

MULTIELEMENT ANALYSIS OF MACEDONIAN WINES BY INDUCTIVELY COUPLED PLASMA–MASS SPECTROMETRY (ICP-MS) AND INDUCTIVELY COUPLED PLASMA–OPTICAL EMISSION SPECTROMETRY (ICP-OES) FOR THEIR CLASSIFICATION

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Major, minor, and trace elements in wines from the Republic of Macedonia were determined in this study. Both inductively coupled plasma–mass spectrometry (ICP-MS) and inductively coupled plasma–optical emission spectrometry (ICP-OES) were used for accurate determination of the concentration of 42 elements (Ag, Al, Au, B, Ba, Be, Bi, Ca, Cd, Ce, Co, Cu, Dy, Er, Eu, Fe, Ga, Gd, Ge, Ho, La, Lu, Mg, Mn, Mo, Na, Nd, Ni, P, Pb, Pr, S, Sm, Tb, Ti, Tl, Tm, U, V, Yb, Zn, Zr) in 25 Macedonian white, rose, and red wines from different wine regions. By means of factor and cluster analyses, the wines were discriminated according to wine type (white vs. red) and geographical origin. The main discriminant elements were B, Ba, Ca, Fe, Mg, Mn, P, and S.

Keywords: elemental composition; red and white wines; multivariate analysis; ICP-MS; ICP-OES

**МУЛТИЕЛЕМЕНТАРНА АНАЛИЗА НА ВИНА ОД МАКЕДОНИЈА СО ПРИМЕНА
НА ИНДУКТИВНО СПРЕГНАТА ПЛАЗМА-МАСЕНА СПЕКТРОМЕТРИЈА (ICP-MS) И
ИНДУКТИВНО СПРЕГНАТА ПЛАЗМА-ОПТИЧКА ЕМИСИОНА СПЕКТРОМЕТРИЈА
(ICP-OES) ЗА НИВНА КЛАСИФИКАЦИЈА**

Во ова истражување е извршено определување на елементите во траги присутни во вина од Република Македонија. За таа цел беа применети две техники, индуктивно спрегната плазма-масена спектрометрија (ICP-MS) и индуктивно спрегната плазма-оптичка емисиона спектрометрија (ICP-OES). Определувана е концентрацијата на 42 елементи (Ag, Al, Au, B, Ba, Be, Bi, Ca, Cd, Ce, Co, Cu, Dy, Er, Eu, Fe, Ga, Gd, Ge, Ho, La, Lu, Mg, Mn, Mo, Na, Nd, Ni, P, Pb, Pr, S, Sm, Tb, Ti, Tl, Tm, U, V, Yb, Zn, Zr) во 25 македонски бели, розе и црвени вина од различни вински региони. Со примена на факторна и кластер-анализа е извршено разликување на вината според видот (бели наспроти црвени) и според географското потекло. Главните елементи според кои е извршено разликување на вината се B, Ba, Ca, Fe, Mg, Mn, P и S.

Клучни зборови: елементарен состав; црвени и бели вина; мултивариетна анализа; ICP-MS; ICP-OES

1. INTRODUCTION

Wine is a very complex matrix, consisting mainly of water (80 %), ethanol (12 %), and other compounds (8 %). The following compounds are present in small quantities and are considered important for wine quality: polyphenols (anthocyanins, flavan-3-ols, flavonols, phenolic acids, stilbenes), carbohydrates, proteins, organic acids, amino acids, vitamins, as well as minerals (sodium, calcium, iron, magnesium, phosphorous, potassium, zinc, copper, manganese, selenium, etc). The knowledge of the mineral composition and content in wine is an important factor influencing its quality and nutritional value. In fact, the determination of the elemental composition of wines is very important not only from the toxicological point of view – since it could contain harmful elements, such as Pb, As, and Cd – but also from the nutritional point of view, since wine contains essential elements for the human organism, such as Ca, Cr, Co, K, Se, and Zn [1].

The presence of metals (e.g. Al, Zn, Cu, Fe, Pb) in wine is important for efficient alcoholic fermentation and for its sensorial characteristics (flavor, aroma, freshness), and therefore, their concentrations in wine must be monitored. The element composition of wines may be influenced by many factors – such as elemental levels in the soil, fertilization practices, as well as processing conditions. The multielement composition of the soil is influenced, not only by the solubility of inorganic compounds present in the soil [2], but also by other factors, such as environmental contamination of the vineyards, agricultural practices, climate changes, vine variety, etc. Pesticides are commonly used in viticulture for vine plant protection from diseases, and its application to the vines could influence the level of some elements in wines, such as Cd, Cu, Pb, and Zn [3]. During vinification, element content changes as a result of wine making processes and conditions, including the addition of yeasts, maceration, content of proteins, using fining agents (i.e., bentonite). The concentration of some elements – such as Al, Cd, Co, Cr, Cu,

Fe, Mn, Pb, V, and Zn – decreases during the fermentation, stabilization, and fining of wines [4] as a result of precipitation or coprecipitation with organic complexing agents, such as polyphenols and tannins [5], while the content of rare earth elements could increase from young to finished wines due to the use of bentonite [6].

Atomic absorption spectroscopy (AAS) is a technique of choice for food authentication, and for wine analysis too, suitable for direct determination of trace elements in wine [7]. AAS instruments can analyze only one or few elements simultaneously. Although the electrothermal atomic absorption spectroscopy (ETAAS) technique is not suitable for multielement analysis, this technique offers high sensitivity and selectivity for determination of low levels of metals. The most versatile techniques for wine multielement analysis are inductively coupled plasma–optical emission spectrometry (ICP-OES) and inductively coupled plasma–mass spectrometry (ICP-MS), providing high detection power, high selectivity, and high sensitivity. Due to the high temperature of the plasma, less matrix interferences are observed [8]. However, wine is a complex matrix, containing high ethanol content and other organic compounds. Therefore, sample pretreatments, such as dilution [9, 10, 11], extraction, including conventional microextraction techniques with solvents for extraction [12], single-drop microextraction (SDME) [13], and dispersive liquid-liquid microextraction (DLLME) [14] have been used, in order to extract the metal ions bound in stable complexes [7]. Heating the samples with HNO_3 , HClO_4 , and H_2SO_4 or mixtures of these acids are commonly used digestion methods [9, 11, 15]. A newer technique uses microwave heating for sample digestion in high-pressure digestion vessels using the following reagents: HNO_3 , HCl , H_2SO_4 , and H_2O_2 [16].

Recently, the interest in certification of the geographic origin of wines significantly increased. Analytical support of the certification system could assist to prove and prevent the deliberate adulteration of wine. Therefore, characterization and classification of wines using trace element data can be practical and suit-

able, and wine can be classified by means of multivariate data analysis. Controlled Denomination of Origin (DOC) has been established in some European countries (France, Italy, Spain) [17] as well as in some Balkan countries (Croatia) [18]. Classification of wines as table, regional, controlled denomination of origin, and controlled and guaranteed denomination of origin has also been established in the Republic of Macedonia in order to guarantee the wine quality and prevent frauds [19]. But information on the trace elements profile of Macedonian wines is rather scarce.

Macedonian wines produced with different vinification procedures have been studied mainly for their phenolic and aromatic content, applying spectrophotometry, HPLC, MALDI, and GC techniques [20–26], and only a few research papers are dealing with individual metals in wines (cadmium, thallium, lead, mercury, arsenic, iron), applying atomic absorption spectrometry [27–37]. Only one publication is dealing with the application of the neutron activation analysis of about 30 elements for several wines from Macedonia [38]. To the best of our knowledge, there is no systematic study performed and published on the elemental composition of Macedonian wines and its applicability in distinguishing white and red and the determination of their geographical origin. Therefore, the aim of this work was to analyze the elemental composition of different wines (red, rose, and white from different regions) and to study the relationship between elemental concentrations and two variables: wine type (white vs. red) and geographical indication, applying two techniques, ICP-OES and ICP-MS, for analyses.

2. EXPERIMENTAL

2.1. Reagents and materials

Purified water ($18 \text{ M}\Omega \text{ cm}^{-1}$, Barnstead Nanopure, Thermo Fisher Scientific, USA) and high purity acid (HNO_3 , Suprapur, Merck, Ger-

many) were used throughout. Standards were prepared from 1 000 mg/l single-element solutions (Alfa Aesar, Germany) in 1 % HNO_3 (v/v). For ICP-OES analysis, a multielement stock solution (28 elements Roti Star, 100 mg/l, Roth, Germany) was used.

2.2. Wine samples

A total of 25 wine samples (10 white wines, 14 red wines, and 1 rose wine) were analyzed in this work. All wines used in this study were from the 2011 vintage and were collected directly from the wineries in the Republic of Macedonia in 500 mL glass bottles. Wineries were located in three different areas: Skopje, Negotino, and Demir Kapija. The wine varieties under this study and the production areas are presented in Table 1. Samples were kept in a cooling room at 4 °C before analysis.

2.3. Instrumentation

For analyte quantification, a simultaneous, axially viewed ICP-OES (CIROS VISION EOP, SPECTRO, Germany) and an ICP-MS (Elan DRC II, PerkinElmer, USA) were used. All relevant instrumental conditions are given in Table 2. The following emission lines were used in ICP-OES: Al 394.401 nm, B 249.773 nm, Ca 396.847 nm, Fe 238.204 nm, Mg 285.213 nm, Mn 257.611 nm, Na 589.592 nm, Ni 231.604 nm, P 177.495 nm, S 180.731 nm, Ti 336.121 nm, Zn 213.856 nm. For the ICP-MS analysis, the following mass-to-charge ratios (m/z) were recorded: 107 Ag, 197 Au, 137 Ba, 9 Be, 209 Bi, 111 Cd, 140 Ce, 59 Co, 65 Cu, 163 Dy, 166 Er, 151 Eu, 69 Ga, 157 Gd, 74 Ge, 165 Ho, 139 La, 175 Lu, 98 Mo, 146 Nd, 208 Pb, 141 Pr, 147 Sm, 159 Tb, 205 Tl, 169 Tm, 238 U, 51 V, 174 Yb, 90 Zr. Instrument drifts were corrected by using internal standards (ICP-OES: 1 mg l⁻¹Sc; ICP-MS: 1 µg l⁻¹In and Re).

Table 1

Wine varieties and production area

No	Type	Variety	Area of production	Abbreviation of wines with wine area
1	White	Temjanika	Demir Kapija	W1-DK
2	White	Žilavka	Demir Kapija	W2-DK
3	White	Riesling	Demir Kapija	W3-DK
4	White	Sauvignon Blanc	Demir Kapija	W4-DK
5	White	Smederevka	Demir Kapija	W5-DK
6	Rose	Stanušina Rose	Demir Kapija	Rose-DK
7	Red	Stanušina Red	Demir Kapija	R1-DK
8	Red	Vranec	Demir Kapija	R2-DK
9	White	Temjanika	Negotino	W6-N
10	White	Žilavka	Negotino	W7-N
11	White	Riesling	Negotino	W8-N
12	White	Sauvignon Blanc	Negotino	W9-N
13	White	Chardonnay	Negotino	W10-N
14	Red	Vranec	Negotino	R3-N
15	Red	Merlot	Negotino	R4-N
16	Red	Cabernet Sauvignon	Negotino	R5-N
17	Red	Syrah	Negotino	R6-N
18	Red	Tempranillo	Negotino	R7-N
19	Red	Pinot Noir	Negotino	R8-N
20	Red	Petit Verdot	Negotino	R9-N
21	Red	Sangiovese	Negotino	R10-N
20	Red	Vranec	Skopje	R11-S
21	Red	Merlot	Skopje	R12-S
22	Red	Cabernet Sauvignon	Skopje	R13-S
23	Red	Syrah	Skopje	R14-S

W – white; R – red; DK – Demir Kapija, N – Negotino, S – Skopje

Table 2

ICP-OES and ICP-MS operating conditions

Parameter	ICP-OES	ICP-MS
RF Power	1350 W	1350 W
Cooling gas flow	12.5 l min ⁻¹	14 l min ⁻¹
Auxiliary gas flow	0.6 l min ⁻¹	1.3 l min ⁻¹
Nebulizer gas flow	0.83 l min ⁻¹	0.91 l min ⁻¹
Nebulizer	Cross flow	Meinhard Type A
Spray chamber	Scott type	Cyclonic
Integration time	24 s	1000 ms for each <i>m/z</i> , 50 ms dwell time, peak hopping
Replicates	5	4

2.4. Sample preparation

For the sample preparation procedure, 5 ml wine and 2 ml nitric acid were digested at 240 °C in a commercial microwave-assisted sample digestion system (Multiwave 3000, Anton Paar, Austria). The microwave power was ramped to 1400 W within 17 min and maintained for an additional 15 min, limited by a maximum permissible pressure of 40 bar. After cooling to room temperature, the samples were reduced to a final volume of 50 ml.

2.5. Validation

One wine sample was spiked with a 10 µg/l multielement solution consisting of Ag, Au, Be, Bi, Cd, Ce, Co, Cu, Dy, Er, Eu, Ga, Gd, Ge, Ho, La, Lu, Mo, Nd, Pb, Pr, Sm, Tb, Tl, Tm, U, V, Yb, Zr for the ICP-MS analysis. Spike recoveries between 93 and 109 % were considered adequate for the purpose of this study. Spike recoveries for the ICP-OES analysis were determined from a 1 mg/l spike in the same wine that was spiked for the ICP-MS analysis. For Al, B, Ca, Fe, Mg, Mn, Na, Ni, P, S, Ti, and Zn, spike recoveries between 91 and 102 % were obtained by ICP-OES.

Due to the lack of sufficient certified reference material on wines, the accuracy of the procedure was evaluated by analyzing a CRM (trace elements in water, NIST SRM 1643e) as the digested wine samples were considered comparable. With exception of Mo (8 % deviation), no difference was found between the certified and the determined values on the 95 % confidence level.

2.6. Statistical analysis

Statistical treatments – including descriptive analysis, factor analysis, and cluster analysis – were performed using the XLSTAT software, version 2012.6.09, Copyright Addinsoft 1995–2012, applied to the data set in order to extract important information and to represent the pattern of similarities or differences between the studied wines in order to make a conclusion about the possible classification.

3. RESULTS AND DISCUSSION

Direct injection of the wine samples into the plasma could cause side effects, such as carbon deposition on the sample introduction system, and alter considerably the plasma stability and troublesome spectral interferences due to the high ethanol content in wines. Therefore sample pretreatment is necessary to eliminate these effects. In the present work, microwave digestion was used for the preparation of the wine samples, using concentrated nitric acid for the dilution of the wines.

The limit of quantification for each element was calculated using the equation $LOQ = (x_0 + 10 SD_0) * f_d$ (x_0 the mean of the blank; SD_0 , standard deviation of the blank; f_d , dilution factor of the sample due to the digestion procedure). The values for LOQ are presented in Table 3, together with the analytical technique used for the analysis of each meal as well as its concentration.

Table 3

LOQ of the elements and the analytical technique used

Element	Unit	Analytical technique	LOQ
Ag	µg/l	ICP-MS	0.2
Al	mg/l	ICP-OES	0.7
Au	µg/l	ICP-MS	0.4
B	mg/l	ICP-OES	0.5
Ba	µg/l	ICP-MS	0.5
Be	µg/l	ICP-MS	0.5
Bi	µg/l	ICP-MS	0.2
Ca	mg/l	ICP-OES	0.3
Cd	µg/l	ICP-MS	0.2
Ce	µg/l	ICP-MS	0.5
Co	µg/l	ICP-MS	0.2
Cu	µg/l	ICP-MS	0.5
Dy	µg/l	ICP-MS	0.4
Er	µg/l	ICP-MS	0.4
Eu	µg/l	ICP-MS	0.4
Fe	mg/l	ICP-OES	0.3
Ga	µg/l	ICP-MS	0.5
Gd	µg/l	ICP-MS	0.2
Ge	µg/l	ICP-MS	0.6
Ho	µg/l	ICP-MS	0.2
La	µg/l	ICP-MS	0.2
Lu	µg/l	ICP-MS	0.2
Mg	mg/l	ICP-OES	0.2
Mn	mg/l	ICP-OES	0.4
Mo	µg/l	ICP-MS	0.7
Na	mg/l	ICP-OES	0.2
Nd	µg/l	ICP-MS	0.4
Ni	mg/l	ICP-OES	0.2
P	mg/l	ICP-OES	2
Pb	µg/l	ICP-MS	0.2
Pr	µg/l	ICP-MS	0.3
S	mg/l	ICP-OES	2
Sm	µg/l	ICP-MS	0.4
Tb	µg/l	ICP-MS	0.2
Ti	mg/l	ICP-OES	0.3
Tl	µg/l	ICP-MS	0.2
Tm	µg/l	ICP-MS	0.2
U	µg/l	ICP-MS	0.09
V	µg/l	ICP-MS	2
Yb	µg/l	ICP-MS	0.3
Zn	mg/l	ICP-OES	0.2
Zr	µg/l	ICP-MS	2.3

In general, the elements in wine are present due to their extraction during the maceration period from the grapes. These elements are present in grapes as a result of their accumulation in the vine plant through the root from the soil, or they could originate from the agents used in spraying and for protecting the vines from diseases. During the maceration, extracted elements are absorbed at the cell membrane of the yeast, and afterward, their content declines as a result of precipitation together with the yeast cell or participation in complexation reactions. The contact of wine with the equipment, the addition of fining agents, or the changing of filters during postfermentation processes could increase the content of elements.

The results related to the determination of the 42 elements (Ag, Al, Au, B, Ba, Be, Bi, Ca, Cd, Ce, Co, Cu, Dy, Er, Eu, Fe, Ga, Gd, Ge, Ho, La, Lu, Mg, Mn, Mo, Na, Nd, Ni, P, Pb, Pr, S, Sm, Tb, Ti, Tl, Tm, U, V, Yb, Zn, Zr) in Macedonian white, red, and rose wines from different geographic areas are summarized as mean values and standard deviations in Tables 4, 5, and 6. All are the average of duplicate measurements. Elemental analysis showed that B, Ba, Ca, Fe, Mg, Mn, P, and S were the most abundant elements in the studied wines, followed by Cu, V, Pb, and Na. Al was detected only in four white wines from the Demir Kapija wine region: Temjanika, Žilavka, Smederevka, and Sauvignon Blanc. The elements Ag, Au, Bi, Dy, Er, Eu, Ge, Ho, Lu, Ni, Pr, Sm, Tb, Ti, Tm, and Yb were detected to have concentrations lower than the LOQ, and therefore they were not included in the statistical treatment of the data and were also not presented in the tables.

The descriptive statistics of the 26 determined elements in the wine samples are presented in Table 7. The five elements that have the highest medians in the wines are P, S, Ba, Mg, and Ca, followed by Cu, Na, and Pb. Factor analysis (FA) was performed on the basis of the matrix of correlation coefficients (Table 8), which describes the linear dependence between two random variables or sets of random variables [39] to identify and characterize the element associations offering reliable classification of the metals. When the correlation coefficient is >1 between variables, it reveals that there exists a strong correlation. A

correlation coefficient > 0.7 is interpreted as the existence of a strong relationship between two parameters, whereas values between 0.5 and 0.7 represent a moderate relationship. Thus, the concentration of every detected element was correlated to the concentration of the other elements separately, and the values of the correlation coefficients are presented in the matrix. The matrix of dominant rotated factor loadings is shown in Table 9. Six factors were identified. The biggest association was composed of Al-B-Be-Ce-Gd-La-Mg-Mn-Mo-Nd-P-U-V-Zr, interpreted as Factor 1. The other five associations were interpreted as Factor 2 (Al-Ba-Be-Ga-Pb-Tl-Zn), Factor 3 (Ca-Gd-Nd), Factor 4 (Ba-Cd-Ga-Na), Factor 5 (Al-Be-S), and Factor 6 (Co-P). The associations of elements were determined assuming that the absolute value of the linear correlation coefficients over 0.5 represents a good association between the elements. With respect to the factor analysis, two components were obtained that explained 46.36 % of the total variability of the original data: 30.02 % was assigned to the first factor and 16.34 % to the second factor. Figure 1 shows the dispersion between factors 1 and 2 and the loading for each variable (chemical element).

Dispersion of the scores of the factor analysis associated with each wine and grouping of the wines are presented in Figure 2. Projection of the wines on the first two factors showed a clear separation of the samples according to wine type (white vs. red). White wines were located in the positive part of F1, and red wines were in the negative part of F2. As presented in Fig 1, rare earth elements (REEs) Ce and La prevail in the first factor together with Mo, Zr, and V, as well as Be, Al, and Pb. B, P, Mg, and Mn were shown as dominant elements in the second factor and were characteristic for the red wines located in the positive part of F2, richer in these elements. The discriminant elements allowing separation of the wines according to wine type were Ba, Ca, Cu, P, Na, and S, the wines grouped according to the region. Thus, a clear separation of the red and white wines produced in the Negotino region was observed, as well as a separation of the red and white wines from the Demir Kapija region.

Table 4

Concentration of elements in red wines from different wine regions

Wine Element	Vranec			Merlot		Cabernet Sauvignon		Syrah	
	Negotino	Demir Kapija	Skopje	Negotino	Skopje	Negotino	Skopje	Negotino	Skopje
B (mg/l)	6.7 ± 0.2	5.34 ± 0.09	6.4 ± 0.1	8.8 ± 0.4	8.6 ± 0.2	9.0 ± 0.6	8.5 ± 0.7	8.4 ± 0.1	7.04 ± 0.09
Ba (µg/l)	171 ± 32	191 ± 2	116 ± 2	243 ± 16	140 ± 5	232 ± 23	114 ± 15	204 ± 2	154 ± 3
Be (µg/l)	< LOQ	< LOQ	0.5 ± 0.1	0.54 ± 0.01	< LOQ	< LOQ	< LOQ	< LOQ	0.5 ± 0.1
Ca (mg/l)	68 ± 2	76.0 ± 0.5	48.8 ± 0.5	71 ± 3	56 ± 1	78.0 ± 0.5	49.1 ± 0.5	65.5 ± 0.5	51 ± 2
Cd (µg/l)	< LOQ	< LOQ	< LOQ	0.25 ± 0.001	1.19 ± 0.08	< LOQ	0.24 ± 0.04	0.252 ± 0.002	1.05 ± 0.08
Co (µg/l)	5.4 ± 0.1	3.3 ± 0.1	2.1 ± 0.01	8.1 ± 0.2	4.1 ± 0.9	3.9 ± 0.3	2.1 ± 0.2	7.04 ± 0.05	3.9 ± 0.2
Cu (µg/l)	39 ± 2	1027 ± 8	24.2 ± 0.7	44 ± 5	63.5 ± 0.9	18 ± 1	36 ± 2	72.6 ± 0.5	102 ± 3
Fe (mg/l)	1.63 ± 0.07	1.70 ± 0.01	0.669 ± 0.004	1.99 ± 0.09	1.25 ± 0.01	1.39 ± 0.01	0.48 ± 0.03	1.59 ± 0.02	1.27 ± 0.02
Ga (µg/l)	4.8 ± 0.8	5.36 ± 0.02	3.1 ± 0.3	6.6 ± 0.4	4.25 ± 0.03	6.6 ± 0.5	3.3 ± 0.3	5.75 ± 0.06	4.39 ± 0.07
Mg (mg/l)	93.8 ± 0.4	94 ± 3	114 ± 3	101 ± 4	103 ± 1	95 ± 2	107 ± 5	104.2 ± 0.8	96 ± 1
Mn (mg/l)	1.33 ± 0.05	1.43 ± 0.01	1.62 ± 0.02	1.89 ± 0.09	1.94 ± 0.03	1.37 ± 0.01	1.37 ± 0.01	1.28 ± 0.01	1.69 ± 0.08
Mo (µg/l)	< LOQ	1.3 ± 0.2	0.9 ± 0.1	< LOQ	1.2 ± 0.2	1.19 ± 0.08	< LOQ	0.7 ± 0.1	0.7 ± 0.1
Na (mg/l)	10.2 ± 0.1	2.9 ± 0.08	16.2 ± 0.4	8.0 ± 0.3	20.2 ± 0.4	6.9 ± 0.3	20.1 ± 0.9	7.21 ± 0.05	22.2 ± 0.2
P (mg/l)	176 ± 9	107 ± 1	122 ± 3	244 ± 11	149 ± 4	189 ± 3	107 ± 3	205 ± 2	156 ± 7
Pb (µg/l)	5.2 ± 0.1	46.0 ± 0.5	5.7 ± 0.3	7.1 ± 0.2	68 ± 1	6.0 ± 0.4	4.9 ± 0.2	5.93 ± 0.06	54 ± 3
S (mg/l)	147 ± 4	111 ± 4	93 ± 6	199 ± 19	125 ± 2	169 ± 10	119 ± 10	157 ± 2	136 ± 1
Tl (µg/l)	0.67 ± 0.05	0.657 ± 0.002	0.67 ± 0.04	0.455 ± 0.009	0.85 ± 0.03	0.539 ± 0.004	0.36 ± 0.06	0.99 ± 0.02	1.8 ± 0.1
U (µg/l)	0.119 ± 0.006	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ
V (µg/l)	< LOQ	< LOQ	< LOQ	< LOQ	2.7 ± 0.2	< LOQ	2.8 ± 0.2	< LOQ	2.0 ± 0.4
Zn (mg/l)	0.454 ± 0.004	0.978 ± 0.02	0.287 ± 0.005	0.56 ± 0.03	0.844 ± 0.005	< LOQ	0.430 ± 0.005	0.646 ± 0.004	0.87 ± 0.02

Results are presented as average of two samples ± SD (standard deviation)
 < LOQ – lower than the limit of quantification

Table 5

Concentration of elements in white wines from different wine regions

Element	Temjanika		Žilavka		Riesling		Sauvignon Blanc	
	Negotino	Demir Kapija	Negotino	Demir Kapija	Negotino	Demir Kapija	Negotino	Demir Kapija
Al (mg/l)	<LOQ	1.38 ± 0.02	<LOQ	1.16 ± 0.05	<LOQ	<LOQ	<LOQ	0.8 ± 0.2
B (mg/l)	4.81 ± 0.07	5.4 ± 0.6	4.19 ± 0.06	5.3 ± 0.2	5.84 ± 0.05	5.21 ± 0.07	8.69 ± 0.09	4.42 ± 0.01
Ba (μg/l)	97 ± 3	324 ± 24	78 ± 8	90 ± 12	106 ± 6	145 ± 7	101 ± 11	109 ± 3
Be (μg/l)	0.91 ± 0.05	9.3 ± 0.6	0.8 ± 0.1	7.91 ± 0.09	1.15 ± 0.09	0.56 ± 0.01	1.3 ± 0.1	2.11 ± 0.04
Ca (mg/l)	56 ± 1	82 ± 5	69.1 ± 0.5	78.4 ± 0.5	84.5 ± 0.5	87 ± 1	78 ± 1	63.2 ± 0.7
Cd (μg/l)	0.43 ± 0.05	0.31 ± 0.03	0.22 ± 0.04	0.218 ± 0.001	0.47 ± 0.02	<LOQ	0.27 ± 0.06	<LOQ
Ce (μg/l)	0.606 ± 0.002	<LOQ	1.9 ± 0.4	1.78 ± 0.02	0.6 ± 0.2	1.3 ± 0.2	0.8 ± 0.1	3.7 ± 0.2
Co (μg/l)	8.01 ± 0.05	5.9 ± 0.3	6 ± 1	5.3 ± 0.1	11 ± 2	7 ± 1	6 ± 1	4.38 ± 0.01
Cu (μg/l)	49 ± 2	443 ± 16	24 ± 1	137 ± 2	21.5 ± 0.8	25 ± 1	123 ± 1	215 ± 2
Dy (μg/l)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.68 ± 0.01
Er (μg/l)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.51 ± 0.02
Fe (mg/l)	5.29 ± 0.05	0.5 ± 0.2	0.84 ± 0.01	0.71 ± 0.03	3.08 ± 0.02	2.11 ± 0.05	3.77 ± 0.04	0.855 ± 0.005
Ga (μg/l)	3.5 ± 0.1	10.9 ± 0.2	2.9 ± 0.4	3.2 ± 0.3	3.73 ± 0.07	4.9 ± 0.2	3.6 ± 0.5	3.4 ± 0.7
Gd (μg/l)	<LOQ	<LOQ	0.22 ± 0.02	<LOQ	<LOQ	<LOQ	<LOQ	0.567 ± 0.001
La (μg/l)	0.209 ± 0.008	<LOQ	0.7 ± 0.1	0.82 ± 0.02	0.22 ± 0.04	0.47 ± 0.05	0.29 ± 0.03	1.49 ± 0.07
Mg (mg/l)	73 ± 1	89 ± 6	66 ± 1	75.8 ± 0.5	72.3 ± 0.7	66.4 ± 0.5	74 ± 1	77.1 ± 0.4
Mn (mg/l)	1.14 ± 0.02	0.96 ± 0.06	1.00 ± 0.03	0.721 ± 0.005	0.901 ± 0.001	1.098 ± 0.005	0.794 ± 0.008	1.06 ± 0.01
Mo (μg/l)	2.56 ± 0.01	2.8 ± 0.1	1.8 ± 0.1	3.6 ± 0.1	3.9 ± 0.7	3.9 ± 0.8	4.4 ± 0.5	1.40 ± 0.04
Na (mg/l)	22.6 ± 0.2	18.8 ± 0.9	5.82 ± 0.04	14.5 ± 0.1	15.9 ± 0.1	6.76 ± 0.02	22.9 ± 0.2	29.45 ± 0.05
Nd (μg/l)	<LOQ	<LOQ	0.7 ± 0.1	0.768 ± 0.003	<LOQ	0.63 ± 0.08	<LOQ	2.10 ± 0.04
P (mg/l)	139 ± 4	181 ± 12	65 ± 2	93 ± 2	168 ± 2	89.2 ± 0.8	89 ± 2	66 ± 1
Pb (μg/l)	22.1 ± 0.2	79 ± 6	10.1 ± 0.1	37.9 ± 0.1	21.2 ± 0.3	11.9 ± 0.3	24.7 ± 0.6	8.2 ± 0.5
Pr (μg/l)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.48 ± 0.01
S (mg/l)	133 ± 0.3	206 ± 31	141 ± 3	193 ± 1	132 ± 1	109 ± 1	122 ± 2	149 ± 2
Sm (μg/l)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.53 ± 0.03
Th (μg/l)	0.459 ± 0.001	1.12 ± 0.04	0.347 ± 0.002	0.409 ± 0.006	0.27 ± 0.01	0.26 ± 0.01	0.36 ± 0.01	0.34 ± 0.01
U (μg/l)	0.194 ± 0.008	0.43 ± 0.04	0.226 ± 0.02	0.348 ± 0.001	0.174 ± 0.007	0.13 ± 0.02	0.290 ± 0.002	0.475 ± 0.005
V (μg/l)	23.7 ± 0.2	14.9 ± 0.5	15.6 ± 0.1	68.5 ± 0.1	28.9 ± 0.1	17 ± 1	31.3 ± 0.1	22 ± 3
Yb (μg/l)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.597 ± 0.001
Zn (mg/l)	0.48 ± 0.01	0.65 ± 0.06	0.236 ± 0.003	0.431 ± 0.006	0.389 ± 0.001	0.293 ± 0.004	0.26 ± 0.01	0.449 ± 0.005
Zr (μg/l)	2.9 ± 0.2	9 ± 1	<LOQ	8 ± 2	<LOQ	6 ± 1	4.5 ± 0.9	7.76 ± 0.01

Results are presented as average of two samples ± SD (standard deviation)

<LOQ – lower than the limit of quantification

Table 6
Concentration of elements in red and white wines from one wine region

Wine Element	Smederevka		Chardonnay		Stanušina Rose		Stanušina Red		Pinot Noir		Tempranilo		Petit Verdot		Sangiovese	
	Demir Kapija		Negotino		Demir Kapija		Demir Kapija		Negotino		Negotino		Negotino		Negotino	
Al (mg/l)	0.7 ± 0.1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
B (mg/l)	4.8 ± 0.1	4.95 ± 0.08	4.88 ± 0.05	4.88 ± 0.05	5.6 ± 0.2	5.6 ± 0.2	5.6 ± 0.2	5.6 ± 0.2	7.11 ± 0.05	7.11 ± 0.05	7.42 ± 0.06	7.42 ± 0.06	7.42 ± 0.06	8.5 ± 0.3	8.5 ± 0.3	8.5 ± 0.3
Ba (µg/l)	97 ± 4	94 ± 7	166 ± 7	166 ± 7	272 ± 13	272 ± 13	272 ± 13	272 ± 13	136 ± 2	136 ± 2	168 ± 2	168 ± 2	168 ± 2	n.d.	n.d.	n.d.
Be (µg/l)	0.53 ± 0.04	1.0 ± 0.1	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	0.67 ± 0.01	0.67 ± 0.01	<LOQ	<LOQ	<LOQ	n.d.	n.d.	n.d.
Ca (mg/l)	89 ± 1	56 ± 1	87.3 ± 0.4	87.3 ± 0.4	77 ± 2	77 ± 2	77 ± 2	77 ± 2	73.9 ± 0.5	73.9 ± 0.5	50.5 ± 0.4	50.5 ± 0.4	50.5 ± 0.4	75.5 ± 0.7	75.5 ± 0.7	61 ± 2
Cd (µg/l)	<LOQ	0.40 ± 0.02	0.21 ± 0.04	0.21 ± 0.04	<LOQ	<LOQ	<LOQ	<LOQ	0.206 ± 0.004	0.206 ± 0.004	<LOQ	<LOQ	<LOQ	n.d.	n.d.	n.d.
Ce (µg/l)	0.97 ± 0.04	0.60 ± 0.02	1.58 ± 0.04	1.58 ± 0.04	<LOQ	<LOQ	<LOQ	<LOQ	1.043 ± 0.008	1.043 ± 0.008	<LOQ	<LOQ	<LOQ	n.d.	n.d.	n.d.
Co (µg/l)	3.1 ± 0.1	7.79 ± 0.05	4.14 ± 0.08	4.14 ± 0.08	3.2 ± 0.1	3.2 ± 0.1	3.2 ± 0.1	3.2 ± 0.1	12.0 ± 0.1	12.0 ± 0.1	4.4 ± 0.2	4.4 ± 0.2	4.4 ± 0.2	n.d.	n.d.	n.d.
Cu (µg/l)	21.4 ± 0.7	46 ± 3	87 ± 1	87 ± 1	85 ± 3	85 ± 3	85 ± 3	85 ± 3	136 ± 1	136 ± 1	39.6 ± 0.3	39.6 ± 0.3	39.6 ± 0.3	n.d.	n.d.	n.d.
Fe (mg/l)	6.65 ± 0.08	5.3 ± 0.2	0.59 ± 0.03	0.59 ± 0.03	1.59 ± 0.04	1.59 ± 0.04	1.59 ± 0.04	1.59 ± 0.04	1.151 ± 0.001	1.151 ± 0.001	0.647 ± 0.004	0.647 ± 0.004	0.647 ± 0.004	1.1 ± 0.1	1.1 ± 0.1	0.979 ± 0.001
Ga (µg/l)	3.5 ± 0.1	3.0 ± 0.2	5.0 ± 0.2	5.0 ± 0.2	7.9 ± 0.5	7.9 ± 0.5	7.9 ± 0.5	7.9 ± 0.5	3.36 ± 0.01	3.36 ± 0.01	4.43 ± 0.06	4.43 ± 0.06	4.43 ± 0.06	n.d.	n.d.	n.d.
La (µg/l)	0.32 ± 0.01	0.205 ± 0.003	0.57 ± 0.01	0.57 ± 0.01	<LOQ	<LOQ	<LOQ	<LOQ	0.421 ± 0.005	0.421 ± 0.005	<LOQ	<LOQ	<LOQ	n.d.	n.d.	n.d.
Mg (mg/l)	74 ± 1	72 ± 3	81.9 ± 0.5	81.9 ± 0.5	84 ± 3	84 ± 3	84 ± 3	84 ± 3	91.8 ± 0.5	91.8 ± 0.5	105.3 ± 0.5	105.3 ± 0.5	105.3 ± 0.5	117.1 ± 0.5	117.1 ± 0.5	117 ± 3
Mn (mg/l)	0.715 ± 0.009	1.15 ± 0.02	1.122 ± 0.004	1.122 ± 0.004	1.26 ± 0.03	1.26 ± 0.03	1.26 ± 0.03	1.26 ± 0.03	2.011 ± 0.004	2.011 ± 0.004	1.22 ± 0.01	1.22 ± 0.01	1.22 ± 0.01	1.399 ± 0.004	1.399 ± 0.004	1.82 ± 0.06
Mo (µg/l)	2.7 ± 0.2	2.9 ± 0.7	0.9 ± 0.1	0.9 ± 0.1	1.10 ± 0.09	1.10 ± 0.09	1.10 ± 0.09	1.10 ± 0.09	1.1 ± 0.1	1.1 ± 0.1	<LOQ	<LOQ	<LOQ	n.d.	n.d.	n.d.
Na (mg/l)	4.67 ± 0.09	22.7 ± 0.9	3.32 ± 0.02	3.32 ± 0.02	2.05 ± 0.04	2.05 ± 0.04	2.05 ± 0.04	2.05 ± 0.04	10.3 ± 0.1	10.3 ± 0.1	17.39 ± 0.05	17.39 ± 0.05	17.39 ± 0.05	15.7 ± 0.3	15.7 ± 0.3	10.5 ± 0.4
Nd (µg/l)	0.439 ± 0.003	<LOQ	0.74 ± 0.03	0.74 ± 0.03	<LOQ	<LOQ	<LOQ	<LOQ	0.46 ± 0.01	0.46 ± 0.01	<LOQ	<LOQ	<LOQ	n.d.	n.d.	n.d.
P (mg/l)	63.3 ± 0.6	143.7 ± 0.5	102.0 ± 0.3	102.0 ± 0.3	73 ± 3	73 ± 3	73 ± 3	73 ± 3	288 ± 2	288 ± 2	217 ± 2	217 ± 2	217 ± 2	174 ± 2	174 ± 2	158 ± 7
Pb (µg/l)	12.3 ± 0.2	21.6 ± 0.4	11.5 ± 0.1	11.5 ± 0.1	5.5 ± 0.1	5.5 ± 0.1	5.5 ± 0.1	5.5 ± 0.1	9.98 ± 0.08	9.98 ± 0.08	6.28 ± 0.04	6.28 ± 0.04	6.28 ± 0.04	n.d.	n.d.	n.d.
S (mg/l)	91 ± 2	170 ± 39	148 ± 1	148 ± 1	96 ± 4	96 ± 4	96 ± 4	96 ± 4	145 ± 1	145 ± 1	148 ± 1	148 ± 1	148 ± 1	391 ± 4	391 ± 4	164 ± 2
Tl (µg/l)	<LOQ	0.45 ± 0.02	0.74 ± 0.01	0.74 ± 0.01	0.96 ± 0.05	0.96 ± 0.05	0.96 ± 0.05	0.96 ± 0.05	0.39 ± 0.002	0.39 ± 0.002	0.40 ± 0.01	0.40 ± 0.01	0.40 ± 0.01	n.d.	n.d.	n.d.
U (µg/l)	0.102 ± 0.006	0.199 ± 0.003	0.189 ± 0.004	0.189 ± 0.004	<LOQ	<LOQ	<LOQ	<LOQ	0.27 ± 0.02	0.27 ± 0.02	<LOQ	<LOQ	<LOQ	n.d.	n.d.	n.d.
V (µg/l)	15 ± 1	28 ± 5	4.5 ± 0.8	4.5 ± 0.8	<LOQ	<LOQ	<LOQ	<LOQ	8.8 ± 0.3	8.8 ± 0.3	<LOQ	<LOQ	<LOQ	n.d.	n.d.	n.d.
Yb (µg/l)	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	n.d.	n.d.	n.d.
Zn (mg/l)	<LOQ	0.49 ± 0.01	0.311 ± 0.001	0.311 ± 0.001	<LOQ	<LOQ	<LOQ	<LOQ	0.597 ± 0.001	0.597 ± 0.001	0.272 ± 0.001	0.272 ± 0.001	0.272 ± 0.001	0.298 ± 0.002	0.298 ± 0.002	0.378 ± 0.009
Zr (µg/l)	11 ± 2	3.1 ± 0.2	8.3 ± 0.4	8.3 ± 0.4	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	n.d.	n.d.	n.d.

Results are presented as average of two samples ± SD (standard deviation)

n.d. – not detected

<LOQ – lower than the limit of quantification

Table 7

Descriptive statistic of measurements for wine samples ($n = 26$ elements)

Statistic	No. of observations	Min	Max	Md	X	X_g	V	SD	A	E
Al (mg/l)	25	0.60	1.38	0.60	0.67	0.65	0.04	0.19	3.19	9.83
B (mg/l)	25	4.19	9.00	6.30	6.49	6.30	2.55	1.60	0.30	-1.43
Ba (μ g/l)	25	0.00	324.00	136.00	141.92	46.03	5660.99	75.24	0.41	0.65
Be (μ g/l)	25	0.00	9.30	0.50	1.20	0.31	5.22	2.29	3.12	9.03
Ca (mg/l)	25	48.80	89.00	71.00	69.27	68.07	166.17	12.89	-0.20	-1.21
Cd (μ g/l)	25	0.00	1.19	0.21	0.26	0.11	0.08	0.29	2.36	5.69
Ce (μ g/l)	25	0.00	3.70	0.25	0.72	0.26	0.68	0.83	2.28	6.29
Co (μ g/l)	25	0.00	12.00	4.40	5.13	2.12	8.48	2.91	0.47	0.49
Cu (μ g/l)	25	0.00	1027.00	46.00	115.15	22.31	44436.14	210.80	3.79	15.67
Fe (mg/l)	25	0.48	6.65	1.27	1.89	1.41	2.77	1.66	1.76	2.33
Ga (μ g/l)	25	0.00	10.90	3.73	4.30	1.87	4.98	2.23	0.79	2.68
Gd (μ g/l)	25	0.00	0.57	0.10	0.12	0.07	0.01	0.10	3.61	15.73
La (μ g/l)	25	0.00	1.49	0.10	0.28	0.11	0.11	0.33	2.43	6.90
Mg (mg/l)	25	66.00	117.10	91.80	89.79	88.41	257.90	16.06	0.17	-1.18
Mn (mg/l)	25	0.72	2.01	1.25	1.29	1.24	0.14	0.37	0.41	-0.55
Mo (μ g/l)	25	0.00	4.40	1.19	1.62	0.61	1.78	1.33	0.76	-0.66
Na (mg/l)	25	2.05	29.45	14.50	13.49	10.94	58.73	7.66	0.20	-1.02
Nd (μ g/l)	25	0.00	2.10	0.20	0.36	0.16	0.18	0.42	3.25	12.65
P (mg/l)	25	63.30	288.00	143.70	142.57	130.92	3479.68	58.99	0.59	-0.01
Pb (μ g/l)	25	0.00	79.00	10.10	19.40	5.42	454.34	21.32	1.66	1.99
S (mg/l)	25	91.00	391.00	145.00	151.76	144.28	3439.44	58.65	2.96	11.68
Tl (μ g/l)	25	0.00	1.80	0.45	0.54	0.25	0.15	0.39	1.45	3.37
U (μ g/l)	25	0.00	0.48	0.10	0.14	0.06	0.02	0.13	1.12	0.48
V (μ g/l)	25	0.00	68.50	2.80	11.75	2.19	249.61	15.80	2.14	5.93
Zn (mg/l)	25	0.02	0.98	0.43	0.43	0.31	0.06	0.25	0.39	0.14
Zr (μ g/l)	25	0.00	11.00	1.15	3.07	0.99	10.53	3.25	1.25	0.19

Min – minimum, *Max* – maximum, *Md* – median, X – mean, X_g – geometrical mean, V – Variance, *SD* – standard deviation, A – skewness, E – kurtosis

Table 8

Matrix of correlation coefficients (*r*)

Variables	Al	B	Ba	Be	Ca	Cd	Ce	Co	Cu	Fe	Ga	Gd	La	Mg	Mn	Mo	Na	Nd	P	Pb	S	Tl	U	V	Zn	Zr		
Al	1.00																											
B	-0.29	1.00																										
Ba	0.30	0.11	1.00																									
Be	0.98	-0.28	0.27	1.00																								
Ca	0.27	-0.24	0.20	0.26	1.00																							
Cd	-0.03	0.18	0.07	0.02	-0.34	1.00																						
Ce	0.23	-0.55	-0.20	0.23	0.21	-0.14	1.00																					
Co	0.03	-0.11	0.23	0.14	0.21	0.20	0.19	1.00																				
Cu	0.29	-0.23	0.34	0.27	0.18	-0.06	0.01	-0.04	1.00																			
Fe	-0.19	-0.23	-0.20	-0.17	0.17	0.07	-0.04	0.29	-0.12	1.00																		
Ga	0.42	0.00	0.98	0.39	0.29	0.09	-0.14	0.25	0.34	-0.13	1.00																	
Gd	0.13	-0.41	0.04	0.09	-0.02	-0.05	0.87	0.09	0.11	-0.14	0.06	1.00																
La	0.27	-0.53	-0.19	0.28	0.19	-0.14	0.99	0.18	0.02	-0.09	-0.13	0.86	1.00															
Mg	-0.17	0.64	-0.02	-0.25	-0.47	-0.04	-0.60	-0.53	-0.02	-0.50	-0.15	-0.38	-0.56	1.00														
Mn	-0.40	0.48	0.04	-0.43	-0.46	0.27	-0.42	-0.09	-0.02	-0.37	-0.11	-0.25	-0.40	0.69	1.00													
Mo	0.35	-0.38	-0.05	0.45	0.47	0.10	0.31	0.47	0.06	0.51	0.11	0.05	0.30	-0.80	-0.69	1.00												
Na	0.20	0.09	-0.28	0.24	-0.55	0.42	0.18	0.05	-0.13	0.07	-0.23	0.29	0.20	0.00	-0.05	0.18	1.00											
Nd	0.24	-0.48	-0.09	0.21	0.13	-0.14	0.97	0.09	0.06	-0.15	-0.05	0.93	0.97	-0.45	-0.31	0.16	0.21	1.00										
P	-0.08	0.55	0.24	-0.07	-0.17	0.09	-0.47	0.40	-0.10	-0.19	0.13	-0.37	-0.45	0.49	0.58	-0.38	0.01	-0.42	1.00									
Pb	0.56	-0.09	0.33	0.57	0.02	0.67	-0.10	0.10	0.50	-0.03	0.42	-0.06	-0.08	-0.13	-0.03	0.33	0.30	-0.09	-0.03	1.00								
S	0.22	0.28	-0.22	0.20	0.11	-0.16	-0.13	-0.18	-0.10	-0.22	-0.22	-0.19	-0.10	0.34	0.07	-0.24	0.13	-0.13	0.35	-0.06	1.00							
Tl	0.16	0.08	0.61	0.16	-0.23	0.59	-0.22	0.03	0.23	-0.25	0.59	0.01	-0.21	0.10	0.20	-0.16	0.07	-0.13	0.11	0.56	-0.21	1.00						
U	0.66	-0.48	0.06	0.70	0.28	-0.04	0.72	0.42	0.18	0.02	0.19	0.60	0.73	-0.61	-0.51	0.56	0.40	0.66	-0.21	0.30	0.00	-0.04	1.00					
V	0.51	-0.40	-0.26	0.62	0.26	0.04	0.50	0.36	-0.02	0.29	-0.13	0.17	0.53	-0.66	-0.64	0.78	0.31	0.36	-0.34	0.23	0.00	-0.23	0.67	1.00				
Zn	0.12	0.10	0.14	0.14	-0.32	0.57	-0.14	0.19	0.55	-0.19	0.11	-0.01	-0.11	0.22	0.43	-0.14	0.27	-0.08	0.32	0.64	0.03	0.49	0.04	-0.08	1.00			
Zr	0.62	-0.52	0.11	0.57	0.51	-0.12	0.57	0.04	0.11	0.26	0.24	0.39	0.55	-0.55	-0.65	0.53	0.05	0.54	-0.46	0.26	-0.12	-0.06	0.64	0.51	-0.19	1.00		

Table 9

Matrix of dominant rotated factor loadings

Element	F1	F2	F3	F4	F5	F6
Al	0.563	0.552	-0.028	-0.078	0.588	0.002
B	-0.650	0.096	0.042	0.079	0.130	0.222
Ba	-0.019	0.711	-0.179	-0.570	-0.250	0.143
Be	0.591	0.563	-0.064	0.040	0.547	0.060
Ca	0.422	0.007	-0.553	-0.303	0.070	0.119
Cd	-0.104	0.486	0.274	0.516	-0.340	-0.145
Ce	0.828	-0.300	0.413	-0.144	-0.102	0.102
Co	0.302	0.239	-0.176	0.280	-0.458	0.721
Cu	0.142	0.461	0.059	-0.224	0.000	-0.234
Fe	0.198	-0.182	-0.454	0.398	-0.299	-0.077
Ga	0.130	0.742	-0.251	-0.505	-0.210	0.086
Gd	0.623	-0.148	0.603	-0.294	-0.236	0.050
La	0.822	-0.267	0.441	-0.140	-0.047	0.116
Mg	-0.815	0.058	0.310	-0.146	0.417	-0.022
Mn	-0.725	0.119	0.396	-0.021	-0.098	0.166
Mo	0.704	0.104	-0.463	0.407	-0.120	-0.013
Na	0.187	0.112	0.484	0.578	0.137	0.012
Nd	0.745	-0.231	0.535	-0.269	-0.091	0.081
P	-0.546	0.300	0.041	0.075	0.111	0.714
Pb	0.208	0.836	0.083	0.292	0.021	-0.296
S	-0.158	-0.035	0.055	0.062	0.594	0.234
Tl	-0.138	0.705	0.204	-0.107	-0.282	-0.191
U	0.866	0.195	0.174	0.063	0.130	0.244
V	0.754	0.001	-0.149	0.436	0.181	0.075
Zn	-0.161	0.642	0.475	0.280	-0.113	-0.032
Zr	0.779	0.090	-0.148	-0.124	0.121	-0.141

F1, F2, F3, F4, F5, and F6 – factor loadings

Figures in bold are factor loading values with the largest squared cosine

The result of the cluster analysis is depicted in Figure 3, which highlights the similarity of the wine groupings according to wine type and observing two main clusters in the dendrogram, confirming the clear separation between red and white wines. This observation was in agreement with the results from the factor analysis. The cluster analysis was performed taking the Euclidean distance as the metric and Ward's method as the amalgamation rule.

The content of rare earth elements (REE) is presented for the first time in Macedonian wines (Figure 4). In fact, the concentration of

rare earth elements (REE) was very low for most of these elements in the wines, observing that total content ranged from 2.5 to 11.6 $\mu\text{g/l}$. In comparison to results reported by other authors [40, 41], the concentrations of REEs in our studied wines were lower. In fact, the presence of REEs is brought mainly by the application of bentonites for wine stabilization [40]. Results from this study were in agreement with this statement since it was observed that the white wines contained higher amounts of REEs than in the red ones, probably as a result of the higher amounts of agents added for stabilization and finalization in white wines.

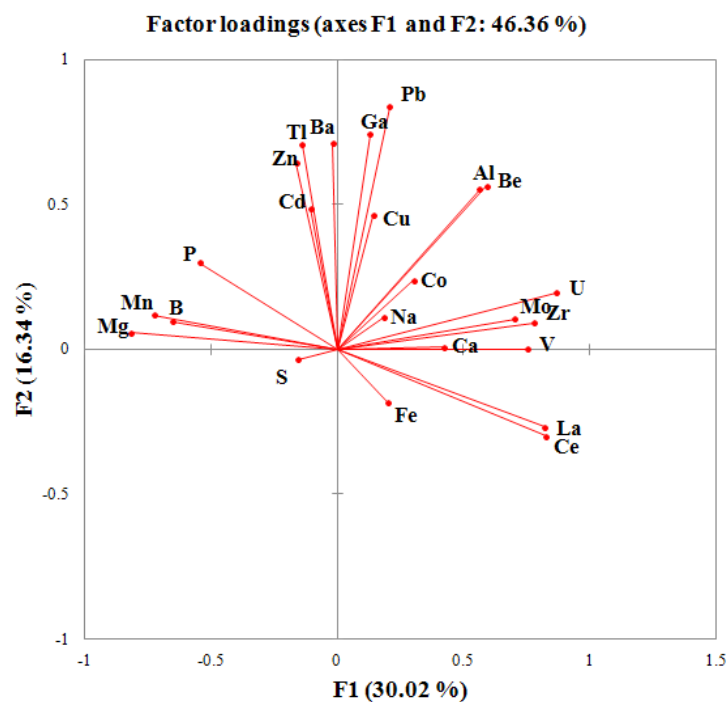


Fig. 1. Factor loadings with F1 and F2 of the variables based on element concentrations in wines

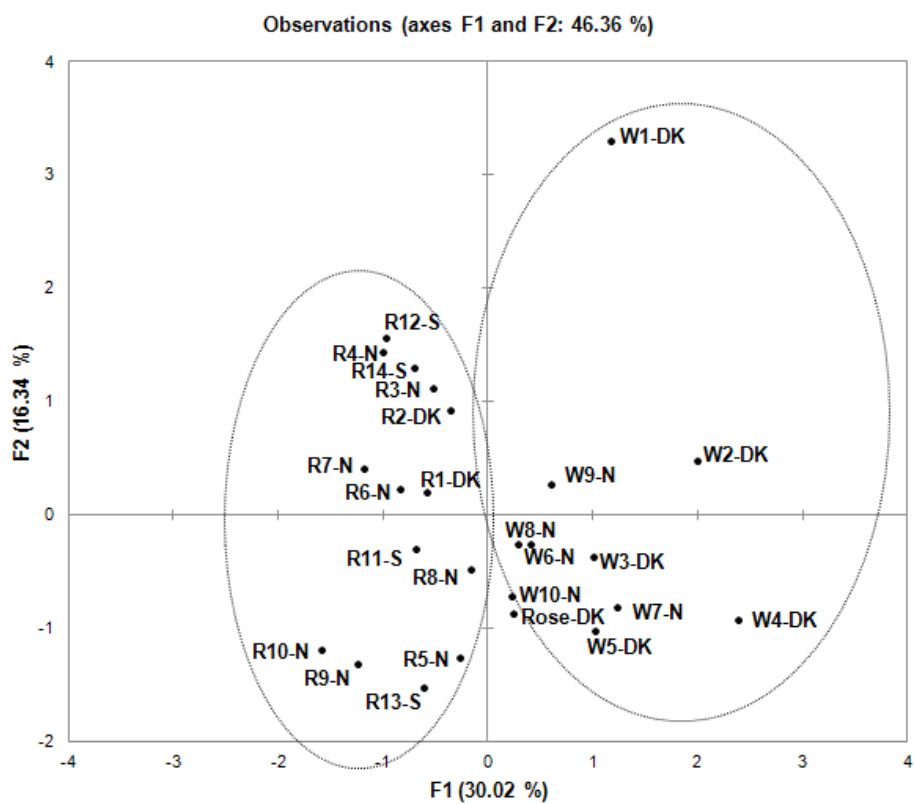


Fig. 2. Observations with F1 and F2 of the variables based on element concentrations in wines and grouping of the wines according to wine type

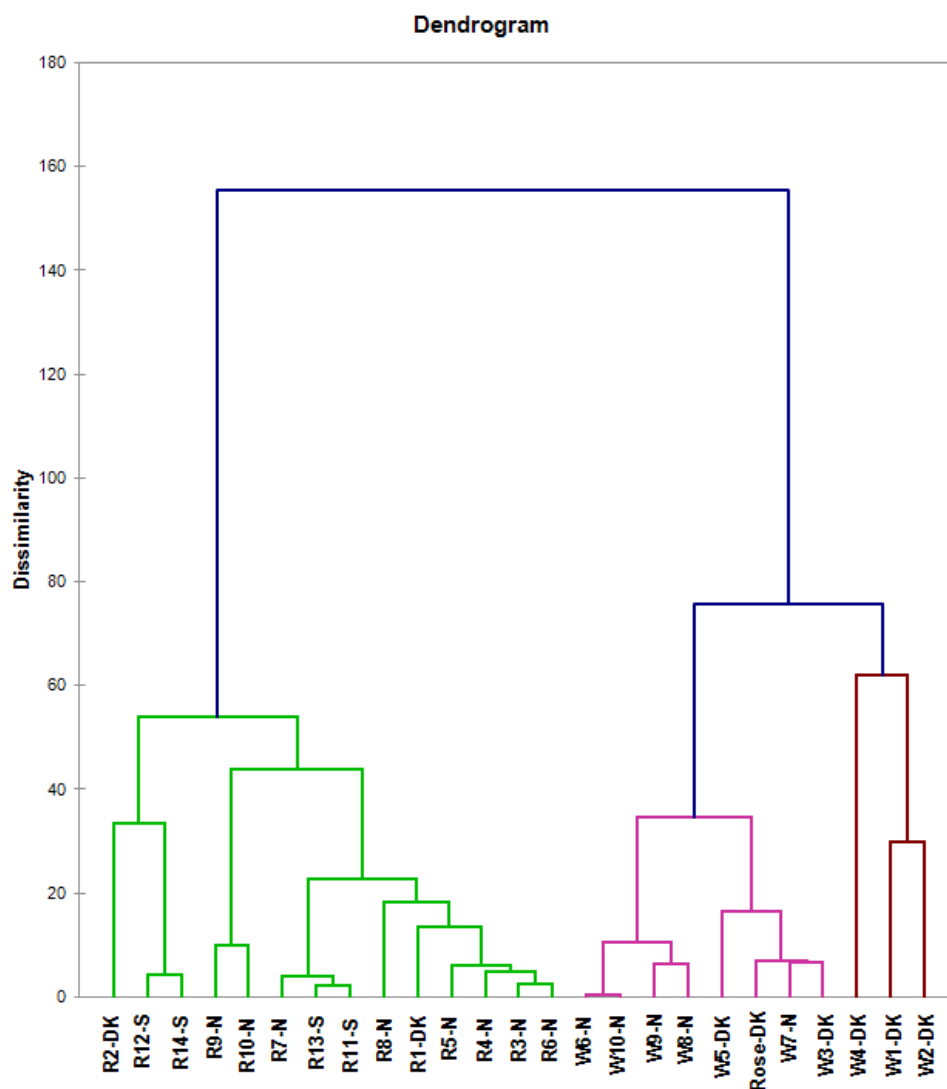


Fig. 3. Dendrogram obtained after the agglomerative cluster analysis performed on all elements quantified in the wine samples (codes as in Table 1)

Ba and P were the dominant elements in both red and white wines. Ba is naturally present in the soil as a macroelement, mainly as a result of natural phenomena, and its presence is strongly correlated to the nature of vineyards, rock weathering, or chemical processes in soil [39]. Also, P is a naturally present element, also considered as a macroelement, and is essential for life. From the results, it was observed that the wines contained high levels of P, ranging from 63 to 288 mg/l, confirming the nutritional value of Macedonian wines. S was detected in the wines (91 to 206 mg/l for the white wines and 93 to 163 mg/l for the red wines, except

for wine R9-N, which contained 391 mg/l) since SO_2 is usually used in winemaking as an antioxidant and a protective agent from the enzymatic and nonenzymatic oxidation of phenolics, sugars, and amino acids, which could cause browning of the wine (especially evident for white wines).

The presence of Pb in wine could indicate anthropogenic influence on the area where the vines are grown (especially if the vineyard is located near roads), or this element could appear as a contaminant during the winemaking process, originating from the materials used in the production of wine equipment because of large

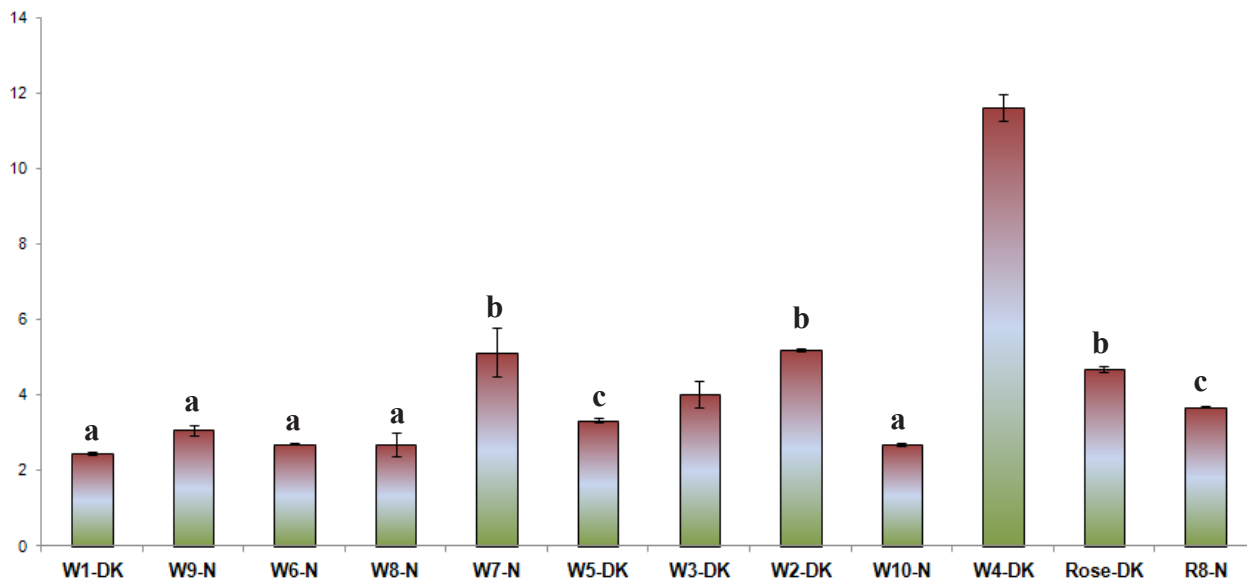


Fig. 4. Content of total rare earth elements in wines ($\mu\text{g/l}$)
Abbreviations of the wine samples are presented in Table 1.
Error bars represent standard deviations.
Bars with the same superscripts have values that
are not significantly different ($p > 0.05$).

tactile surfaces and long contact time. The presence of Cu was confirmed in the wines, ranging from 21 to 1027 $\mu\text{g/l}$. The Cu in wines could have originated from a few sources, such as agents used for vine protection (i.e. fungicides that contain Cu or CuSO_4) or wine equipment produced from bronze and brass. Heavy metals are toxic for biological systems because of their capacity to deactivate enzymes. Therefore their maximum allowed content in food must be controlled. Thus, the maximum allowed concentrations of Cu and Pb according to the International Organization of Vine and Wine (OIV) are 1 mg/l and 0.3 mg/l, respectively. According to the obtained results, the Cu and Pb content in the Macedonian wines was lower than the maximum allowed concentrations. Bi and Cd, both heavy and harmful elements, were not detected in the studied wines. As a conclusion, the studied Macedonian wines did not contain hazardous heavy metals. Instead their nutritional value was confirmed, showing high levels of macroelements such as P, Na, and Ba.

4. CONCLUSION

In this study, 25 Macedonian white, red, and rose wines from three different wine regions – Demir Kapija, Negotino, and Skopje, were discriminated mainly according to wine type (white vs. red) and geographical origin, applying factor and cluster analyses to the element concentrations. Element analysis was performed by inductively coupled plasma–mass spectrometry (ICP-MS), a methodology that allowed the determination of the concentration of 42 elements (Ag, Al, Au, B, Ba, Be, Bi, Ca, Cd, Ce, Co, Cu, Dy, Er, Eu, Fe, Ga, Gd, Ge, Ho, La, Lu, Mg, Mn, Mo, Na, Nd, Ni, P, Pb, Pr, S, Sm, Tb, Ti, Tl, Tm, U, V, Yb, Zn, Zr) in the wines. The main discriminant elements allowing separation of the wines were B, Ba, Ca, Fe, Mg, Mn, Na, P, and S. For the first time, the concentrations of rare earth elements were determined in wines from the Republic of Macedonia, which were observed to be lower compared to those of other wines from other parts of the world.

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REFERENCES

- [1] G. Grindlay, J. Mora, L. Gras, M. T. C. de Loos-Vollebregt, Atomic spectrometry methods for wine analysis: A critical evaluation and discussion of recent applications – review, *Anal Chim Acta*, **691**, 18–32 (2011).
- [2] J. D. Greenough, H. P. Longerich, S. E. Jackson, Element fingerprinting of Okanagan Valley wines using ICP-MS: relationships between wine composition vineyard and wine colour, *Aust J Grape Wine Res*, **3**, 75–83 (2008).
- [3] F. Salvo, L. la Pera, G. di Bella, M. Nicotina, G. Dugo, Influence of different mineral and organic pesticide treatments on Cd(II), Cu(II), Pb(II), and Zn(II) contents determined by derivative potentiometric stripping analysis in Italian white and red wines, *J Agric Food Chem*, **51**, 1090–1094 (2003).
- [4] H. Eschnauer, L. Jakob, H. Meierer, R. Neeb, Use and limitations of ICP-OES in wine analysis, *Mikroc Acta*, **3**, 291–298 (1989).
- [5] M.R.C. Almeida, T. S. D. Vasconcelos, Multielement composition of wines and their precursors including provenance soil and their potentialities as fingerprints of wine origin, *J Agric Food Chem*, **51**, 4788–4798 (2003).
- [6] N. Jakubowski, R. Brandt, D. Stuewer, H. Eschnauer, S. Gortges, Analysis of wines by ICP-MS: Is the pattern of the rare earth elements a reliable fingerprint for the provenance, *Fresen J Anal Chem*, **364**, 424–428 (1996).
- [7] T. Stafilov, I. Karadjova, Atomic absorption spectrometry in wine analysis, *Maced J Chem Chem Eng*, **28**, 17–31 (2009).
- [8] G. Thiel, K. Danzer, Direct analysis of mineral components in wine by inductively coupled plasma optical emission spectrometry (ICP-OES), *Fresen J Anal Chem*, **357**, 553–557 (1997).
- [9] M. M. Castiñeira Gómez, I. Feldmann, N. Jakubowski, J.T. Andersson, Classification of German white wines with certified brand of origin by multielement quantification and pattern recognition techniques, *J Agric Food Chem*, **53**, 2962–2974 (2004).
- [10] S. Catarino, A. S. Curvelo-Garcia, R. Bruno de Sousa, Measurements of contaminant elements of wines by inductively coupled plasma-mass spectrometry: A comparison of two calibration approaches, *Talanta*, **70**, 1073–1080 (2006).
- [11] A. Gonzalez, S. Armenta, A. Pastor, M. de la Guardia, Searching the most appropriate sample pretreatment for the elemental analysis of wines by inductively coupled plasma-based techniques, *J Agric Food Chem*, **56**, 4943–4954 (2008).
- [12] S. Sounderajan, A. C. Udas, B. Venkataramani, Characterization of arsenic(V) and arsenic(III) in water samples using ammonium molybdate and estimation by graphite furnace atomic absorption spectroscopy, *J Hazard Mater*, **149**, 238–242 (2007).
- [13] E.M. Martinis, R. G. Wuilloud, Cold vapor ionic liquid-assisted headspace single-drop microextraction: A novel preconcentration technique for mercury species determination in complex matrix samples, *J Anal Atom Spectrom*, **25**, 1432–1439 (2010).
- [14] P. Berton, R. G. Wuilloud, An online ionic liquid-based microextraction system coupled to electrothermal atomic absorption spectrometry for cobalt determination in environmental samples and pharmaceutical formulations, *Anal Methods*, **3**, 664–672 (2011).
- [15] M. Álvarez, I. M. Moreno, M. J. Jos, A. M. Cameám, A. Gustavo González, Study of mineral profile of Montilla-Moriles “fino” wines using inductively coupled plasma atomic emission spectrometry methods, *J Food Compos Anal*, **20**, 391–395 (2007).
- [16] G. Somer, A. Nakişci Ünlü, The effect of acid digestion on the recoveries of trace elements: recommended policies for the elimination of losses, *Turk J Chem*, **30**, 745–753 (2006).
- [17] S. Frías, J. E. Conde, J. J. Rodríguez-Bencomo, F. Garcia-Montengolo, J. P. Pérez Trujillo, Classification of commercial wines from de Canary Islands (Spain) by chemometric techniques using metallic contents, *Talanta*, **59**, 335–344 (2003).
- [18] I. Vinković Vrčak, M. Bojić, I. Žuntar, G. Mendaš, M. Medić-Šarić, Phenol content, antioxidant activity and metal composition of Croatian wines deriving from organically and conventionally grown grapes, *Food Chem*, **124**, 354–361 (2011).
- [19] Wine Law, Official Gazette of R. Macedonia, No 50, from 13.4. 2–25 (2010).

- [20] V. Ivanova, M. Stefova, B. Vojnoski, Assay of the phenolic profile of Merlot wines from Macedonia: effect of maceration time, storage, SO₂ and temperature of storage, *Maced J Chem Chem Eng*, **28**, 141–149 (2009).
- [21] V. Ivanova, M. Stefova, F. Chinnici, Determination of polyphenol contents in Macedonian grapes and wines assessed by standardized spectrophotometric methods, *J Serb Chem Soc*, **75**, 45–59 (2010).
- [22] V. Ivanova, B. Vojnoski, M. Stefova, Effect of the winemaking practices and aging on phenolic content of Smederevka and Chardonnay wines, *Food Bioprocess Tech*, **4**, 1512–1518 (2011).
- [23] V. Ivanova, Á. Dörnyei, M. Stefova, T. Stafilov, B. Vojnoski, F. Kilár, L. Márk, Rapid MALDI-TOF-MS Detection of Anthocyanins in Wine and Grape Using Different Matrices, *Food Anal Method*, **4**, 108–115 (2011).
- [24] V. Ivanova, Á. Dörnyei, L. Márk, B. Vojnoski, T. Stafilov, M. Stefova, F. Kilár, Polyphenolic content of Vranec wines produced by different vinification conditions, *Food Chem*, **124**, 316–325 (2011).
- [25] V. Ivanova, M. Stefova, B. Vojnoski, T. Stafilov, I. Bíró, A. Bufa, A. Felinger, F. Kilár, Volatile composition of Macedonian and Hungarian wines assessed by GC-MS, *Food Bioprocess Tech*, **6**, 1609–1617 (2013).
- [26] V. Ivanova, B. Vojnoski, M. Stefova, Effect of winemaking treatment and wine aging on phenolic content in Vranec wines, *J Food Sci Tech*, **49**, 161–172 (2012).
- [27] J. Cvetković, T. Stafilov, D. Mihajlović, Nickel and strontium nitrates as modifier for determination of selenium in wine by Zeeman electrothermal atomic absorption spectrometry, *Fresen J Anal Chem*, **370**, 1077–1081 (2001).
- [28] J. Cvetković, S. Arpadjan, I. Karadjova, T. Stafilov, Determination of thallium in wine by electrothermal atomic absorption spectrometry after extraction preconcentration, *Spectrochim Acta B*, **57**, 1101–1106 (2002).
- [29] J. D. Cvetković, S. H. Arpadjan, I. B. Karadjova, T. Stafilov, Determination of selenium in wine by electrothermal atomic absorption spectrometry, *Bulg Chem Commun*, **34**, 50–57 (2002).
- [30] J. Cvetković, S. Arpadjan, I. Karadjova, T. Stafilov, Determination of chromium in macedonian wine by electrothermal atomic absorption spectrometry, *J Instit Sci Tech Balikesir Uni*, **4**, 80–84 (2002).
- [31] J. Cvetkovic, S. Arpadjan, I. Karadjova, T. Stafilov, On the problems of the ETAAS determination of arsenic in wine, *Annual of University of Sofia, Faculty of Chemistry*, **96**, 173–178 (2004).
- [32] J. Cvetković, S. Arpadjan, I. Karadjova, T. Stafilov, Determination of nickel in wine by electrothermal atomic absorption spectrometry, *Ovidius University, Annals of Chemistry*, **16**, 31–34 (2005).
- [33] J. Cvetković, S. Arpadjan, I. Karadjova, T. Stafilov, Determination of cadmium in wine by electrothermal atomic absorption spectrometry, *Acta Pharmaceut*, **56**, 69–77 (2006).
- [34] I. Karadjova, S. Arpadjan, J. Cvetković, T. Stafilov, Sensitive method for trace determination of mercury in wines using electrothermal atomic absorption spectrometry, *Microchim Acta*, **147**, 39–43 (2004).
- [35] I. Karadjova, J. Cvetković, T. Stafilov, S. Arpadjan, On the determination of lead in wine by electrothermal atomic absorption spectrometry, *Cent Eur J Chem*, **5**, 739–747 (2007).
- [36] K. Tašev, I. Karadjova, T. Stafilov, Determination of inorganic and total arsenic in wines by hydride generation atomic absorption spectrometry, *Microchim Acta*, **149**, 55–60 (2005).
- [37] K. Tašev, I. Karadjova, S. Arpadjan, J. Cvetković, T. Stafilov, Liquid/liquid extraction and column solid phase extraction procedures for iron species determination in wines, *Food Control*, **17**, 484–488 (2006).
- [38] J. Cvetković, R. Jaćimović, T. Stafilov, S. Arpadjan, I. Karadjova, Determination of major and trace elements in wine by k₀-instrumental neutron activation analysis, *Bull Chem Technol Maced*, **21**, 187–192 (2002).
- [39] B. Balabanova, T. Stafilov, R. Šajn, K. Bačeva, Distribution of chemical elements in attic dust as reflection of their geogenic and anthropogenic sources in the vicinity of the copper mine and flotation plant, *Arch Environ Con Tox*, **61**, 173–18 (2011).
- [40] E. Rossano, Z. Szilágyi, A. Malorni, G. Pocsfalvi, Influence of winemaking practices on the concentration of rare earth elements in white wines studied by inductively coupled plasma mass spectrometry, *J Agric Food Chem*, **55**, 311–317 (2007).
- [41] S. Catarino, I.M. Trancoso, M. Madeira, F. Monteiro, R. Bruno de Sousa, A.S. Curvelo-Garcia, Rare Earths Data for Geographical Origin Assignment of Wine: A Portuguese Case Study. *Le Bulletin l'OIV*, 84: N° 965–966–967, 333–346 (2011).

