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Elemental composition as a tool for the assessment of type, seasonal variability, and geographical origin of wine and its contribution to daily elemental intake†

S. Đurđić,[a] M. Pantelić,[b] J. Trifković,[a] V. Vukojević,[a] M. Natić,[a] Ž. Tešić[a] and J. Mutić*[ac]

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The elemental profiles of 63 red and white wine samples from four different regions in Serbia were investigated. Twenty-one elements were analysed (Ca, Mg, Na, K, Fe, Mn, Cu, Zn, Co, Se, Cr, V, Ni, Cd, As, Al, Sb, Pb, Ba, Rb, and Be) by inductively coupled plasma quadrupole mass spectrometry (ICP-Q-MS) and inductively coupled plasma with optical emission spectrometry (ICP-OES). A pattern recognition method was applied in order to classify and differentiate type, seasonal variability, and geographical origin of the wine. Dietary mineral intake for elements was calculated in order to assess their contribution to daily intake. The most important descriptors for discrimination among red and white wine samples were Be, Al, Rb, Mg, K, Cu, Mn, and Na, in descending order. The variables Cd, Pb, As, Sb, V, Na, K, and Zn have the highest influence on vintage-to-vintage classification of red wines. Furthermore, the model revealed the existence of three groups of descriptors for different regions of production. All obtained statistical models confirmed that data from the elemental content of wine samples could be used for accurate prediction of wine type, seasonal variability, and regional origin.

Introduction

Determination of the elemental composition of wines is useful for many reasons. Firstly, the concentration of elements in wine is useful information to viticulturists and oenologists for controlling the process of obtaining high-quality wine.[1] Secondly, the elemental composition could be used as a wine fingerprint and represents one of the criteria for evaluating the authenticity of wine.[2] Currently, determination of food authenticity is an important issue in quality control. However, the elemental composition of wines is influenced by numerous factors such as soil in the vineyard, wine processing equipment, and vinification.[3] Based on these factors, the elements in wine can be classified into two groups: endogenous and exogenous.[4] The most abundant are endogenous elements (Ca, Mg, Zn, Fe, P, Na, and K) that are related to the grape variety and maturity, type of soil in the vineyard, and climatic conditions. The exogenous elemental content (Al, Cd, Cr, Cu, Fe, and Zn) depends on external impurities during the growth of grapes and vinicultural and winemaking practices.[1,5,6] Anthropogenic factors, such as application of pesticides, fungicides, and fertilisers during the growing season, can lead to an increase in Cd, Cu, Mn, As, and Zn in the resulting wine. The presence of Pb in wines can originate from sources like traffic, fertilisers, vessels, and pumps used during vinification.[7] Finally, recent data indicate that some beverages, including wine, contribute to the total dietary intake of certain trace elements (B, Ba, Co, Mn, Ni, Rb, Sb, Tl, and V)[8] and iron as well.[9] Thus, determination of the elemental concentrations in wine allows calculation of their daily dietary intake from wine, which is of particular importance for toxic elements. Various instrumental techniques have been used to classify wine variety such as infrared spectroscopy (IR),[10] nuclear magnetic resonance (NMR),[11] gas chromatography (GC),[12] high-performance liquid chromatography (HPLC),[13,14] inductively coupled plasma optical emission spectrometry (ICP OES),[5,15] and inductively coupled plasma mass spectrometry (ICP MS).[4,16] The advantages of ICP-based techniques for the determination of trace elements are numerous: high sensitivity, a wide range of linearity, and simultaneous multielement analysis. Furthermore, a simple sample dilution has been proposed as the most advantageous because it is time-efficient, prevents contamination, and reduces the number of operation steps. According to Grindlay *et al.*,[2] matrix effects were almost eliminated when 1 : 5 and 1 : 10 (v/v) dilution factors were employed for ICP OES and ICP MS analyses, respectively. In recent years, chemometric pattern-recognition techniques have also been applied, contributing to characterizing and classifying wine samples.[15]

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[a] *University of Belgrade, Faculty of Chemistry, P.O. Box 51, 11158 Belgrade, Serbia*

[b] *Innovation Centre of Faculty of Chemistry Ltd, Studentski trg 12-16, 11000, Belgrade, Serbia*

[c] *Ghent University Global Campus, Incheon, South Korea. E-mail: jelena.mutic@ghent.ac.kr*

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However, wine quality control is still highly dependent on wine tasters.[17] A human sensory panel can provide information about the sensory properties of wine that is far richer than can be provided by instrumental devices alone.

Serbia has a very long tradition of wine production due to its favourable climate and good geographical conditions, but export capacity is very small due to the lack of competitiveness in price as a consequence of the high cost of production in relatively small wineries. The elevated prices are justified by the wine quality and by the relationship between the quality and the region of origin. The evaluation of the quality and authenticity of the wine is a key factor in establishing cost-effective wine, which would help numerous small Serbian wineries to enter this highly competitive market. In that sense, the aim of this work was, firstly, to obtain basic information about the elemental composition of genuine Serbian wines. The second objective was to apply chemometric techniques, such as principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA), in order to establish parameters that could be used for classification and differentiation of two types of wine (white and red), to determine which elements could be affected by seasonal variability between wines from one winery, and to try to establish the regional origin of red wine samples. A significant number of samples supplied by different wineries were collected from three different regions of Serbia. To the best of our knowledge, this is the first report on the influence of vintage on the elemental content of red wine samples analysed with chemometrics. The third objective was to evaluate the content of essential and toxic elements in wine samples in order to study their nutritional significance and to estimate the daily mineral intake provided by the consumption of this beverage.

Experimental

Site description and wine samples

A total of 63 wine samples were analysed (46 red wines and 17 white wines). Samples originated from four different regions in Serbia: Belgrade ($n = 39$), Central Serbia ($n = 9$), Vojvodina ($n = 6$), and South Serbia ($n = 9$). The regions, vineyards, wineries, and vintages are presented in Table S1 (ESI†). The geographic distribution of wine samples is shown on the map in Fig. 1. Wines from the Belgrade region originated from "Radmilovac", an experimental station which belongs to the Faculty of Agriculture, University of Belgrade. The location belongs to the Šumadija–Velika Morava wine region (Central Serbia – 44°45′24.66″N–20°34′54.50″E, elevation 153 m above sea level). All wines were provided by the wineries as finished wines in 750 mL glass bottles with cork stoppers and were stored at 3–4 °C before analysis. One bottle was used for each sample, and three replicates were taken.

Fig. 1 Map of the geographic distribution of the wine samples.

Reagents and chemicals

ICP multi-element stock solutions (VHG standards, Manchester, UK) containing 1000 mg L^{-1} were used for calibration. Distilled, deionised water (Milli-Q Water System, Millipore Corporation, Bedford, MA) was used throughout. The isotopes ^{6}Li, ^{45}Sc, ^{115}In, and ^{159}Tb (VHG standards, Manchester, UK) were used as internal standards to cover the full mass range. All other chemicals were supplied by Merck (Darmstadt, Germany).

Sample preparation

For determination of the elemental content in wine, samples were diluted (1 : 10) with water containing 2% (v/v) nitric acid (Merck, Germany). Standards were prepared with 1% (v/v) ethanol and 2% (v/v) nitric acid in order to provide the same concentrations of ethanol and nitric acid as the samples.[18] The certified reference material (CRM 1640a, trace elements in natural water) was prepared in the same manner as the standards. The samples were taken from their packages and filtered in a G3 Gooch filter to guarantee the absence of solid particles in order to avoid clogging the nebuliser.[19]

Instrumentation

ICP-OES (iCAP 6500 Duo Thermo Scientific, UK) was used for determination of calcium, sodium, potassium, magnesium, rubidium, iron, and arsenic. For the determination of arsenic, the system was equipped with an integrated unit for hydride generation.[20] Table S2 (ESI†) shows the analytical lines used for each element and the instrumental conditions. The limit of detection (LOD) for each element was calculated[21] and is shown in Table S2.† An inductively coupled plasma quadrupole mass spectrometer (ICP-QMS, Thermo Scientific Xseries 2, UK) was used for measurements of trace elements in wine. Fourteen elements (Ba, Be, Cd, Co, Cr, Ni, Sb, Pb, Se, V, Al, Mn, Zn, and Cu) were measured in no gas and/or collision cell mode.[22] High-purity He and H_2 (99.9999% H_2, Messer, Serbia) were used as a collision gas (7% hydrogen in helium). The use of a cooled spray chamber was required in order to minimize the effect of the ethanol matrix. Measured isotopes and instrument operating conditions are given in Table S2 (ESI†).

Due to the lack of a reference material for wine, the accuracy of the measurements of elements was verified by CRM 1640a (trace elements in natural water) which contained all analysed elements. The results of the analysis showed good agreement with the certified levels of the standard (±10%).

Statistical analysis

Descriptive statistics and the Mann–Whitney *U*-test were performed by a demo version of the NCSS statistical software.[23] The Mann–Whitney *U*-test was carried out at a significance level of $P = 0.05$. PCA and PLS-DA were carried out by PLS_Tool Box, v.6.2.1, for MATLAB 7.12.0 (R2011a).

All data were auto-scaled prior to any multivariate analysis to bring values to compatible units. Metal content served as the input data matrix. The PCA was performed using a singular value decomposition algorithm with a 0.95 confidence level for Q and T2 Hotelling limits for outliers. The analysis was based on a correlation matrix, and factors with eigenvalues greater than 1 were retained. The PLS-DA used the SIMPLS algorithm without forcing orthogonal conditions on the model in order to condense Y-block variance into the first latent variables. The models were validated using a venetian blinds validation procedure. The quality of the models was monitored with two different sets of parameters. The values of R_{cal}^2, the cumulative sum of squares of the Ys explained by all extracted components, R_{CV}^2, the cumulative fraction of the total variation of the Ys that can be predicted by all extracted components, and R_{PRED}^2, the cumulative fraction of the total variation of the Ys that can be predicted by test components should be as high as possible. In contrast, RMSEC (Root Mean Square Errors of Calibration), RMSECV (Root Mean Square Errors of Cross-Validation), and RMSEP (Root Mean Square Errors of Prediction) should be as low as possible, with minimal differences between them.[24]

Calculation of daily elements intake

A volume of 100 mL of wine and the 70 kg body weight of an average consumer (European Food Safety Authority, EFSA)[25] were used to calculate elemental intakes per day. Daily mineral intake (DMI, %) and recommended daily allowance (RDA) values were calculated according to the European Economic Community (EEC).[26] DMI was calculated using $\mathrm{DMI} = C \times 100/\mathrm{RDA}$; where C is the elemental content (mg) in 100 mL of wine. According to the regulation EEC 90/496, the contribution is important if 100 mL contains at least 15% of the RDA. Daily Intake (DI, %) of As, Cd and Pb was calculated for 100 mL of red and white wine using $\mathrm{DI} = C \times 100/\mathrm{MDI}$ where MDI corresponds to the maximum tolerable daily intake established by the EFSA.

Results & discussion

Major and trace elements in wines

Twenty one elements, including major (Ca, Mg, Na, K, Rb, Fe, and Mn) and trace (Al, Cu, Zn, Be, V, Co, Ni, Cd, Sb, Ba, Cr, As, Se, and Pb) elements, were analysed in the set of 63 Serbian wine samples. The summarized parameters (mean, median, standard deviation, minimum and maximum value) obtained for the elemental content of wine samples of different types (red and white), seasonal variability, and geographical origin are presented in Tables 1–3. The concentration of major and trace metals in wines from different viticulture countries are presented in Table S3 (ESI†). As expected, potassium was the most abundant element in all investigated red and white wine samples (Tables 1–3) since K is an essential element for the growth and development of plants and is often a component of fertiliser.[21] According to our results, the K concentration was higher in red compared to white wines. The measured values ranged between 413 and 1170 mg L^{-1} for red wines and from 298 to 560 mg L^{-1} for white wines (Table 1). Similar K content was found in all investigated regions (Table 3). These results agree with values reported in the literature.[15,27] Magnesium and calcium were the second most abundant elements found in our study (Tables 1–3). The magnesium concentration was higher in red wines (mean 94.9 mg L^{-1}, values between 53.0 to 124.8 mg L^{-1}) than in white wines (mean 84.6 mg L^{-1}, values between 38.0 to 89.4 mg L^{-1}), while calcium concentrations were similar in both types of wine (mean values of 83.1 mg L^{-1} and 84.6 mg L^{-1} for red and white wines, respectively) (Table 1). Paneque *et al.*[28] observed that the Ca concentration depended on wine maturity. The young wines had slightly lower Ca content (mean 64.1 ± 16.1 mg L^{-1}) than aged wines (mean 88.4 ± 17.7 mg L^{-1}). The values obtained for the Mg and Ca contents in our selected wines were in good agreement with the results for Macedonian,[5] Serbian,[29] Croatian,[13] and Czech[30] wines. On the other hand, our Ca and Mg contents were significantly higher than published data for wines from Argentina[31] and Belgium[32] (Table S3, ESI†). In contrast with Mg, the average content of Na was significantly higher in white wines than in red wines (Table 1). In the studied wines, Na content differed among the regions and decreased in the order Vojvodina > Central Serbia > South Serbia. The highest concentration of Na (mean 23.0 ± 11.7 mg L^{-1}) (Table 3) was found in wines from Vojvodina region compared with the other three wine production areas. These results agree with Ražić and Onia[29] where the obtained concentration of sodium in wine from North Serbia was 33 ± 11 mg L^{-1}. The sodium content in our study agreed with results published on Serbian[29] and Czech[30] wines, but was lower compared to Spanish[27] red wines. The higher content of sodium in Spanish wines has been attributed to the vicinity of the vineyards to the ocean.[1] The rubidium concentration was significantly higher in red wines (mean values 2.57 mg L^{-1}) compared to white wines (1.23 mg L^{-1}) (Table 1). Similar data was obtained by Geana *et al.*[33] for Romanian white wines (ranged from 0.22 mg L^{-1} to 2.066 mg L^{-1}) and by Kment for Czech wine[30] (ranged from 0.09 to 2.47 mg L^{-1}), but rubidium content found in Belgian[32] wines

Table 1 Descriptive statistics obtained from the analysis of metal content in two wine types

Wine type		K (mg L^{-1})	Mg (mg L^{-1})	Ca (mg L^{-1})	Na (mg L^{-1})	Fe (mg L^{-1})	Rb (mg L^{-1})	Mn (μg L^{-1})	Al (μg L^{-1})	Zn (μg L^{-1})	Cu (μg L^{-1})	V (μg L^{-1})	Ba (μg L^{-1})
Red wine ($n = 46$)	Mean	690	94.9	83.1	8.48	1.32	2.58	1194	225	635	131	1.54	90.3
	Stdev	173	15.7	21.3	7.45	0.93	0.72	681	186	263	135	2.58	66.4
	Median	656	97.3	80.4	5.70	1.23	2.44	994	175	598	81	0.34	73.5
	Min	413	53.0	41.2	2.94	0.01	1.25	385	6	287	24	0.02	20.6
	Max	1167	124.8	131.2	37.0	6.01	4.70	3310	800	1499	646	14.62	301.2
White wine ($n = 17$)	Mean	396	66.6	84.6	17.6	1.54	1.24	550	1145	977	558	3.07	79.9
	Stdev	71	14.5	16.2	12.0	0.68	0.28	178	469	693	515	1.75	33.7
	Median	400	68.1	83.8	13.7	1.37	1.20	562	1004	837	501	3.40	70.5
	Min	298	38.0	55.3	4.5	0.36	0.79	294	514	215	33	0.83	35.4
	Max	560	89.4	115.7	34.3	2.80	1.71	885	2189	2585	1684	7.22	157.4
Man–Whitney *U*-test[a]	*P* value	<0.0001	<0.0001	0.5562	0.0013	0.1371	<0.0001	<0.0001	<0.0001	0.0829	0.0008	0.0001	0.8892
	H_0	Reject	Reject	Accept	Reject	Accept	Reject	Reject	Reject	Accept	Reject	Reject	Accept

Wine type		Pb (μg L^{-1})	Ni (μg L^{-1})	Cr (μg L^{-1})	Sb (μg L^{-1})	As (μg L^{-1})	Co (μg L^{-1})	Be (μg L^{-1})	Cd (μg L^{-1})	Se (μg L^{-1})
Red wine ($n = 46$)	Mean	47.8	37.5	5.49	8.78	16.1	3.89	0.129	1.99	3.40
	Stdev	40.4	28.4	4.90	4.76	10.9	2.28	0.196	2.53	3.02
	Median	26.3	27.8	4.73	8.50	18.1	3.59	0.023	0.82	2.31
	Min	9.3	8.5	0.10	0.66	1.2	0.40	0.001	0.07	0.06
	Max	168.5	123.6	20.67	18.65	37.9	9.16	1.028	11.1	10.52
White wine ($n = 17$)	Mean	83.8	88.0	1.85	8.95	21.2	3.96	4.34	1.31	4.98
	Stdev	52.5	122.9	1.72	5.92	9.46	2.60	1.55	1.47	2.07
	Median	68.8	41.4	1.34	10.54	23.0	3.65	4.00	0.71	5.39
	Min	16.0	8.58	0.10	0.06	2.00	0.50	0.39	0.14	1.19
	Max	188.2	420.5	6.59	17.77	35.7	9.60	7.01	4.75	8.00
Man–Whitney *U*-test	*P* value	0.0051	0.0492	0.0024	0.7217	0.0945	0.9938	<0.0001	0.9629	0.0132
	H_0	Reject	Reject	Reject	Accept	Accept	Accept	Reject	Accept	Reject

[a] Differences between two set of data is significant when *P* value is less or equal to $P = 0.05$.

was lower (Table S3†). Al, Cu, Mn, and Zn were also present in amounts similar to previously published results.[13,27,30,33,34] These elements in our wines were significantly higher than in Belgian[32] and Argentinian[31] wines. The average values obtained for V were 3.07 μg L^{-1} and 1.54 μg L^{-1} in white and red wines, respectively (Table 1). These results indicated that Serbian wines are moderately rich in vanadium, have less vanadium than Macedonian,[5] Spanish[27] or Czech[30] wines, and have more vanadium than Belgian[32] ones. Higher concentrations of V in wine could be associated with storage.[35] The content of trace elements (Be, Co, Sb, and Se) found in Serbian wines agreed with literature data (Table S3†).[5,16,30,33]

Regarding the content of toxic elements (As, Cd, and Pb) (Tables 1–3), the As content was higher than in published data (Table S3†)[16,27,30,31,36] while the Pb content was lower than that in Czech[30] and Romanian[33] wines. The average content of Cd was slightly higher than in wines from Serbia's neighbouring countries Croatia[16] and Macedonia.[5] Only one sample contained elevated concentrations of this element. Analysing data from Table S3,† Serbian wines were concluded to contain higher amounts of essential elements (Cu, Mn, and Zn) and major elements (Ca and Mg) in comparison to Belgian[32] or Argentinian[31] wines. Additionally, our wines contained less Ni, Pb, and V than Turkish,[36] Czech,[30] and Romanian[33] wines, respectively. Although Serbian wines contained higher amounts of arsenic compared to other wines, its concentrations were under the maximum acceptable limit. Due to significant deviations from the normal distribution for each of the studied variables, statistical evaluation of the differences in the elemental content in the two wine types was evaluated by the Mann–Whitney *U*-test, while the differences in the elemental content according to the vintage and regional origin were determined by the Kruskal–Wallis test. Tests were employed for each variable taking the appropriate variety as a single factor. The results are presented in Tables 1–3. Differences between two or three sets of data were considered significant when the *P* value was less than or equal to $P = 0.05$. In the cases where the Kruskal–Wallis test has indicated a statistically significant difference between the medians, the Kruskal–Wallis multiple-comparison *Z*-value test was also performed. Vintages and regions with different contents of a given metal are denoted in parentheses with a letter or number (Tables 2 and 3).

The Mann–Whitney *U*-test revealed that the variables K, Mg, Na, Rb, Be, Cr, Ni, Pb, Se, V, Al, Mn, and Cu governed the differences between white and red wines (Table 1) and suggest a totally different elemental profile for the two wine types. The Kruskal–Wallis test revealed that the macro elements K, Mg, Ca, and Na and trace elements Al, Cu, Ba, Ni, and Sb had statistically significant differences between vintages (Table 2). Additionally, the statistically significant differences between the

Table 2 Descriptive statistics obtained from the analysis of metal content in wine samples from different vintages

Vintage		K (mg L^{-1})	Mg (mg L^{-1})	Ca (mg L^{-1})	Na (mg L^{-1})	Fe (mg L^{-1})	Rb (mg L^{-1})	Mn (μg L^{-1})	Al (μg L^{-1})	Zn (μg L^{-1})	Cu (μg L^{-1})	V (μg L^{-1})	Ba (μg L^{-1})
2012 (A)	Mean	539	110.9	89.7	5.87	1.77	2.38	776	250	1013	230.2	0.82	66.4
	Stdev	155	9.7	14.7	0.92	1.77	0.72	225	127	271	208.5	1.20	15.5
	Median	493	110.3	84.2	6.09	1.13	2.25	784	187	920	131.3	0.20	61.5
	Min	413	97.0	73.3	4.66	0.70	1.58	496	124	738	80.9	0.03	48.1
	Max	903	124.8	112.9	7.30	6.01	3.69	1169	488	1499	645.8	3.32	88.6
2013 (B)	Mean	635	94.5	78.0	4.41	1.42	2.53	966	112	420	63.1	0.88	43.5
	Stdev	188	13.6	14.0	1.22	0.37	0.72	240	112	134	21.5	0.87	17.9
	Median	613	95.4	75.7	4.01	1.36	2.58	995	66	357	71.1	0.34	38.1
	Min	430	59.3	55.0	2.94	0.86	1.25	614	6	292	24.4	0.09	20.6
	Max	1167	109.9	100.7	7.08	2.26	4.22	1384	356	742	89.2	2.33	75.4
2014 (C)	Mean	897	99.1	120.1	3.98	1.23	3.37	1036	221	671	153.0	0.59	73.2
	Stdev	73	4.9	7.9	0.57	0.24	1.03	125	160	97	116.8	0.80	7.9
	Median	915	99.3	118.3	3.80	1.23	3.12	1015	165	690	137.4	0.23	76.1
	Min	797	93.2	112.7	3.55	0.99	2.51	907	99	549	49.1	0.10	61.9
	Max	961	104.5	131.2	4.78	1.49	4.70	1207	455	757	287.9	1.79	78.8
Kruskal–Wallis test	P value	0.0120	0.0181	0.0047	0.0133	0.6873	0.1717	0.1144	0.0918	0.0002	0.0041	0.6565	0.0127
	Z-Test	C(A, B)	A(B)	C(A, B)	B(A, C)	—	—	—	—	A(B)	A(B)	—	A(B, C)

Vintage		Pb (μg L^{-1})	Ni (μg L^{-1})	Cr (μg L^{-1})	Sb (μg L^{-1})	As (μg L^{-1})	Co (μg L^{-1})	Be (μg L^{-1})	Cd (μg L^{-1})	Se (μg L^{-1})
2012 (A)	Mean	44.1	34.5	3.63	13.56	19.5	3.65	0.049	1.07	2.88
	Stdev	39.2	28.7	1.76	2.57	4.8	1.98	0.052	1.43	1.70
	Median	26.0	21.9	3.59	12.46	19.0	3.28	0.023	0.55	3.57
	Min	15.8	16.9	0.22	10.42	12.6	0.50	0.011	0.07	0.42
	Max	128.6	102.1	5.89	17.51	26.7	7.35	0.138	4.44	4.76
2013 (B)	Mean	49.4	18.6	2.33	6.18	23.2	3.12	0.042	2.84	3.40
	Stdev	34.7	8.9	1.92	3.13	4.9	2.35	0.040	2.39	2.61
	Median	44.2	15.8	2.12	6.56	22.1	2.49	0.023	2.82	3.57
	Min	9.3	8.8	0.10	0.66	15.2	0.50	0.023	0.07	0.42
	Max	100.3	41.1	5.10	11.13	30.7	6.81	0.131	6.43	6.67
2014 (C)	Mean	55.7	26.2	4.27	6.89	20.2	4.03	0.103	3.33	6.03
	Stdev	75.4	3.4	2.21	5.86	3.4	2.90	0.096	5.19	2.46
	Median	20.8	25.5	4.26	4.95	21.5	3.65	0.087	0.87	6.67
	Min	12.6	23.3	2.15	2.20	15.2	1.01	0.023	0.49	2.82
	Max	168.5	30.7	6.42	15.45	22.7	7.80	0.214	11.10	7.96
Kruskal–Wallis test	P value	0.8704	0.0249	0.1533	0.0022	0.2887	0.7373	0.4723	0.3384	0.1319
	Z-Test	—	A(B, C)	—	B(A, C)	—	—	—	—	—

metal content of samples from different geographical regions could only be observed for the elements Mg, Na, Fe, and Cu with the differentiation in samples arising from the Vojvodina region (Table 3). Pearson correlation coefficients between all elemental contents in the wine samples were determined separately for white and red wines due to the observed differences in relationships between elements (Tables S4 and S5, ESI†). Significant r values at the 99% confidence level[37] are represented in bold. A significant correlation between two or more elements indicates a similar ability to penetrate the grape or the existence of the same source.[16] Correlation matrices (Tables S4 and S5†) showed that the different elements were mutually correlated for the two wine types indicating different sources of elements in red and white wines. Due to the high number of samples and the fact that t value for the correlation is directly proportional to the number of samples, a significant r value was very low in both cases. For that reason, herein we will discuss only dependencies with $r > 0.700$. For white wine samples, correlations were obtained between the following elements: Pb and Zn ($r = 0.9177$), Pb and Cu ($r = 0.8824$), Zn and Cu ($r = 0.9247$), and Mn with Ca ($r = 0.7401$) (Table S4†). The correlations between Pb and these two essential metals could be explained by their similarity as micronutrients or by anthropogenic influences. Furthermore, the existence of a correlation between Mn and Ca could be attributed to the type of soil, which is rich in manganese, iron oxide minerals, and hydrated silicate of Na, Ca, Al, and Mg. In red wine samples, significant correlations were observed between Ba and Mn ($r = 0.7519$), Ba and Ni ($r = 0.7054$), Cd and Pb ($r = 0.7527$), and Mn and Na ($r = 0.7001$) (Table S5†). Mn, Ba, and Ni together with alkali and alkaline earth metals belong to the lithophile elements which form the main components of the corresponding soil. Therefore, their origin in wine was likely mostly from the soil. On the contrary, Cd and Pb are toxic elements with similar chemical properties, and they predominately come from anthropogenic sources.

Table 3 Parameters of descriptive statistics obtained from metal content analysis of wine samples from different regions

Regional origin		K ($\mathrm{mg\ L^{-1}}$)	Mg ($\mathrm{mg\ L^{-1}}$)	Ca ($\mathrm{mg\ L^{-1}}$)	Na ($\mathrm{mg\ L^{-1}}$)	Fe ($\mathrm{mg\ L^{-1}}$)	Rb ($\mathrm{mg\ L^{-1}}$)	Mn ($\mathrm{\mu g\ L^{-1}}$)	Al ($\mathrm{\mu g\ L^{-1}}$)	Zn ($\mathrm{\mu g\ L^{-1}}$)	Cu ($\mathrm{\mu g\ L^{-1}}$)	V ($\mathrm{\mu g\ L^{-1}}$)	Ba ($\mathrm{\mu g\ L^{-1}}$)
Central Serbia (1)	Mean	762	88.2	74.5	11.00	0.64	2.63	1632	325	617	176.4	2.73	175.2
	Stdev	104	14.9	12.4	5.82	0.54	0.64	1056	247	178	184.4	4.56	76.5
	Median	783	92.9	74.6	8.98	0.50	2.32	1172	305	601	110.7	1.72	138.9
	Min	573	53.1	53.8	3.07	0.01	1.99	385	9	287	44.7	0.20	94.3
	Max	877	102.6	93.9	21.26	1.62	3.69	3310	800	948	619.2	14.62	301.2
Vojvodina (2)	Mean	678	101.9	78.0	23.02	2.01	2.13	1884	312	586	142.1	2.80	89.4
	Stdev	74	16.3	7.3	11.68	0.52	0.32	718	201	207	38.4	2.62	47.4
	Median	677	107.6	78.9	23.44	1.83	2.19	1921	321	511	150.0	3.20	79.1
	Min	607	75.6	68.7	5.65	1.42	1.77	774	9	375	92.6	0.20	27.9
	Max	798	118.7	86.0	37.05	2.81	2.51	2609	574	834	182.3	6.28	155.1
South Serbia (3)	Mean	735	78.0	71.4	7.33	0.91	2.72	1141	148	567	68.3	0.67	99.6
	Stdev	179	11.4	29.3	5.81	0.45	0.74	645	138	132	15.0	0.70	81.3
	Median	621	76.5	67.2	4.82	0.69	2.35	895	122	550	72.5	0.20	62.8
	Min	550	66.9	41.2	4.02	0.52	2.02	594	10	403	40.1	0.19	30.9
	Max	998	100.1	127.4	20.30	1.87	4.20	2456	437	766	80.2	1.90	260.0
Kruskal–Wallis test	*P* value	0.4768	0.0484	0.4668	0.0293	0.0058	0.1533	0.2232	0.2862	0.8237	0.0224	0.2558	0.0817
	Z-Test	—	2(3)	—	2(3)	2(1)	—	—	—	—	2(3)	—	—

Regional origin		Pb ($\mathrm{\mu g\ L^{-1}}$)	Ni ($\mathrm{\mu g\ L^{-1}}$)	Cr ($\mathrm{\mu g\ L^{-1}}$)	Sb ($\mathrm{\mu g\ L^{-1}}$)	As ($\mathrm{\mu g\ L^{-1}}$)	Co ($\mathrm{\mu g\ L^{-1}}$)	Be ($\mathrm{\mu g\ L^{-1}}$)	Cd ($\mathrm{\mu g\ L^{-1}}$)	Se ($\mathrm{\mu g\ L^{-1}}$)
Central Serbia (1)	Mean	61.9	67.7	10.44	9.78	5.1	4.75	0.292	2.38	2.02
	Stdev	50.6	39.2	6.25	3.27	8.7	2.05	0.313	2.80	3.29
	Median	61.5	52.1	10.19	10.59	2.3	4.39	0.262	0.35	0.42
	Min	11.5	8.5	0.10	4.02	1.2	2.44	0.023	0.15	0.06
	Max	154.7	123.6	20.67	14.48	28.3	9.16	1.028	7.31	10.52
Vojvodina (2)	Mean	53.6	30.1	6.74	6.51	13.1	4.61	0.120	1.87	2.64
	Stdev	31.1	17.7	4.23	7.23	10.8	2.61	0.156	2.37	2.36
	Median	43.9	18.4	6.15	2.89	9.5	5.00	0.023	1.13	1.62
	Min	27.9	15.8	1.06	0.69	5.0	0.40	0.023	0.13	0.93
	Max	107.0	52.7	12.88	18.65	32.1	7.59	0.382	5.85	6.67
South Serbia (3)	Mean	21.8	49.7	6.11	10.14	12.3	4.24	0.135	0.54	4.38
	Stdev	11.9	12.9	6.15	3.97	16.4	2.29	0.227	0.96	4.50
	Median	16.8	49.0	7.70	9.38	3.5	4.53	0.023	0.13	1.64
	Min	14.5	33.1	0.10	4.72	1.4	0.59	0.001	0.07	0.47
	Max	47.4	70.5	15.15	16.91	37.9	7.84	0.609	2.68	10.52
Kruskal–Wallis test	*P* value	0.0916	0.1984	0.5340	0.3049	0.0802	0.8596	0.3375	0.1035	0.2027
	Z-Test	—	—	—	—	—	—	—	—	—

Discrimination of wines by type, seasonal variability, and regional origin

The chemometrics technique PCA was applied in order to classify and differentiate wine type (red *vs.* white), seasonal variability, and wine from diverse regional sites. The PCA was carried out at the exploratory level to provide insight into the structure of data and reveal some logical pattern in the data.[24] In addition, PLS-DA was used as a multivariate classification model method aimed to find mathematical models that can assign each sample to an appropriate class of wine type.

Red *vs.* white wines

A PCA model applied to classify samples according to wine type resulted in a seven-component model that explained 76.09% of the total variance. Obtaining low overall data variance captured by a few PCs is not unusual, especially when the variability among the samples is relatively high (naturally occurring objects, samples), and a diverse set of parameters (variables) is considered. The parameters of the PCA model, eigenvalues, and percentage variance captured by each principal component (PC) are presented in Table S6 (ESI†). Mutual projections of the scores and their loadings for the first two PCs are presented in Fig. 2. The score plot (Fig. 2a) of the model revealed the existence of two distinctive groups of characteristics belonging to different wine types (white and red) separated alongside PC1. The loading plot (Fig. 2b) implies that the variables Al, Be, and Cu have the most positive impact on the direction of PC1 and denote a group of white wine samples. Additional chemical indicators of this group were Zn, Pb, Na, V, Ni, As, and Se that had a lower influence on the classification. The variables K and Rb had the highest negative influence on PC1, and Mg, Mn and Cr had lower negative influence on PC1. Together, these variables characterize the red wine type. The results of differentiation of wine type agreed with previously published articles dealing with the elemental composition of red and white

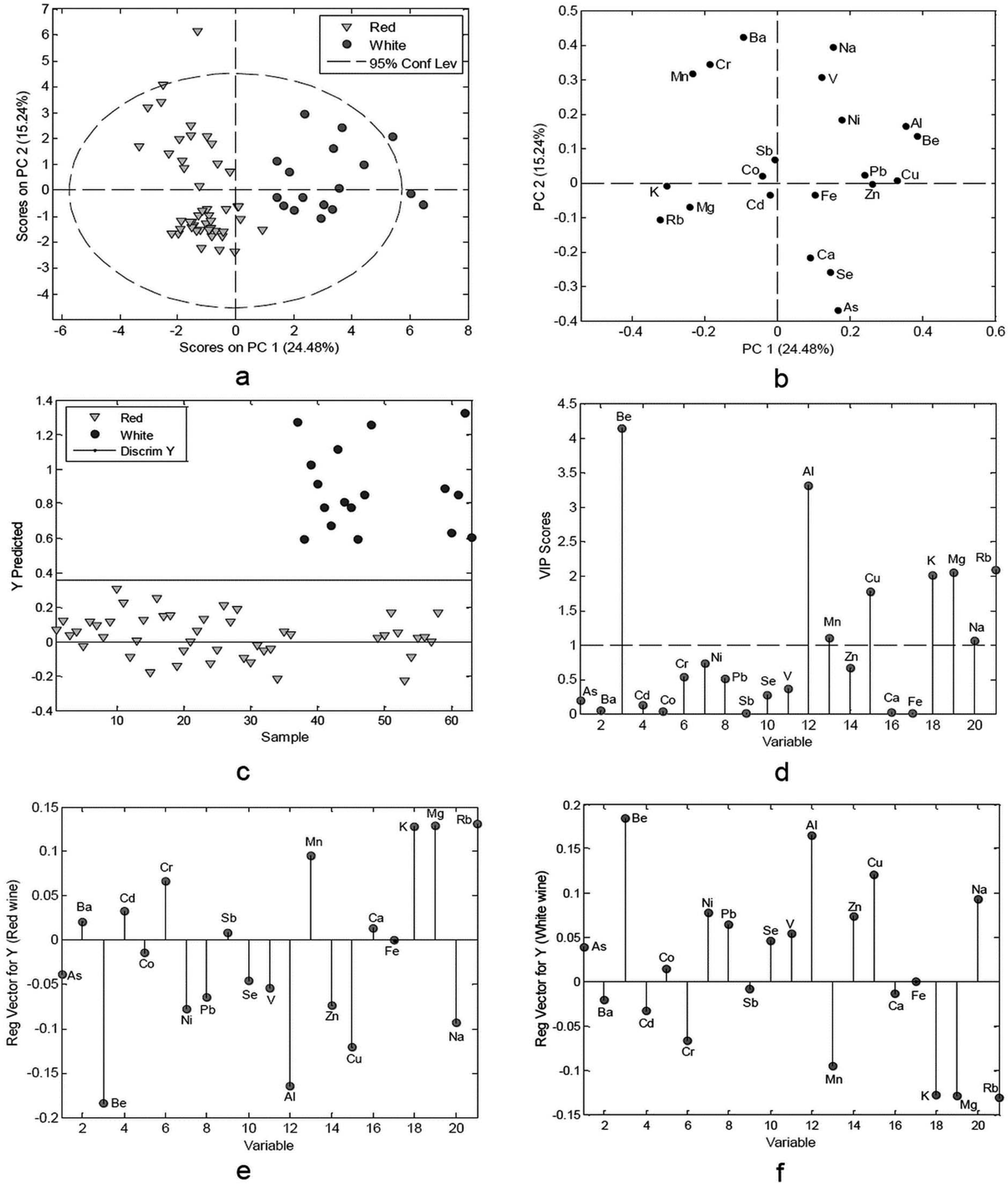

Fig. 2 Classification models for wine. (a) PCA scores plot for red and white wine samples, (b) PCA loadings plot for red and white wine samples, (c) PLS-DA score plot, (d) VIP scores for the PLS-DA model, (e) regression coefficients of the PLS-DA model for red wine samples, and (f) regression coefficients of the PLS-DA model for white wine samples.

wines.[29,38] Such classification could be attributed to differences in the winemaking process but could also be ascribed to grape variety.[1,21] In addition, the largest influence on the differentiation of wine type was the lithophile elements (K, Mg, Rb, Mn, Cr, Ba, Al, and Be), which indicates their origin from vineyard soils, similar to the results obtained by other authors.[3,16,21,39] On the other hand, a different elemental composition in wine may be the result of differences in the process of vinification, *i.e.* the difference between red and white wines occurs due to prolonged contact of skin and seeds of grapes with the must (juice) during the production of red wines (maceration).[39,40] An increase in the Fe and Cr contents in red wines could also be the result of long maceration (7–21 days), which causes a more efficient extraction of chemical elements from the grapes.[1,21,41]

Also, an increased As concentration may occur due to the use of groundwater for irrigation of agricultural areas, which are contaminated with arsenic.[42] Some authors emphasized that groundwater with increased concentration of As was detected in Vojvodina, which is one of our investigated regions.[16] The high impact of Cu on differentiation could be the result of its accumulation in soil after the application of Bordeaux mixture or other copper-based fungicides or the use of equipment based on this metal.[5,15,29] Also, the increase of Cu concentration may occur due to adding copper salts to remove H_2S.[1] The endogenous origin of zinc in wines is based on easy absorption of this element by plants due to its high mobility in soil.[43] In contrast, the exogenous origin of zinc in wines is the consequence of long contact of wine with stainless steel wine-making machinery and pipes which were used for handling and storing the wine.[31]

Samples of different wine types were also modelled simultaneously using PLS-DA. The number of the latent variables (LVs) was selected based on the minimum value of RMSECV, which was achieved with one LV. Classification and validation results were expressed as R_{cal}^2, R_{CV}^2, R_{pred}^2, RMSEC, RMSECV, and RMSEP values. Two models were statistically significant, with relatively high values of R_{cal}^2, R_{CV}^2, and R_{pred}^2 (0.8484, 0.8094 and 0.8382, respectively), as well as low differences between RMSEC, RMSECV, and RMSEP values (0.169, 0.189, and 0.193, respectively). Hence, data from the elemental content of wine samples allows accurate prediction of wine type and provides a useful tool for their determination (Fig. 2c). The contribution of variables that had the strongest influence on differentiation between types was analysed using the variable importance in projection (VIP) value (Fig. 2d). The variables with a VIP score higher than one were considered the most relevant for classification of the wine samples by type. The most important descriptors for discrimination among red and white wine samples were Be, Al, Rb, Mg, K, Cu, Mn, and Na, in descending order. The standardized regression coefficients that indicate in which direction (*i.e.* positively or negatively) the element is contributing to the model are shown on Fig. 2e and f. The regression coefficients indicated identical but opposite influence of the variables in the two models. The highest positive impacts on classification of red wines arose from the elements Rb, K, Mg, Mn, and Cr. The model for white wines indicated high positive regression coefficients for Be, Al, Cu, Na, Ni, Pb, Zn, V, and Se. These differences could be attributed to differences in winemaking techniques between red and white grapes such as long maceration, length of time in contact with the grape skins, and the filtration process typically undergone by white wines.[1,38–40,44]

Vintage-to-vintage differences in red wines

The types of grapes that can be grown and overall wine quality that certain regions produce are a result of the baseline climate, while climate variability determines vintage-to-vintage quality differences.[45] Many individual weather and climate factors can affect grape growth and wine quality, such as solar radiation, heat accumulation, temperature extremes, precipitation, wind, and extreme weather events such as hail. However, the growing season length and temperatures are critical aspects due to their major influence on the ability to ripen grapes to optimum levels of sugar, acid, and flavour in order to maximize a quality of wine.[45]

Annual reports for 2012, 2013, and 2014 about average air temperature, total rainfall, and total sunshine hours in Serbia were obtained from the Republic Hydrometeorological Service of Serbia (RHSS).[46] According to these data, the three chosen vintages differed by rainfall and number of sunny days. The year 2012 had a high number of sunny days, an average temperature of 14.0 °C, and low rainfall (563 mm); in 2013, the rainfall was within normal limits (680.0 mm) and the average temperature was 11.6 °C. The average rainfall in the Serbia in 2014 was 1017.9 mm, which was the rainiest year in 60 years. The effect of vintage on the average content of some selected elements is presented in Fig. 3. Significant differences were found between the average content of Zn and Fe in the wine samples in 2012 where they were higher in comparison with other two years. The same trend was observed for the trace elements Sb and Ni. The content of Cr, Co, Cd, and Se observed in samples from 2014 was higher than in those from the other two other vintages. Similar results were also found for the content of Pb and Ba (Fig. 3). The variation of soil pH is known to be highly dependent on precipitation amount,[14] and the (bio)availability of some elements from soil is influenced by water (in this case from rain). Therefore, a high amount of precipitation changed soil conditions and led to leaching of some elements dependent on their bound in soil substrate to make them available for plants. The changes in ionic strength of water and pH affect leaching of elements which are usually in ion-exchangeable forms in soil such as: Cu, Pb, Ni, As, Co, Cd, and Mg.[47]

The influence of seasonal variability on the elemental content of red wine samples obtained from the same vineyard and with the same production procedure in the winery Radmilovac (Smederevo, Belgrade region) was analysed by PCA. To our knowledge, this is the first report of vintage-to-vintage influence on the elemental profile of wine. The PCA model resulted in a seven-component model that explained 78.99% of the total variance. The parameters of the PCA model, eigenvalues, and percentage variance captured by each PC are presented in Table S6 (ESI†), and mutual projections of factor scores and their loadings are presented in Fig. 4. Score plots (Fig. 4a and b, seasonal classification) of the model revealed the

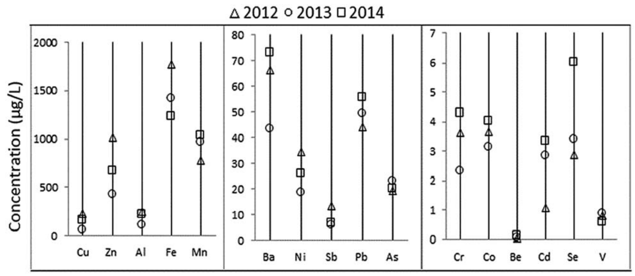

Fig. 3 Results from different vintages represented as an average of selected measured variables of the wines.

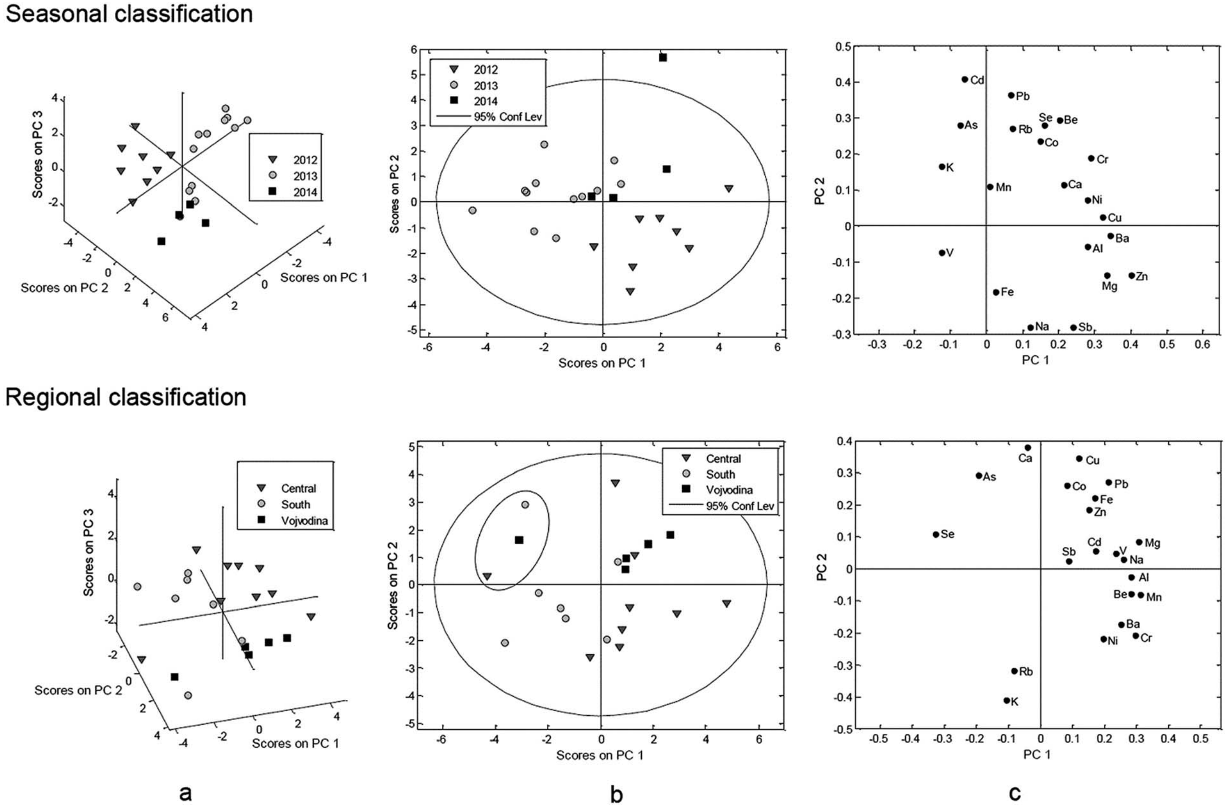

Fig. 4 PCA for seasonal and regional classifications: (a) 3D score plots, (b) 2D score plots, and (c) loading plots.

existence of three distinctive groups of characteristics belonging to different vintages with some overlap of samples from 2013 and 2014. The loading plot (Fig. 4c) implies that variables Cd, Pb, As, Sb, V, Na, K, and Zn have the highest influence on the classification. Interestingly, Cd, Pb, As, and Sb are most probably derived from anthropogenic sources rather than from natural sources. Pb and Cd come predominately from atmospheric pollution or from fungicide treatment. Among these elements, Na, K and Zn are elements that derived from both natural and artificial sources with their concentration determined by many different factors. The results of the PCA confirm that seasonal variability does have a significant influence on the elemental profile of red wines.

Regional origin of red wines

Previously presented results revealed that the elemental composition of wine is closely related to the vineyard soil of its grapes.[39,48] Namely, the same wine type might have quite different chemical composition, as accumulation of phytochemicals depends on climatic conditions (sunlight, moisture), soil characteristics, and the presence of different minerals in the soil.[33,43]

We tried to determine regional origin of red wine samples collected from three statistical regions of Serbia (Vojvodina, Central region, and South region) and from different wineries within each region, by using the elemental content and chemometrics. The PCA model resulted in a seven-component model that explained 82.92% of the total variance. Parameters of the PCA model, eigenvalues, and percentage variance captured by each PC are presented in Table S6.† The score plots (Fig. 4a and b, regional classification) of the model revealed the existence of three groups of characteristics associated with different regions of production. Using this classification, we could assume that the regional origin of red wine from Serbia could be determined. However, we should emphasize that the three samples in the left upper part of the score plot (circled, Fig. 4b) represented samples whose grapes originated from the observed regions but were produced in Radmilovac, a winery that did not belong to the regions included in this analysis. Since each of the observed regions includes several different wineries whose production procedure does not have influence on classification, we assumed that the winery Radmilovac differed from all other wineries in Serbia. A similar conclusion was found by Atanacković *et al.*[49] These authors studied the influence of winemaking techniques on the resveratrol content, total phenolic content, and antioxidant potential of red wines made at the oenological station Radmilovac from the grape varietals Merlot (M), Cabernet Sauvignon (KS), Pinot noir (PN) and Prokupac (P). Their results implied that the use of thermovinification procedures could enhance the phenolic composition. Obviously, besides the composition of soil, vintage, and grape varietal, the winery also contributes to the fingerprint of wine. The existence of three groups of characteristics associated with different regions of production could be explained by different types of soil in Serbian regions.

Table 4 The daily mineral intake of essential and toxic elements for red and white wines

Essential element	Average content in 100 mL wine (mg)		RDA (mg per day)	DMI (%)	
	Red	White		Red	White
K	67.5	39.6	2000	3.38	1.98
Mg	9.74	6.66	375	2.60	1.78
Ca	8.18	8.46	800	1.02	1.06
Na	0.88	1.76	1500	0.06	0.12
Fe	0.131	0.15	14	0.94	1.10
Cu	0.0014	0.068	1	0.14	6.75
Zn	0.0065	0.098	10	0.07	0.98
Se	0.0003	0.0005	0.055	0.49	0.71
Mn	0.014	0.087	2	0.71	4.37
Cr	0.0010	0.0008	0.04	2.60	1.96

Toxic element	Average content in 100 mL wine (μg)		MDI (μg per day)	DI (%)	
	Red	White		Red	White
Pb	5.15	8.97	250	2.06	3.59
As	1.40	2.12	150	0.96	1.41
Cd	0.19	0.13	25	0.78	0.52

Samples from the Central region form a cluster in the right lower part of score plot and showed higher content of the elements Al, Mn, Be, Ba, Cr, and Ni (Fig. 4b and c). In central Serbia, high concentrations of Cr and Ni in the soil on serpentine rocks was recorded.[50] Compared to other soil types, the Rankers on serpentine stands out for having significantly higher concentrations of Ni and Cr, which is characteristic for soil on this substrate. Wines from Vojvodina, grouped in the right upper part of score plot, were characterized by higher content of Ca, Na, and Mg (Fig. 4b and c). The dominant soil type in Vojvodina is chernozem that is rich in humus and minerals such as hydrated silicate of Ca, Na, and Mg. Samples from the South region were positioned in the left lower part of the score plot and showed higher content of K and Rb (Fig. 4b and c). Limestone type soil in Southeast Serbia is rich in alkaline metals such as Rb and K. Rubidium content in soils is largely inherited from the parent rocks, as indicated by the highest mean Rb content in soils over granites, limestones, and calcareous rocks.[51] Rubidium is very closely linked with K as their properties are similar, and it may partially substitute for K sites in plants.[51]

Estimation of dietary elemental intake

Calculated RDA and DMI values of essential elements and DI and MDI values of toxic elements for wine samples are given in Table 4.

Essential elements

Recommended daily allowance for K is 2000 mg according to the EEC.[26] The Scientific Committee on Food (SCF) established the acceptable range for Na from 1400 to 8800 mg per day for adults. A tolerable upper intake level (UL) for K and Na was not established.[52] In the analysed wines, the calculated DMI for K was 3.38% and 1.98% of the RDA in red and white wines, respectively (Table 4). The DMI for Na was 0.06% and 0.12% in red and white wines, respectively. Based on the results of Na, a low percentage is suitable because increased concentration of Na in the body causes hypertension and diabetes.[53] For Mg, the RDA for men aged 19 to 30 years is 400 mg per day (310 mg per day for women) and 420 mg per day for over 30 years old (320 mg per day for women).[52] An UL for Mg could not be established due to lack of available data.[52] However, the RDA according to EEC is 375 mg per day. In our wines, the calculated DMI for Mg was higher in red wines (2.60%) than in white wines (1.78%). The UL for Ca is proposed to be 2500 mg per day. In contrast to Mg, similar values of DMI obtained for Ca in both type of wines indicated that the consumption of red and white wines would lead to intake of almost identical amounts of Ca. Iron has important metabolic functions such as transfer of oxygen.[52] Based on our results, its contribution to dietary intake was 0.94% and 1.10% for red and white wines, respectively (Table 4). Based on different intakes of Cu, the EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA) set Adequate Intakes (AI) values for men and women. For men, the AI for Cu is 1.6 mg per day, and for women it is 1.3 mg per day.[54] The RDA for Cu according to the EEC is 1 mg per day.[26] Calculated DMI for Cu was 0.14% and 6.75% in red and white wines, respectively (Table 4), which indicated that the white wine significantly contributes to the daily mineral intake for this element. Copper in the human body preferably has a role as a catalyst but is also an integral part of the enzymes involved in the electron transport chain. This metabolic process plays an important role in the synthesis of ATP which is the main source of energy in cells.[52] The European PRI for Zn for adult males and females is 9.5 mg per day and 7.0 mg per day, respectively.[55] This value applies to pregnant and lactating women.[52] The RDA for Zn according to the EEC is 10 mg per day.[26] The DMI for Zn was 0.07% and 0.98% in red and white wines, respectively (Table 4). Compared with Cu, Mg and Zn, the non-metal Se is needed in much smaller doses; the current adequate dose of Se for humans is 1 μg per kg body weight (b.w.) per day, which corresponds 70 μg per day for an average consumer,[56] with UL of 300 μg per day.[52] At least eleven selenoproteins containing the amino acid selenocysteine have been identified in mammals. In addition, Se has been related to immune function, viral infection, and reproduction. In the analysed wines, the DMI for Se was 0.49% in red wines and was 1.71% in white wines (Table 4). Adequate Intake for Mn is 3 mg per day, while UL for Mn is not established.[57] The RDA for Mn according to the EEC is 2 mg per day. From our research, it seems that one glass of white wine is a good source of Mn as it provides 4.37% of the recommended daily allowance. The RDA for Cr according to EEC is 40 μg per day.[26] One glass of red wine contributes to 2.60% of the Cr-RDA, and one glass of white wine contributes to 1.96% of the RDA.

A 100 mL glass of analysed wine will provide significant amounts of essential elements including Cu (white wine), Mn (white wine), K (red wine) and seem to be good source of these metals. These results highlight that no element could be labelled as significant in a single dose, since none achieves 15% of the RDA, as recommended for labelling purposes.[26] Importantly, the presence of other bioactive compounds in the beverages can influence the bioavailability of the minerals.

Toxic elements

Concentrations of As, Cd, and Pb in analysed wine samples were under Maximum Acceptable Limits (MAL) at 0.2 mg L^{-1}, 0.01 mg L^{-1}, and 0.15 mg L^{-1}, respectively, as published by the Organization of Vine and Wine.[58] Provisional tolerable weekly intake (PTWI) for inorganic As is 15 μg per kg b.w., which corresponds to an intake of 2.1 μg per kg b.w per day. This daily intake is equivalent to an intake of 150 μg per day for the average consumer.[59]

For Cd, the EFSA's Panel on Contaminants in the Food Chain (CONTAM Panel) set the Tolerable Weekly Intake (TWI) of 2.5 μg per kg b.w.[60] in order to ensure a high level of protection for all consumers, which corresponds 25 μg per day for an adult person of 70 kg. The provisional tolerable weekly intake for Pb of 25 μg per kg b.w. is no longer appropriate.[61] The respective benchmark dose lower confidence limit (BMDL) derived from blood lead levels in μg L^{-1} (corresponding to dietary intake values in μg per kg b.w. per day) were the following: developmental neurotoxicity $BMDL_{01}$ 12 μg L^{-1} (0.50 μg per kg b.w. per day), effects on systolic blood pressure $BMDL_{01}$ 36 μg L^{-1} (1.50 μg per kg b.w. per day), and effects on kidney in adults $BMDL_{10}$ 15 μg L^{-1} (0.63 μg per kg b.w. per day).[55] Lead has the greatest concentration in wine of the toxic elements (3.59% and 2.06% of DI for white and red wines, respectively). In the studied wines, the contribution to the daily intake decreased in the order Pb > As > Cd. Estimated daily intake of toxic elements from consumption of 100 mL wine hence poses no toxicological risk.

Conclusions

Characterisation of Serbian wines according to their elemental composition was performed, and the obtained results were similar to literature data. Potassium, magnesium, and calcium were the most abundant elements in all investigated red and white wine samples.

PCA and PLS-DA were applied in order to establish parameters for classification and differentiation of two types of wine (white and red). Data from the elemental content of wine samples allows accurate prediction of wine type and provides a useful tool for their determination. The most important descriptors for discrimination among red and white wine samples were Be, Al, Rb, Mg, K, Cu, Mn, and Na, in descending order. In addition, PCA was applied to determine which elements affected seasonal variability between wines from one winery. The obtained model revealed the existence of three distinctive groups of characteristics associated with three vintages with the highest influence from the variables Cd, Pb, As, Sb, V, Na, K and Zn. The entire elemental profile of wine is influenced by rain and number of sunny days during the vintage. A pattern recognition technique was also applied for the determination of regional origin of red wine samples. A significant number of samples were supplied by different wineries from three different regions of Serbia. The PCA model revealed the existence of three groups of characteristics associated with different regions of production which could be explained by different soil types in the selected Serbian regions.

A 100 mL glass of the analysed wines will provide significant amount of Cu (white wine, 6.7% of RDA), Mn (white wine, 4.4% of RDA), and K (red wine, 3.4% of RDA). These results highlight that no element could be labelled as significant in a single dose and that the estimated daily intake of toxic elements hence posed no toxicological risk.

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