

SHORT REPORT

**Distribution of Selected Elements as Wine Origin Markers
in the Wine-making Products***MILAN SUHAJ and MÁRIA KOREŇOVSKÁ**Food Research Institute, Bratislava, Slovak Republic***Abstract**

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The analysis of the trace elements has been shown to be a valuable tool to discriminate wines according to their region of origin. As, Ba, Ca, Co, Cr, Li, Mg, Rb, Sn, Sr, and V were selected as specific markers indicating the origin of Slovak wines according to the vineyard regions. Several factors, such as the environmental contamination, agricultural practices, climatic changes, and others, may markedly change the multielement composition of the wine and may endanger the relationship between the wine and the soil composition. The effect was studied of the viticulture process on the distribution of selected markers in the winemaking products. The main markers pass from the vineyard soil to the grape, and the main portion leaves the winemaking process in the press cake and yeast lees. Very significant correlation of the wine origin markers was found between changed the wine making products and the vineyard soils. The sugar addition to grape juice to some extent the total element compositions of wines but did not result in substantial changes of the markers determining the wine origin.

Keywords: elements; atomic absorption spectrometry; winemaking process; factor analysis; correlation

The recent outbreak of food-borne diseases, and scares in different parts of the world have heightened consumers' awareness of food quality and safety concerning the aspects of geographical origin, agricultural practices, and accurate labelling of food products as well. Wine adulteration, mainly in terms of varieties and regions of geographical origin, has been very widespread. Commission Regulation (EC) No. 1429/2004 of 9 August 2004, amending Regulation (EC) No. 753/2002, lays down certain rules for applying Council Regulation (EC) No. 1493/1999 as regards the description, designa-

tion, presentation, and protection of certain wine sector products.

The development of reliable methods for traceability is strongly required in grapevines, in conformity with the market needs for the knowledge of the product source and the protection of the origin declared. For the identification of the wine origin, some new rapid, robust, accurate and cost effective sophisticated techniques are searched, out of which namely the analysis of trace elements and the ratios of stable isotopes have been shown to be valuable tools to discriminate wines according

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to their geographical origin and the technological processes applied during the manufacture. Elements can be considered as good indicators of the wine origin since they are not metabolised or modified during the vinification process (ARVANITZANNIS *et al.* 1999). The identification of the geographical origin of wines is of great interest for the wine consumers and producers since it may provide determinant criteria for the guarantee of quality.

The possibility of the discrimination of wines by regions through their micro- and macro-element patterns suggests that the elements are regulated mainly by their movement from rock to soil and from soil to grape. Wine multielemental composition is strongly influenced by the solubility of inorganic compounds of the soil. In principle, the pattern of the respective wine will reflect the geochemistry of the provenance soil. However, several factors, such as environmental contamination, agricultural practices, climatic changes, and vinification processes, may markedly change the multielement composition of the wine and may endanger the relationship between the wine and soil compositions. Another factor that may condition that relationship is related with the plant uptake, which can vary with the wine variety. All of these factors may imperil the usefulness of the wine multielement composition as a fingerprint of the wine region of origin (ALMEIDA & VASCONCELOS 2003; KMENT *et al.* 2005).

The wine-making process can considerably alter the mineral content of wine, mainly the grape processing and the skin maceration time. Some information in the literature shows that during fermentation and fining of wines the concentrations of several elements (Al, Cd, Co, Cr, Cu, Fe, Mn, Pb, V, and Zn) decrease (ESCHNAUER *et al.* 1989) or, to the contrast, increase because of the contamination of the respective wine by Cd, Cr, and Pb (KRISTL *et al.* 2002), or Al, Cr, Fe, Ni, Pb, and V (ALMEIDA & VASCONCELOS 2003). By the not stainless steel wine making equipments, wines can be contaminated with Fe, Cu, Zn coming from bronze or brass, or with lead. The aimed wine treatment with potassium ferrocyanide or with calcium phytate removes the excess of Fe and of other metals, namely Zn, Mn and Cu. Using technological additives or processes, such as cold treatment, can lower the content of potassium. Potassium, calcium, and sodium are elements that can vary widely, both with the process, and from wine to wine. Potassium and calcium can

combine with tartaric acid and form potassium bitartrate and calcium tartrate; these molecules can precipitate potassium by the temperature influence. The deacidification of wine can be carried out with calcium carbonate or potassium bicarbonate, thus producing an increase in the contents of those elements. Bentonite fining adds sodium and aluminium to the wine and increases the concentrations of rare earth elements in the course from young to finished wines (JAKUBOWSKI *et al.* 1999). This treatment is forbidden in red wines making in France. The effects due to the variations in the sugar content of the must for characterising the origin of wines are quantified by MARTIN *et al.* (1988) and vary according to the composition of the sugar added. Some elements, such as Ni, Cr, and Li, can migrate with time into the wine from the glass of the bottles (MÉDINA 1996). Car exhaust emissions have been carefully studied since vineyards are invariably bordered by roads and, in a few cases, by high vehicle density motorways. Therefore, because the vinification process can influence the concentrations of several elements in wine and in some regions different winemaking processes have been used, the element fingerprints of the wines may be the relics of soil and other environment signatures that survived metabolic and winery processing (ALMEIDA & VASCONCELOS 2003).

In this article we present some results of the study of the winemaking processes effects on the correlation and distribution of some selective elemental markers of wine origin.

MATERIALS AND METHODS

Samples. 37 soil samples from 6 Slovak vineyard regions (S – Middle Slovakian, N – region Nitra, M – Small Carpathian, J – South Slovakian, V – East Slovakian, T – Tokaj), 34 grape samples and 69 commercial wine samples (Müller Thurgau, Veltliner Green, Riesling Wälsch) were analysed for the contents of elemental markers As, Ba, Ca, Co, Cr, Li, Mg, Rb, Sr, Sn, V, and their correlation with vineyard soils, grapes, and wines. The distribution of these elements from soils up to the wine-making products – grape, must, young wine, yeast lees and press cake, was studied in the case of two Veltliner green white wine producers (A and B) from Small Carpathian vineyard region. Both producers adjusted the sugar content of grape juice from 16 to 21 NM (normalised mustmeter degrees

– kg sugar for 1 hl must). At the wine filtration, bentonite was used as the absorption medium. The effect of the sugar addition was studied in the wines prepared under laboratory conditions from Veltliner green grape obtained from Small Carpathian vineyard region. The content of sugar in this wine was adjusted from 16 to 21 NM with sugar originated from the sugar refinery Dunajská Streda (DS – South Slovakian vineyard region), Trnava (TR – Small Carpathian vineyard region), and Rimavská Sobota (RS – Middle Slovakian vineyard region). The wine filtration was carried out using white filtration paper.

Analysis of grape matrix and wines. The samples of wine, grape, must, young wine, yeast lees, and press cake were digested by means of the microwave digestion system MLS 1200 MEGA (Milestone, Italy). The wine (2.5 ml) and 2.0 g of the sample of grape, must, young wine, or sugar, or 1.0 g of yeast lees or press cake were introduced into teflon microwave digestion vessels, to which 2 ml to 4 ml of HNO₃ were added. The microwave digestion program was applied: 250 W (1 min), 0 W (1 min), 250 W (5 min), 400 W (5 min), and 650 W (5 min). The digested samples were adjusted to the volume of 10 ml with ultra pure water.

Analytical techniques. A Perkin Elmer 4100 atomic absorption spectrometer (USA) equipped with a deuterium lamp background-correction system and HGA 700 graphite tube atomiser with

pyrolytically coated graphite tubes and flame was used for the metal determination involving atomic absorption spectrometry. The metals Ca, Mg, and Rb were determined by atomic spectrometry using the air/acetylene flame, while Sr was characterised using the acetylene/nitrous oxide flame. The metals As, Ba, Co, Cr, Li, Sn, Rb, and V were determined by graphite tube atomiser. The instrumental conditions for the determination are shown in Table 1, and the analytical characteristics for the determination of metals in the samples by AAS and X-RFS, in Table 2. All results were expressed as the average of triplicate measurements. The limit of quantification – LOQ was calculated according to EURACHEM recommendation (FLEMING *et al.* 1997). The combined standard uncertainty (U_C) was calculated as $\sqrt{(u_A^2 + u_B^2)}$ (EURACHEM 1995). The accuracy of the results was verified by the standard addition method, because no certified reference materials were available for the determination of elements in wine, grape, must, young wine, yeast lees, and press cake. The recovery of the method was assessed in matrix by analysis of the samples fortified with elements at the level of 0.005 mg/l and 0.020 mg/l. The fortification of the samples was performed before the microwave digestion. The mean recoveries of the elements were from 85 to 107%. The accuracy of the method was tested by the determination of As, Ba, Ca, Co, Cr, Li, Mg, Rb, Sr, and V concentrations in DC 73351

Table 1. Instrumental conditions for the determination of elements

Element	Wavelength (nm)	Lamp current (mA)	Technique by AAS	Gas	Signal type	Suppressor modifier
As	193.7	310	GF	argon	AA-BG	Pd + Mg(NO ₃) ₂
Ba	553.6	25	GF	argon	AA	No
Ca	422.7	15	flame	C ₂ H ₂ -air	AA	0.1% LaCl ₃
Co	240.7	30	GF	argon	AA-BG	No
Cr	357.9	25	GF	argon	AA-BG	No
Mg	285.2	15	flame	C ₂ H ₂ -air	AA-BG	0.1% LaCl ₃
Li	670.8	20	GF	argon	AA	No
Rb	780.0	155	flame	C ₂ H ₂ -air	AA	0.5% CsCl
Rb	780.0	155	GF	argon	AA-BG	No
Sr	460.7	15	flame	N ₂ O-C ₂ H ₂	AA	0.5% CsCl
Sn	286.3	20	GF	argon	AA-BG	Pd + Mg(NO ₃) ₂
V	318.4	25	GF	aArgon	AA-BG	No

GF – graphite furnace; AA-BG – atomic absorption with background correction; AA – atomic absorption

Table 2. Analytical characteristics for the determination of elements in grapes matrix and soil

Element	Grapes matrix			Soil		
	measurement	LOQ (mg/kg)	U _C (%)	measurement	LOQ (mg/kg)	U _C (%)
As	GF-AAS	0.006	11.8	X-RSF	2	5
Ba	GF-AAS	0.005	13.3	X-RSF	10	1
Ca	flame	0.100	6.8	flame	1	2.5
Co	GF-AAS	0.003	7.7	flame	1	10
Cr	GF-AAS	0.004	5.3	X-RSF	5	2.5
Li	GF-AAS	0.003	16.1	flame	1	5
Mg	flame	0.090	3.5	flame	1	2.5
Rb	flame	0.090	9.1	flame	2	1.7
Rb	GF-AAS	0.003	5.6			
Sr	flame	0.080	4.7	flame	10	1
Sn	GF-AAS	0.006	15.7	X-RSF	1	5
V	GF-AAS	0.006	6.3	X-RSF	20	2

LOQ – limit of quantification; U_C – combined uncertainty

Tea (reference material China National Analysis Center), and NCS DC 73319 soil (China National Analysis Center).

Analysis of soil. The sample representing an average from mixed sub-samples of vineyard soil was taken with a probe of an area of 100 m² from the depth soil horizon of 0–20 cm. In this way, composite samples were obtained in the volumes of 2 kg. The samples were first dried to a constant weight. For the determination of the total content of the chemical elements, the fraction ≤ 0.125 mm was used. Ca, Co, Li, and Mg were determined by AAS – flame after microwave digestion using the decomposition mixture of concentrated acids HF + HNO₃ + HCl (Merck, Germany). The elements As, Ba, Cr, Rb, Sr, Sn, and V were determined by X-ray fluorescence spectrometry (X-RFS, SPECTRO LAB 2000, Kleve, Germany) from pressed tablets.

Chemicals. All chemicals used were of analytical grade. Stock solutions of each element (As, Ba, Ca, Co, Cr, Li, Mg, Rb, Sn, Sr, and V at the concentration of 1.00 g/l) were from Merck (Germany). The working standard solutions were prepared by suitable dilution of the stock solution. Nitric acid of suprapure quality was purchased from Merck (Germany), as well as the modification solutions of Pd and Mg (NO₃)₂. Cesium chloride 99% as ionic suppressor was obtained from Serva (USA), and lanthanum chloride 5% as ionic suppressor was

delivered from SMÚ (Slovakia). Ultra pure water from Milli-Q equipment (UK) with conductivity of 18 MΩ was used throughout.

Statistics. For the visualisation of the segregated vineyard regions factor analysis was applied using the statistical programme Unistat[®] (Unistat Ltd., England). Correlation coefficients were calculated using of Microsoft[®] Excel 2002 statistical tools.

RESULTS AND DISCUSSION

The selection of the traceability markers started from the study of the distribution of about 36 different elements in vineyard regions using the Slovakian geochemical atlas of soils (ČURLÍK & ŠEFCÍK 1999). For wines regional differentiation, As, Ba, Ca, Co, Cr, Li, Rb, Sr, Sn, V, and Mg were selected according to their variability. Using these specific elements, an efficient geographical authentication of Slovak and some European wines was achieved (KOREŇOVSKÁ & SUHAJ 2005). In this study, the effect of the viticulture process on the distribution of these selected markers in winemaking products was studied.

In Figure 1–3 are shown the results of the research of the effect of the winemaking processes on the composition and distribution of elemental markers in grape, must, young wine, yeast lees, and press cake. In the case of Co, Cr, Li, Sn, and V

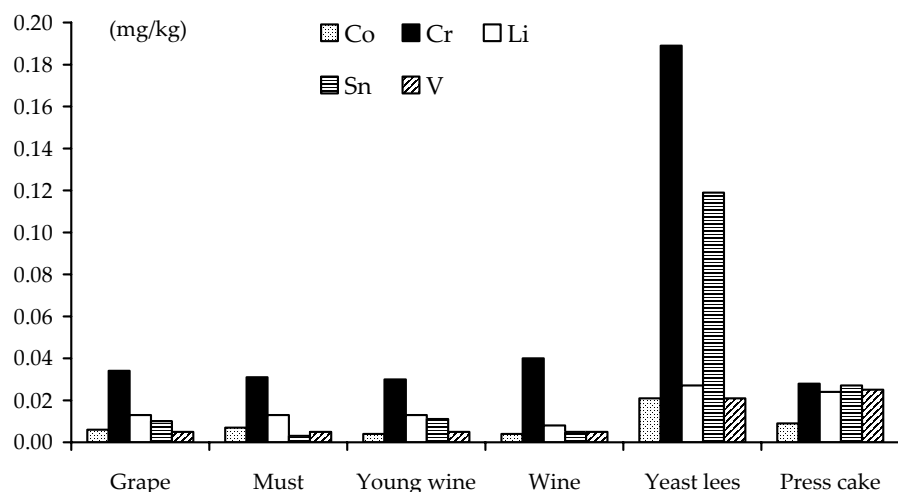


Figure 1. Distribution of Co, Cr, Li, Sn, and V in winemaking procedure of white wine

(Figure 1), the contents of Cr and Sn are cumulated in the yeast lees and the others less in the press cake. The main portion of the markers Ba, Sr, Rb, Ca, and Mg (Figures 2 and 3), belonging to the group of alkaline elements and earths, is cumulated in the press cake. By comparison of the contents of the markers in grapes and wines, we can find a significant decrease of all the markers with the exception of Cr as it was similarly observed in the findings of KRISTL *et al.* (2002), or ALMEIDA and VASCONCELOS (2003).

In spite of significant changes of these markers from grape to wine products, we observed a very

good relation among the marker compositions in wine-making products. The correlation coefficients between the products tested are calculated in Table 3. Very good correlations between the vineyard soil markers and their contents in grapes, musts, new wines, yeast lees, press cakes, and wines were found in all cases. For the confirmation of the high correlation found, further study was realised with the samples from all Slovakian vineyard territories (Table 4). The mean contents of the elements as wine origin markers and their profile correlations between vineyard soils, grapes, and wines from six vineyard regions are

Table 3. Correlation between the wine origin markers in vineyard soils and in winemaking products

Producer	Grape	Must	New wine	Wine	Yeast lees	Press cake
A	0.9500	0.9797	0.9944	0.9885	0.9946	0.8972
B	0.9397	0.9006	0.9387	0.9322	0.9522	0.9990

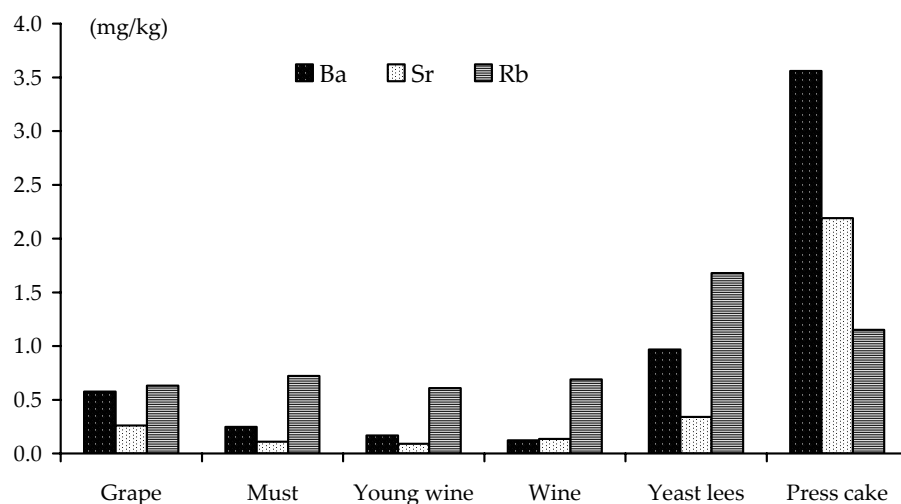


Figure 2. Distribution of Ba, Rb and Sr in winemaking procedure of white wine

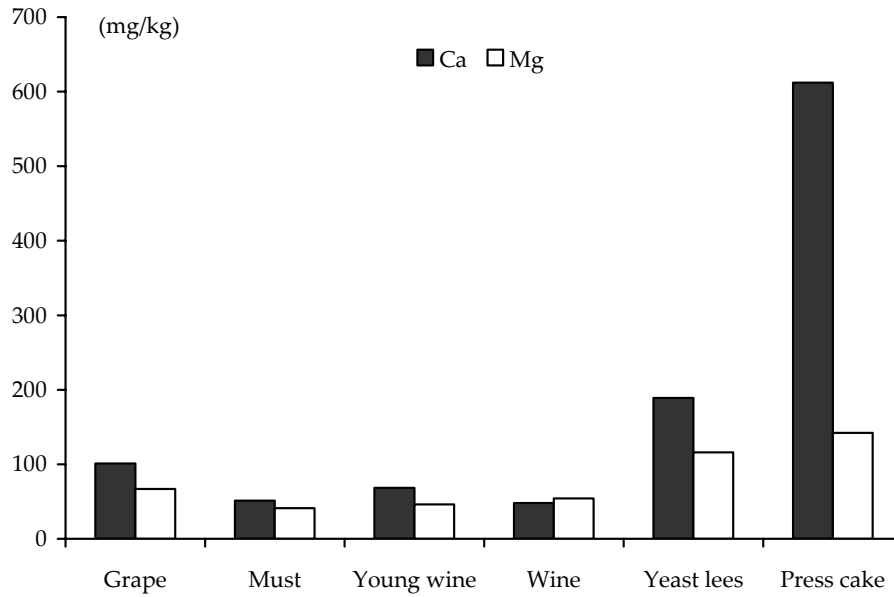


Figure 3. Distribution of Ca and Mg in winemaking procedure of white wine

shown in this table. With all vineyard regions, very good correlations between the marker contents in vineyard soils and wines were found, only for the Tokaj region the correlation between the soil and grape was relatively small. The high correlations found between the sets of indicator markers very strongly support the presumption by the possible use of the selected elements for tracing the Slovakian vineyard regions.

The changes of the selected markers of wine origin during the winemaking process in the case

of the sugar addition to grape must are shown in Table 5. The addition to grape must of sugar, originated from different sugar refineries, was in the case of some elements relatively very significant (for example Li). We found a decrease of Sr of about 25%, Ba of about 11%, Ca of 8%, and Mg of 8% in comparison to the contents of these elements in the original grape must. In total, the contents of the selected markers in wines prepared from sugared musts were similar to their contents in non-sweetened grape juice, thus the sugar ad-

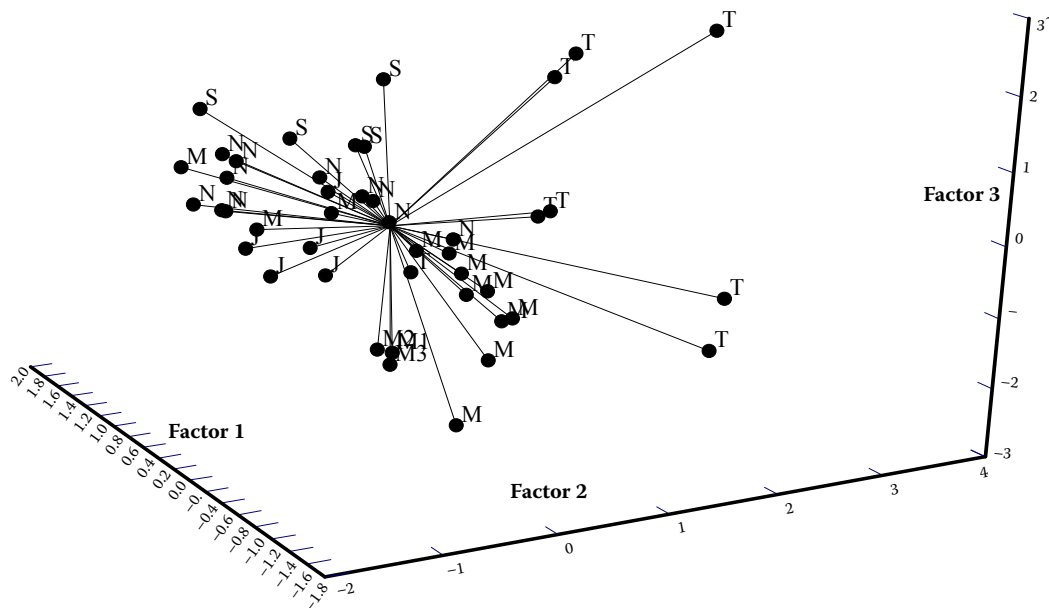


Figure 4. Principal component factoring – segregation of Slovakian wines (Vineyard region: T – Tokaj, N – Nitra, J – South Slovakian, S – Middle Slovakian, M – Small Carpathian; M1, M2 and M3 with addition of sugar from different origin; markers Ca, Mg, Rb, Sr, Ba and V)

Table 4. Correlation and distribution of wine origin markers from vineyard soils up to grapes and wines

Vine- yard	Matrix	<i>n</i>	Element content (mg/kg)													Correlation
			As	Ba	Co	Cr	Li	Rb	Sn	Sr	V	Ca	Mg			
J	soil	6	7.500	300.7	7.00	53.2	16.0	72.3	2.33	186.8	55.0	48800	15416	–		
	grape	5	0.016	0.476	0.009	0.029	0.039	1.05	0.234	1.85	0.065	272.5	66.5	0.9975		
	wine	10	0.009	0.150	0.003	0.016	0.016	0.845	0.005	0.795	0.009	100.8	100.9	0.8662		
M	soil	5	7.60	616.0	5.40	43.8	26.4	97.8	3.20	153.8	57.2	9920	4500	–		
	grape	5	0.008	0.598	0.016	0.146	0.035	0.657	0.135	1.04	0.064	198.0	75.3	0.9967		
	wine	17	0.008	0.208	0.004	0.019	0.028	0.621	0.007	0.116	0.014	97.2	87.3	0.9413		
N	soil	7	24.1	571.7	11.0	70.8	25.7	106.7	3.14	152.0	88.8	11357	6485	–		
	grape	6	0.010	0.757	0.016	0.124	0.044	1.299	0.321	1.12	0.054	233.6	77.2	0.9768		
	wine	16	0.008	0.159	0.004	0.015	0.019	1.019	0.006	0.581	0.014	145.7	106.3	0.9912		
S	soil	9	10.6	407.3	12.4	89.3	26.3	104.4	2.78	104.6	106.3	9188	7078	–		
	grape	8	0.005	0.698	0.008	0.056	0.029	0.909	0.171	0.852	0.050	125.0	81.2	0.9957		
	wine	8	0.005	0.156	0.007	0.016	0.015	1.019	0.007	0.452	0.038	110.9	104.1	0.9935		
T	soil	5	9.00	448.2	12.4	79.6	32.2	101.4	2.80	100.2	80.8	3600	5840	–		
	grape	5	0.002	0.394	0.006	0.033	0.034	1.11	0.109	1.02	0.066	300.4	59.2	0.6200		
	wine	8	0.007	0.245	0.005	0.033	0.035	0.333	0.008	0.961	0.088	171.1	100.8	0.8558		
V	soil	5	12.2	472.6	13.0	85.0	21.6	91.4	3.20	134.0	84.6	28380	7100	–		
	grape	5	0.016	0.331	0.006	0.024	0.026	1.59	0.176	0.930	0.066	273.4	58.7	0.9993		
	wine	10	0.013	0.235	0.006	0.022	0.018	0.429	0.008	0.690	0.020	108.8	82.1	0.9069		

Vineyard region: J – South Slovakian, M – Small Carpathian, N – Nitra, S – Middle Slovakian, T – Tokaj, V – East Slovakian; *n* – number of analysed samples

Table 5. Effect of sugar addition to grape juice on geographical marker composition of must and wine

Element	Content (mg/kg)				
	original must	sugar origin	sugar	must with sugar addition	wine
As	< 0.006	TR	0.018 ± 0.001	< 0.006	0.009 ± 0.000
		DS	0.023 ± 0.002	< 0.006	0.007 ± 0.002
		RS	0.024 ± 0.008	0.011 ± 0.001	0.009 ± 0.000
Ba	0.205 ± 0.002	TR	0.047 ± 0.001	0.176 ± 0.030	0.192 ± 0.012
		DS	0.052 ± 0.001	0.204 ± 0.011	0.177 ± 0.023
		RS	0.053 ± 0.002	0.168 ± 0.021	0.199 ± 0.024
Ca	121.6 ± 10.1	TR	87.0 ± 10.0	119.6 ± 13.0	90.5 ± 3.1
		DS	56.5 ± 4.0	95.1 ± 9.0	83.6 ± 5.2
		RS	53.6 ± 1.0	123.2 ± 10.0	79.6 ± 2.5
Co	0.003 ± 0.001	TR	< 0.003	0.004 ± 0.002	< 0.003
		DS	< 0.003	0.003 ± 0.001	< 0.003
		RS	< 0.003	0.003 ± 0.001	< 0.003
Cr	0.018 ± 0.003	TR	< 0.004	0.017 ± 0.002	0.021 ± 0.000
		DS	< 0.004	0.019 ± 0.001	0.023 ± 0.000
		RS	< 0.004	0.016 ± 0.001	0.022 ± 0.001
Li	0.023 ± 0.008	TR	0.013 ± 0.005	0.013 ± 0.002	0.006 ± 0.001
		DS	0.008 ± 0.002	0.013 ± 0.001	0.007 ± 0.002
		RS	0.016 ± 0.002	0.012 ± 0.001	0.012 ± 0.003
Rb	0.454 ± 0.011	TR	0.015 ± 0.003	0.445 ± 0.002	0.354 ± 0.080
		DS	0.034 ± 0.010	0.446 ± 0.003	0.393 ± 0.003
		RS	0.016 ± 0.002	0.422 ± 0.001	0.398 ± 0.006
Sr	0.217 ± 0.020	TR	< 0.080	0.173 ± 0.004	0.155 ± 0.005
		DS	< 0.080	0.146 ± 0.012	0.150 ± 0.025
		RS	< 0.080	0.168 ± 0.007	0.150 ± 0.031
Sn	0.026 ± 0.006	TR	< 0.006	0.025 ± 0.004	0.004 ± 0.001
		DS	< 0.006	0.026 ± 0.001	0.006 ± 0.001
		RS	< 0.006	0.027 ± 0.007	0.004 ± 0.001
V	< 0.006	TR	0.006 ± 0.002	< 0.006	< 0.006
		DS	0.006 ± 0.001	< 0.006	< 0.006
		RS	0.006 ± 0.002	< 0.006	< 0.006
Mg	56.2 ± 2.2	TR	5.10 ± 0.70	54.0 ± 3.3	53.1 ± 9.3
		DS	1.31 ± 0.31	50.1 ± 11.0	44.7 ± 2.1
		RS	6.41 ± 0.22	51.9 ± 1.1	46.2 ± 1.6

Sugar origin: TR – Trnava, DS – Dunajská Streda, and RS – Rimavská Sobota

dition did not change significantly the main set of wine markers utilised in the differentiation of Slovakian vineyard regions by factor analysis, Figure 4. In this figure we can see that the wines prepared with the sugar addition (M1, M2, and M3) were not significantly different from those from the original Small Carpathian region, and did not overlay with those coming from other Slovakian vineyard regions.

CONCLUSIONS

The study of the effect of the wine-making process on the distribution of the selected markers of wine origin in winemaking products revealed a very significant correlation between the geographical diagnostic markers of soils and the winemaking products. The sugar addition to grape juice partially changed the total elemental composition of wines but did not lead to substantial changes of wine geographical authentication results. The results obtained indicate minimal changes of the determining markers in the wine-making process, and due to the insignificant changes caused by the sugar addition they can be useful for many other authentication procedures with other European regional wines, where similar elemental markers of the wine origin are used.

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