ± 0.03	0.08 ± 0.13	0.11 ± 0.08	0.05 ± 0.08	0.08 ± 0.09
W	0.01	0.17 ± 0.16	0.05 ± 0.04	0.12 ± 0.13	0.97 ± 2.0	0.03 ± 0.02	0.78 ± 2.0	0.04 ± 0.02	0.04 ± 0.02	0.04 ± 0.02
Tl	0.01	0.65 ± 0.30	0.86 ± 0.29	0.75 ± 0.31	0.12 ± 0.10	0.14 ± 0.03	0.13 ± 0.02	0.69 ± 0.33	0.82 ± 0.24	0.76 ± 0.29
Pb	0.14	16.3 ± 7.8	8.39 ± 5.2	13.5 ± 7.6	17.0 ± 12	6.26 ± 4.9	12.5 ± 11	22.6 ± 21	9.68 ± 6.9	16.1 ± 16
U	0.01	0.40 ± 0.40	0.03 ± 0.0	0.27 ± 0.42	0.10 ± 0.36	0.02 ± 0.03	0.06 ± 0.11	0.01 ± 0.02	0.02 ± 0.02	0.01 ± 0.02

^a Concentrations in mg/L.**Figure 2.** Plot of discriminant analysis results for Robertson (Ro), Stellenbosch (St), and Swartland (Sw) wines using the discriminant functions defined in eqs 1 and 2.

discriminant analysis procedure, the pairwise discriminant analysis procedure proposed here, to classify a wine. In this procedure, each step classifies a wine as coming from a particular region or not, using a unique discriminant function for each region. The discriminant functions for the regions Robertson (f_{Ro}), Stellenbosch (f_{St}), and Swartland (f_{Sw}) were

$$f_{Ro} = 0.001 \ln(\text{Se}) + 0.067 \ln(\text{Rb}) - 0.013 \ln(\text{Cs}) + 0.969 \ln(\text{Tl}) \quad (3)$$

$$f_{St} = -0.777 \ln(\text{Al}) + 0.903 \ln(\text{Mn}) - 1.246 \ln(\text{Rb}) + 0.927 \ln(\text{Ba}) + 0.431 \ln(\text{W}) \quad (4)$$

$$f_{Sw} = +0.882 \ln(\text{Al}) - 0.623 \ln(\text{Mn}) + 1.096 \ln(\text{Rb}) + 0.494 \ln(\text{Sr}) - 0.881 \ln(\text{Ba}) - 1.776 \ln(\text{Tl}) \quad (5)$$

The values of the functions at the group centroids are summarized in **Table 4**.

The classification of a wine was done by calculating the Mahalanobis squared distances from the centroid and posterior probabilities for each sample and for each group. A wine is then classified into a region according to the largest posterior probability. Mahalanobis squared distances (d^2) were calculated for each sample using paired equations for each group. The paired equations for the Robertson group, for example, are

$$d_{Ro}^2 = (f_{Ro} + 3.555)^2 \quad (6)$$

$$d_{\text{not } Ro}^2 = (f_{Ro} - 1.226)^2 \quad (7)$$

where +3.555 and -1.226 are the values of the discriminant

Table 4. Values of the Discriminant Functions at the Group Centroids

region	centroid	region	centroid	region	centroid
Robertson	-3.555	Stellenbosch	-3.582	Swartland	-1.727
not Robertson	+1.226	not Stellenbosch	1.791	not Swartland	2.483

function at the centroid (see **Table 4**) of the Robertson and the not Robertson group, respectively. The probabilities associated with the two classes (Robertson and not Robertson) were computed from the pair of equations

$$P_{\text{Ro}} = \frac{\exp(0.5d_{\text{Ro}}^2)}{\exp(0.5d_{\text{Ro}}^2) + \exp(d_{\text{not Ro}}^2)} \quad (8)$$

$$P_{\text{not Ro}} = \frac{\exp(0.5d_{\text{not Ro}}^2)}{\exp(0.5d_{\text{Ro}}^2) + \exp(d_{\text{not Ro}}^2)} \quad (9)$$

Similar equation pairs were used for the other two groups. After this procedure, the classification of the wines into one of the groups was 100% successful. The models were cross-validated by leaving out one observation at a time and predicting the region of that observation. In the case of the Stellenbosch–not Stellenbosch and the Swartland–not Swartland models, the cross-validation was also 100% correct. In the case of the Robertson–not Robertson model, only 1 wine (out of 40) not from Robertson was classified as coming from Robertson. This cross-validated classification was therefore 97.5% correct.

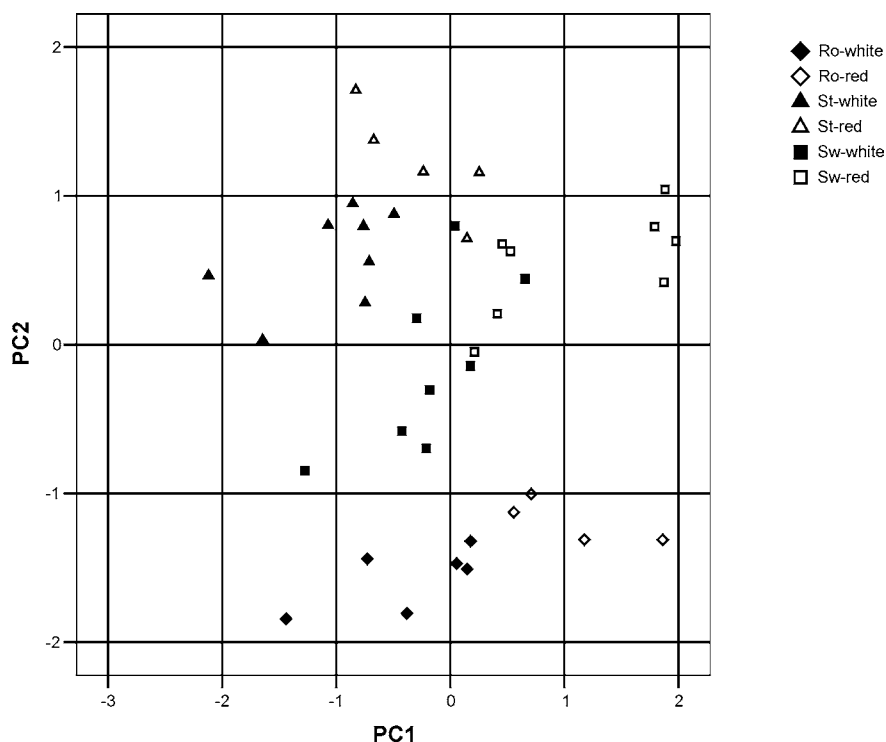
This procedure suggests that it would in principle be possible in the future to classify a wine known to be from one of these regions, as coming from Robertson, Stellenbosch, or Swartland. The success rate will depend on how representative the current database is and on factors such as the variability of the soils within a defined region.

Differences in Elemental Composition between Red and White Wines. Discriminant analysis of the data has shown that red and white wines included in the same data set can be

classified correctly according to the region of origin. However, it is known that differences may exist in the elemental composition of wines made from different grape cultivars, in particular between red and white wine cultivars. An indication of the nature of these differences can be found in **Table 3**, which summarizes the means of elemental concentrations for red and white wines in the regions. For the more abundant lighter elements, red wines seem to have higher elemental concentrations than white wines. This result is consistent with the known fact that the skins of the grapes are enriched in trace elements. In the case of red wines, the crushed grapes remain in contact with the skins for a longer period, allowing more minerals to leach from the skins.

Because of the seemingly large differences observed in concentrations of some elements in red and white wine from the same region, the question arises whether this could affect the reliability of the fingerprinting procedure when mixed white and red data are used. This would be the case if regional differences were smaller than cultivar differences. To validate the use of one model for red and white wine combined, the discriminant scores of the two discriminant functions (eq 1 and 2) were compared by means of a multivariate analysis of variance (MANOVA) test. The difference between the means of the three regions was highly significant (Wilkes' Lambda gave an F value of 98.5 with 4 and 64 degrees of freedom and a p value of <0.001) The difference between the means of the wine types (red and white wine) was not significant ($F = 1.115$ with 2 and 32 degrees of freedom and a p value of 0.340). There was also no significant interaction effect between the region and wine type ($F = 0.204$ with 4 and 64 degrees of freedom and a p value of 0.935).

Principal Component Analysis (PCA). The information for the elements Al, Sc, Mn, Ni, Ga, Se, Ru, Sr, Cs, Ba, W, and Tl were analyzed using PCA. The eigenvalues of the first three components were larger than 1 and together explained 74.3% of the variability in the data. The first principal component (PC1) explained 40.6%, and the second component (PC2) explained

**Figure 3.** Scatterplot of the PC1 and PC2 component scores of red and white wines showing differentiation according to region.

24.7%, while the third component only explained 9% of the variability. In **Figure 3**, a scatterplot of the PC1 and PC2 component scores of the wines shows that the first two components not only distinguish clearly between the three regions but also between the red and white wines within each region.

A discriminant analysis was subsequently performed using the three principal components. Only one wine was classified incorrectly. This model essentially gave the same overall result but did not improve on the models generated by the stepwise and pairwise methods.

CONCLUSION

The excellent results that were achieved in classifying wines from three wine-producing areas in South Africa according to their geographical origin gives further evidence of the ability of multivariate statistical analysis based on trace element data to show provenance. Results obtained by a pairwise discriminant analysis procedure, not previously applied to wine provenance studies, compared favorably with that from a conventional discriminant analysis procedure and essentially gave the same result. Adequately reliable multi-element analytical data were obtained using the semiquantitative mode of ICP-MS analysis and minimum sample preparation in the form of a 1:1 dilution of the wine samples with 1% HNO₃.

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LITERATURE CITED

- (1) Kaufmann, A. Multivariate statistics as a classification tool in the food laboratory. *J. AOAC Int.* **1997**, *3*, 665–675.
- (2) Martinez, I.; Aursand, M.; Erikson, U.; Singstad, T. E.; Veliyulin, E.; van der Zwaag, C. Destructive and non-destructive analytical techniques for authentication and composition analyses of foodstuffs. *Trends Food Sci. Technol.* **2003**, *14*, 489–498.
- (3) Thiel, G.; Geisler, G.; Blechschmidt, I. Determination of trace elements in wines and classification according to their provenance. *Anal. Bioanal. Chem.* **2004**, *378*, 1630–1636.
- (4) Taylor, V. F.; Longrich, H. P.; Greenough, J. D. Multi-element analysis of Canadian wines by ICPMS and multivariate statistics. *J. Agric. Food Chem.* **2003**, *51*, 856–860.
- (5) Almeida, C. M. R.; Vasconcelos, M. D. T. S. Multi-element composition of wines and their precursors including provenance soil and their potentialities as fingerprints of wine origin. *J. Agric. Food Chem.* **2003**, *51*, 4788–4798.
- (6) Baxter, M. J.; Crews, H. M.; Dennis, M. J.; Goodall, I.; Anderson, D. The determination of the authenticity of wine from its trace element composition. *Food Chem.* **1997**, *60*, 443–450.
- (7) Castiñeira Gómez, M. M.; Feldmann, I.; Jakubowski, N.; Andersson, J. T. Classification of German wines with certified brand of origin by multielement quantitation and pattern recognition techniques. *J. Agric. Food Chem.* **2004**, *52*, 2962–2974.
- (8) Rebolo, S.; Pena, R.; Latorre, M. J. Characterization of Galician (NW Spain) Ribeira Sacra wines using pattern recognition analysis. *Anal. Chim. Acta* **2000**, *417*, 211–220.
- (9) Jakubowski, N.; Brandt, R.; Stuewer, D.; Eschauer, H. R.; Gortges, S. Analysis of wines by ICP-MS: Is the pattern of the rare earth elements a reliable fingerprint for the provenance? *Fresenius' J. Anal. Chem.* **1999**, *364*, 424–428.
- (10) Arvanitoyannis, I. S.; Katsota, M. N.; Psarra, E. P.; Soufleros, E. H.; Kallithraka, S. Application of quality control methods for assessing wine authenticity: Use of multivariate analysis (chemometrics). *Trends Food Sci. Technol.* **1999**, *10*, 321–336.
- (11) Wieser, M. E.; Iyer, S. S.; Krouse, H. R.; Cantagallo, M. I. Variations in the boron isotope composition of *Coffea arabica* beans. *Appl. Geochem.* **2001**, *16*, 317–322.
- (12) Moreda-Piñeiro, A.; Fisher, A.; Hill, S. J. The classification of tea according to region of origin using pattern recognition techniques and trace metal data. *J. Food Compos. Anal.* **2003**, *16*, 195–211.
- (13) Day, M. P.; Zhang, B.-L.; Martin, G. J. The use of trace element data to complement stable isotope methods in the characterization of grape musts. *Am. J. Enol. Vitic.* **1994**, *5*, 79–85.
- (14) Ogrinc, N.; Košir, I. J.; Spangenberg, J. E.; Kidric, J. The application of NMR and MS methods for detection of adulteration of wine, fruit juices, and olive oil. *Anal. Bioanal. Chem.* **2003**, *376*, 424–430.
- (15) Horn, P.; Hölzl, S.; Todt, W.; Matthes, D. Isotope abundance ratios of Sr in wine provenance determinations, in a tree-root activity study, and of Pb in a pollution study on tree-rings. *Isot. Environ. Health Stud.* **1998**, *34*, 31–42.
- (16) Rosman, K. J. R.; Chisholm, W.; Jimi, S.; Candelone, J.-P.; Boutron, C. F.; Teissedre, P.-L.; Adams, F. C. Lead concentrations and isotopic signatures in vintages of French wine between 1950 and 1991. *Environ. Res.* **1998**, *78*, 161–167.
- (17) Almeida, C. M. R.; Vasconcelos, M. D. T. S. Does the winemaking process influence the wine ⁸⁷Sr/⁸⁶Sr? A case study. *Food Chem.* **2004**, *85*, 7–12.
- (18) Day, M. P.; Zhang, B. L.; Martin, G. J. Determination of the geographical origin of wine using joint analysis of elemental and isotopic composition: Differentiation of the principal production zones in France for the 1990 vintage. *J. Sci. Food Agric.* **1995**, *67*, 113–123.
- (19) Martin, G. J.; Mazure, M.; Jouitteau, C. Characterization of the geographic origin of Bordeaux wines by a combined use of isotopic and trace element measurements. *Am. J. Enol. Vitic.* **1999**, *50*, 409–417.
- (20) Gremaud, G.; Quail, S.; Piantini, U. Characterization of Swiss vineyards using isotopic data in combination with trace elements and classical parameters. *Eur. Food Res. Technol.* **2004**, *219*, 97–104.
- (21) Castiñeira Gómez, M. M.; Jakubowski, N.; Andersson, J. T. Changes in the metal composition in German wines through the winemaking process. A study of 63 elements by ICP-MS. *J. Agric. Food Chem.* **2004**, *52*, 2953–2961.
- (22) Nicolini, G.; Larcher, R.; Pangrazzi, P. Changes in the contents of micro- and trace-elements in wine due to winemaking treatments. *VITIS* **2004**, *43*, 41–45.
- (23) Eschauer, H.; Jakob, L.; Meierer, H.; Neeb, N. Use and limitations of ICP-OES in wine analysis. *Microchim. Acta* **1989**, *3*, 291–298.
- (24) Smith, F.; Arsenault, E. Microwave-assisted sample preparation in analytical chemistry. *Talanta* **1996**, *43*, 1207–1268.
- (25) Almeida, C. M. R.; Vasconcelos, M. D. T. S. UV-irradiation and MW-digestion pre-treatment of Port wine suitable for the determination of lead isotope ratios by inductively coupled plasma mass spectrometry. *J. Anal. At. Spectrom.* **1999**, *14*, 1815–1821.
- (26) Alimoni, A.; Petrucci, B.; Cristaudo, A.; Caroli, S. Determination of chromium and nickel in human blood by means of inductively coupled plasma mass spectrometry. *Anal. Chim. Acta* **1995**, *306*, 35.
- (27) Perez-Jordan, M. Y.; Soldevila, J.; Salvador, A.; Pastor, A.; de la Gardia, M. Inductively coupled plasma mass spectrometry analysis of wines. *J. Anal. At. Spectrom.* **1998**, *13*, 33–39.
- (28) Castiñeira, M. M.; Brandt, R.; von Bohlen, A.; Jakubowski, N. Development of a procedure for the multi-element determination of trace elements in wine by ICP-MS. *Fresenius' J. Anal. Chem.* **2001**, *370*, 553–558.
- (29) Almeida, C. M. R.; Vasconcelos, M. D. T. S. Advantages and limitations of the semi-quantitative operation mode in an inductively coupled plasma-mass spectrometer for multi-element analysis of wines. *Anal. Chim. Acta* **2002**, *463*, 164–175.

- (30) Laborda, F.; Medrano, J.; Castillo, J. R. Quality of quantitative and semi-quantitative results in ICP–MS. *J. Anal. At. Spectrom.* **2001**, *16*, 732–738.

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