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ORIGINAL PAPER

ELEMENTAL ANALYSIS AS A TOOL FOR CLASSIFICATION OF CZECH WHITE WINES WITH RESPECT TO GRAPEVINE VARIETIES*

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Abstract

The share of adulterated wines on the global market is rising and this trend is also visible in the Czech Republic. The control authorities are confronted with an increasing number of cases of adulterated wine. A characteristic feature of grapevine cultivation in the Czech Republic is a diverse spectrum of the cultivars. The verification of wine's varietal authenticity, next to the confirmation of geographic origin, is the toughest challenge for analytical chemists and control laboratories. The aim of this study was to assess possibilities of the discrimination and classification of Moravian varietal wines based on the elemental composition data. An important objective was to find the variables in elemental composition which are strongly associated with a particular variety. Tests were performed on three popular varieties (Rhine Riesling, Müller-Thurgau and Green Veltliner). Analysis of wine samples was carried out by the combination of ICP-MS and ICP-OES methods. Experimental data were evaluated by univariate and multivariate statistical methods, such as analysis of variance, principal component analysis and discriminant analysis. Statistically significant discriminant fuctions and predictive functions were constructed by the method of canonical discriminant analysis. These fuctions were based on elemental composition parameters: Al, Sn, Gd, Tb, Tm/Yb, Yb/Lu, Mo/Sn, Mn/Cr. The model thus created was capable of classifying known varietal wines at a succes rate of 95.83%. The predictive capability of the model was finally tested by the cross validation method. Classification effectiveness for unknown samples was determined at 70.83%. The results prove that the approach to varietal wine authentification presented in this paper is a promissing option in interregional varietal wine discrimination.

Keywords: wine, elemental analysis, ICP-MS, ICP-OES, ANOVA, PCA, CDA.

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INTRODUCTION

Wine is globally one of the most widespread food commodities. Wines from renowned regions gain a high commercial value with time, which can lead to such problems as wine adulteration. The share of adulterated wines on the global market is rising and this trend is also observable in the Czech Republic. Control institutions are increasingly confronted with improper or falsely labelled products, which are sold as original ones in order to generate higher profits. Experts estimate the global proportion of falsified wines to be approximately 5% (Luykx et al. 2008). The European Union protects the authenticity of regional food in the regulations 2081/82 and 2082/92 (amended in 2006 – 510/2006 and 509/2006) on protection of indications of geographical origin and control of designations of agricultural products and food. These legal measures have encouraged researchers to try and find appropriate methodology for the determination of wine authenticity.

The essence of viticulture and winemaking in the Czech Republic is the production of varietal wines. All comercially grown cultivars in the Czech Republic are tested and registered in the State Varietal Book. Until 2016, 58 wine varieties had been registered in this book. Adulteration of varietal wines is relatively common. Varietal authenticity can be evaluated by determination of the chemical composition of wines with univariate and multivariate data processing. Most of the methods are based on the determination of organic acids, amino acids (Hérnandez-Orte et al. 2002, Pavloušek, Kumšta 2013), phenolic (Brossaud et al. 1999, Gonzáles-Neves et. al. 2004, Makris et al. 2006, Von Baer et al. 2008, Kumšta et. al. 2014) and volatile compounds (Mateo, Jiménez 2000, Nasi et al. 2008), DNA profiling (Baleiras-Couto, Eiras-Diras 2006) and also determination of the elemental composition of wine (Almeida et al. 2003, Greenough et al. 2005, Charlton et al. 2010). Analysis of wine alone is only one part of the work. Selection of a suitable data analysis method is no less important. For the purpose of wine varietal authentification, data exploratory, discriminatory and classification techniques like principal component analysis (PCA), linear discrimination analysis (LDA), hierarchical cluster analysis, soft independent modeling of class analogy (SIMCA) and others are commonly used as basic chemometric tools. These methods yield graphical and numerical representation of discrimination, mostly in the form of discriminatory functions, which can be further used for classification of uknown samples of wine (Gonzálvez, Guardia 2013).

The core of wine varietal discrimination and classification with the use of elemental profiling consists of the knowledge of physiological and metabolical differences between the cultivars. The growth and development of plant roots are mainly determined by the genetic make-up of plants. Differences in root systems of *Vitis vinifera* L. are reflected in different uptake of minerals from soil. The root system of *Vitis vinifera* L. is composed of old and new roots which renew quite frequently. Old woody roots are strongly rooted in

the ground, which brings water and nutrients from deeper parts of soil. Evolutionarily older and autochtonous cultivars of *Vitis vinifera* L. usually have more extensive roots in comparison with newly bred cultivars. New and old cultivars also vary in the ratio of woody and young roots (Siddique 1990). Another difference can be observed in the grape maturation rate, oxygen defficiency sensitivity, waterlogging tolerance and, last but not least, in the accessibility to mycorrhizal symbiosis. This cooperation of root cells with fungal microorganisms has a significant impact on the rate of uptake of inorganic nutrients, esspecially P, Ni, S, Mn, B, Fe, Zn, Cu, Ca and K. It also protects againts the toxic effects of metals like Pb and Cd. Another positive impact of this symbiosis can be observed in dry seasons. Fungal microorganisms produce chelating agents, which increase the bioavalability of inorganic nutrients in dry soil conditions. Beside roots, various cultivars also differ in plant morphology, e.g. height, leaf area, fruit size or density of foliage. All these factors may potentially affect grape must elemental profile and create a characteristic varietal fingerprint of specific *Vitis vinifera* L. cultivars. Mainly mass spectrometry and optical emmision spectrometry with inductivelly coupled plasma (ICP-MS and ICP-OES) were used for the purpose of elemental analysis of wine in previously published studies (Greenough et al. 2005, Kment et al. 2005, Martin et al. 2012).

The aim of this study was to assess possibilities of the discrimination and classification of Moravian varietal wines based on elemental composition data. An important objective was to find the variables in elemental composition which are strongly associated with a particular variety. These variables must not be significantly influenced by other factors, like geographical provenance, environmental influences and effects of wine ageing. Tests were performed on three popular varieties: Rhine Riesling, Müller-Thurgau and Green Veltliner. Analysis of wine samples was carried out by the combination of ICP-MS and ICP-OES methods. Experimental data were evaluated by univariate and multivariate statistical methods, such as analysis of variance, principal component analysis and discriminant analysis.

MATERIAL AND METHODS

Wine samples and preparation

A total of 24 white Moravian wines (Moravia is one of the two wine regions in the Czech Republic) of 3 varieties (Green Veltiner – GV, Rhine Riesling – RR and Müller-Thurgau – MT) were gathered for the purpose of constructing a classification model. Samples were selected with an effort to evenly cover all Moravian wine subregions in order to eliminate influences of geographical origin. To suppress the climatic impact and effects of wine ageing, the sample set contained wines from three different vintages (2011- 2013).

All samples were dilluted with deionized water in a 1:1 ratio (ELGA,

UK) to decrease the concentration of ethanol, which can cause instabillity of inductivelly coupled plasma tests (Jakubowski et al. 1999, Coetzee et al. 2005). Immediately before analyses, all samples were passed through quantitative filtres with the pore diameter of 0.45 μm.

Instrumental methods

For the purpose of the elemental analysis of wine, two different methods (ICP-MS and ICP-OES) were developed and validated. Most of the elemental parameters, except macro-elements (Mg, Ca, K, Na) and elements which were affected by spectral interferences, were determined by ICP-MS Thermo X-series (Thermo Fisher Scientific, USA) with a hexapole collision cell technology (CCT) working with He/H gas. Adverse changes in the signal during the measurement and matrix effects were corrected using internal standards (^{45}Sc, ^{115}In, ^{232}Th), which were added into the system through a mixing device. Other elements were analysed on an ICP-OES Horiba Ultima 2 (Horiba Scientific, France). Instrumental settings are summarised in Table 1. A total

Table 1

ICP-MS and ICP-OES settings

ICP-MS	Parameter	Value	ICP-OES	Parameter	Value
	RF power	1400 W		RF power	1300 W
	Gas	argon		Gas	argon
	Plasma gas	14 L min^{-1}		Plasma gas	13 L min^{-1}
	Auxiliary Gas	0.6 L min^{-1}		Auxiliary Gas	0.1 L min^{-1}
	Nebuliser Gas	0.8 L min^{-1}		Nebuliser Gas	0.85 L min^{-1}
	Plasma view	axial		Plasma view	radial
	Nebuliser	Meinhard		Nebuliser	Meinhard
	CCT gas	He/H		Nebuliser pressure	0.3 MPa
	CCT gas flow	6.5 mL.min^{-1}			

RF power – power of radiofrequency generator, CCT – collision cell technology

of 40 elements were analysed: ^{7}Li, ^{9}Be, ^{111}Cd, ^{118}Sn, ^{121}Sb, ^{137}Ba, ^{139}La, ^{140}Ce, ^{141}Pr, ^{146}Nd, ^{147}Sm, ^{153}Eu, ^{157}Gd, ^{159}Tb, ^{163}Dy, ^{165}Ho, ^{166}Er, ^{169}Tm, ^{172}Yb, ^{175}Lu, ^{208}Pb, ^{209}Bi, ^{51}V, ^{52}Cr, ^{59}Co, ^{60}Ni, ^{65}Cu, ^{75}As, ^{89}Y, ^{95}Mo using ICP-MS and Al (396.152 nm), Fe (259.940 nm), Mg (285.213 nm), Mn (257.61 nm), Sr (421.552 nm), Zn (206.191 nm), Ca (422.673 nm), K (766.490 nm), Na (588.995 nm) using ICP-OES. In addition to the basic input data, elemental ratios were created to expand the dataset. Both instruments were calibrated by the standard addition method using 1 g L^{-1} standards (Analytika Praha, Czech republic), diluted as needed. Recoveries obtained for a spiked wine sample analysed in the same way as the original samples ranged between 93 and 105%.

Statistical analysis

Data analysis and statistical evalutation were performed in Microsoft Excel (Microsoft, USA), Statistica (Statsoft, USA), Unistat (Unistat, UK), XLstat (Addinsoft, France) and IBM SPSS (IBM, USA). Results were sorted out and processed by various statistical approaches. Each wine was represented by 66 parameters. All samples were analysed as duplicates and the ICP-OES and MS methods were set for three scans for every sample. Before the main data analysis, results were tested for outliners and data distribution. The Grubbs test for outliners did not reveal any outlined values within the 3 tested groups of varietal wines and data showed a normal Gaussian distribution.

Analysis of variance (ANOVA) was used for pretreatment of the data to find variables which exhibited statistically significant differences between the varietal groups. Based on the fact that differences in the elemental composition between varietal wines are quite small, and due to the biological nature of the samples, ANOVA was set at a 90% confidence interval. The aim of this approach was to enlarge the dataset by adding the variables which were close to the border of a standard 95% interval.

The number of selected variables was further reduced by PCA into a smaller number of principal components. The importance of original variables for the newly calculated principal components was described as factor loadings. A loading can range from -1 to 1. Numbers close to 0 indicate weak infuence of a variable. This method serves as a characterisation mechanism to find specific links between observed (wine samples) and original variables (elemental composition). The main goal of this analysis was to find similarities and dissimilarities between different varietal wines and to obtain a potential wine grouping according to a corresponding cultivar. This was achieved by projecting the observations onto a 2D plane of the created principal components.

For the purpose of discriminating wines into the corresponding groups, the canonical discriminant analysis method (CDA) was selected. It was used to build a predictive model for wine grouping, based on discriminant fuctions calculated by linear combinations of variables. The discriminant analysis was focused on the maximal separation of wine groups. This method was also used for discarding variables which are not significantly related to group differentiation. Variables were gradually added to the model on the basis of the forward stepwise selection until the point of the highest accuracy was reached. Input parameters were refined by a unidimensional test of equality of the means of the classes. The addition of variables into the model was approved by the F value (confidence interval 90%) and Wilk's lambda criterium, which evaluates the importance of a variable for the accuracy of classification (the smaller a lambda value, the more important a given independent variable is for discrimination). Equality of the vectors was tested by the Rao`s approximation set at a 95% confidence interval. Discriminant functions were described by calculations of the standardised coefficient, factor structure coefficients and canonical correlation coefficient.

RESULTS AND DISCUSSION

Analysis of variance (ANOVA)

Innitially, it was important to determine variables which were specific for selected varietal wines. Analysis of variance (confidence interval 90%) was used for the determination of statistically important variables, which would show differences in mean concentrations between groups of varietal wines. On the basis of ANOVA, 3 statistically significant variables at $P < 0.05$ and 5 variables on $P < 0.1$ were determined (Table 2). These variables (concentrations and ratios) are graphically presented in the form of box plots (Figure 1). The P and F values are shown in Table 2. The tested varietal wine groups differed mainly in the mean concentrations of rare earth elements (REE) and their ratios. Besides, differences were observable on amounts of Al and Sn, and in ratios of Mo/Sn and Mn/Cr.

Table 2

Variables with statistically significant differences between groups of varietal wines – selected by ANOVA

Variable	F	P
Tm/Yb	5.067	0.016
Al	4.363	0.026
Yb/Lu	4.333	0.027
Gd	3.326	0.056
Mo/Sn	3.241	0.059
Sn	3.126	0.065
Mn/Cr	3.005	0.071
Tb	2.603	0.098

The most significant univarietal difference in the elemental composition between cultivars within non-REE elements was found in the concentration of Al ($P = 0.026$ and $F = 4.363$). Plants have developed protective mechanisms from the toxic effects of Al^{3+}. Sensitivity of plants to adverse effects of aluminium differs between plant cultivars. These differences were described by FOY et al. (1992). The protective mechanism is based on releasing organic acids by roots, which causes immobilisation of aluminium by the complexation reaction. This effect was also studied by DELHAIZE (1993), who discovered that organic acid release is mediated by the activation of specific plasmatic membrane channels of root cells in case of their contact with aluminium ions. Minor differences between the cultivars in terms of root structure and genetic make-up of root cells are a probable reason of the Al mean concentration variations.

Other differences between varietal wines were found in the mean concentrations of transition metals Mo, Mn and Cr at $P < 0.1$. All these metals

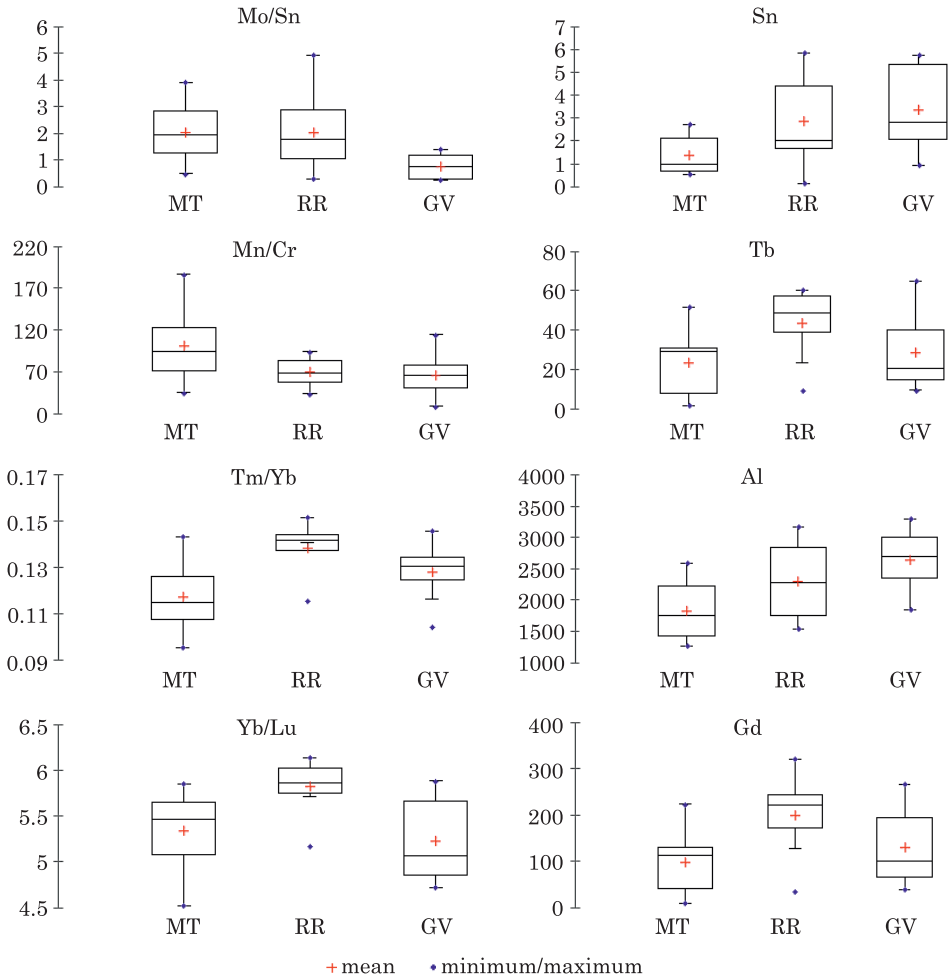

Fig. 1. Box plot of variables selected by ANOVA. Values are presented in μg dm^{-3}, ratios are undimensional. The bottom and top of the box is 25th and 75th percentile. The horizontal line inside the box is the median

have a strong tendency towards forming complexes with plant and microbial ligands, which enhances their mobility from soil into plant. These three metals are usually found in soils together. Especially Mn and Cr are often bound together in one type of soil. Mo, Mn and Cr are highly significant in plant redox reactions by being part of metalloproteins. It can be expected that the way these elements are absorbed by plants is influenced by the activity of the root system, which differs between *Vitis vinifera* cultivars.

In the group of rare earth elements, statistically significant variables proved to be the ratios Tm/Yb, Yb/Lu with $P < 0.05$ and elements Gd and Tb with $P < 0.1$. The REEs in soils are usually bound together, mostly in the form of phosphates, fluorides, silicates and carbonates. These elements occur

in various depths of the litosphere (ALEX et al. 1998). It was confirmed that REEs can have both positive and negative effects on the viability of plants. For example, FASHUI et al. (2002) found that most of the REEs can enter chlorophyll, where they can substitute Mg^{2+} and create REE-chlorophyll. This substitution was observed in spinach and it was reflected in a higher growth activity in comparison with the control. As for the negative effect, it has been found that Gd and other REEs probably block channels for calcium release in the endoplasmatic reticulum (JOHANNES et al. 1992, SCHWENKE,WAGNER 1992, KLÜSENER et al. 1995). These are just a few examples of the effects of REEs on plants. Similarly to the aforementioned significant elements, the transport of REEs through the grapevine is mediated by the structure and activity of the roots. REEs are distributed in plants in decreasing concentrations from roots to fruit, so the final concentration in wine is on an ultratrace level. Howewer, these small nuances seem to be enough for the discrimination of wine according to varieties.

Principal Component Analysis (PCA) and Canonical Discriminant Analysis (CDA)

After pre-sorting of the data by ANOVA, discrimination itself was performed by the PCA and CDA methods. Dimension of the 8 input variables was reduced to 3 principal components with an eigenvalue > 1 (Figure 2). According to the Kaiser's criterion, components with eigenvalue less than one were excluded (F4, F5, F6, F7, F8). Selected principal components F1 (40.01%), F2 (25.65%) and F3 (15.51%) together carried 81.17% of the variability of the original data set (variance distribution of components is presented in Figure 2). Principal components were more or less positively and negatively correlated with the original variables (Figure 3 and Table 3). Component F1 was positi-

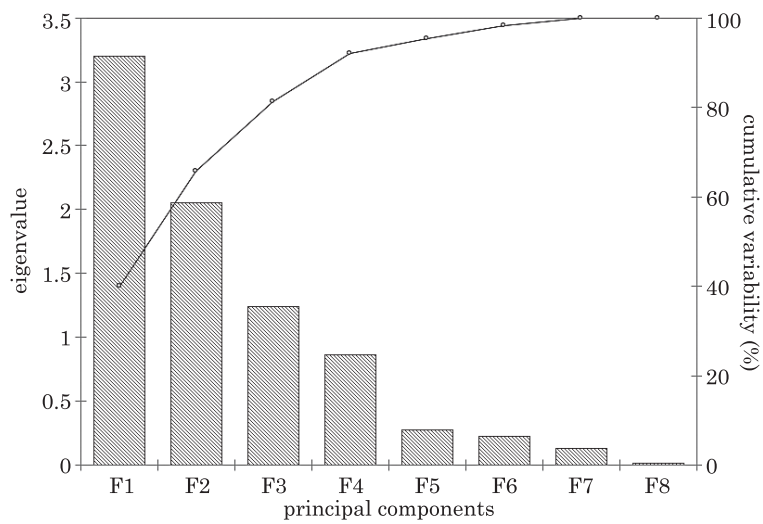

Fig. 2. Graphical summary of the variability of principal components F1 – F8

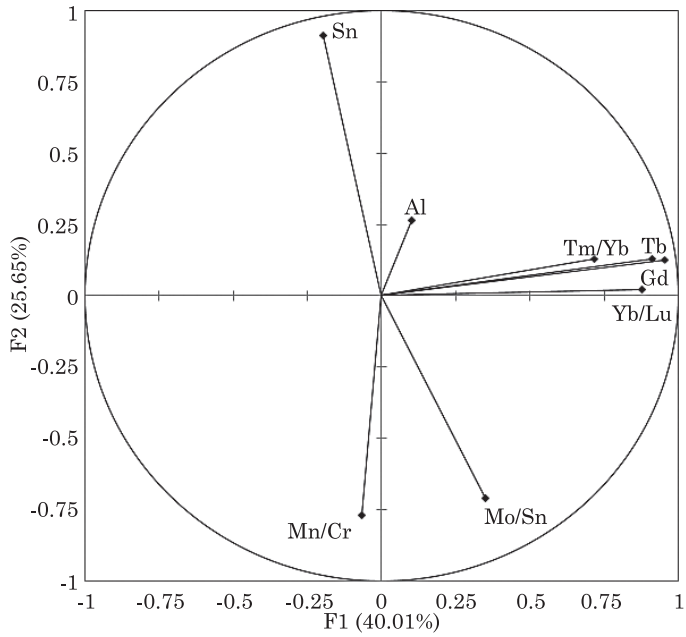

Fig. 3. Projection of variables into the PCA factor plane of principal components F1 and F2 (correlations between variables and factors)

Table 3

Factor loadings

Elements	F1	F2	F3	F4	F5	F6	F7	F8
Sn	-0.195	0.913	-0.151	0.133	0.115	0.151	0.227	0.003
Gd	0.954	0.126	-0.039	-0.211	-0.093	-0.093	0.064	0.084
Al	0.100	0.266	0.788	0.511	-0.108	-0.156	0.033	-0.002
Mn/Cr	-0.067	-0.769	-0.447	0.382	-0.027	-0.143	0.194	-0.003
Mo/Sn	0.350	-0.712	0.514	-0.056	0.070	0.298	0.102	0.006
Tm/Yb	0.717	0.128	-0.361	0.492	-0.196	0.215	-0.113	-0.005
Yb/Lu	0.879	0.020	-0.042	0.197	0.419	-0.084	-0.061	-0.012
Tb	0.911	0.130	0.016	-0.335	-0.145	-0.065	0.102	-0.074

F1 - F8 – are principal components

vely correlated with REE and their ratios. Component F2 negatively correlates with the elemental ratios Mn/Cr, Mo/Sn and strongly positively – with Sn. Another strong correlation was found between component F3 and parameter Al. Variables also correlated between each other. This is represented as a correlation matrix (Table 4). Significant intervariable connections can be

Table 4

Pearson correlation matrix of the variables (PCA)

Elements	Sn	Gd	Al	Mn/Cr	Mo/Sn	Tm/Yb	Yb/Lu	Tb
Sn	1*	-0.058*	0.103*	-0.403*	-0.596*	0.006	-0.100*	-0.023*
Gd	-0.058*	1*	0.059*	-0.206*	0.216*	0.633*	0.757*	0.972*
Al	0.103*	0.059*	1*	-0.362*	0.236*	0.060*	0.001	-0.031*
Mn/Cr	-0.403*	-0.206*	-0.362*	1*	0.135*	0.101*	0.071*	-0.227*
Mo/Sn	-0.596*	0.216*	0.236*	0.135*	1*	0.027*	0.181*	0.220*
Tm/Yb	0.006	0.633*	0.060*	0.101*	0.027*	1*	0.607*	0.540*
Yb/Lu	-0.100*	0.757*	0.001	0.071*	0.181*	0.607*	1*	0.699*
Tb	-0.023*	0.972*	-0.031*	-0.227*	0.220*	0.540*	0.699*	1*

* Values different from 0 at the significance level 95%

observed, as expected, in the REE group. These elements have very similiar chemical and physical properties. Other strong intervariable linear relationships were not evident.

Best possible graphical characterisation of varietal wines was obtained by the dispersion of observations onto a 2D factor plane of principal components F1 and F2 (Figure 4). These two principal components describe 65.66 % of the original data variability. A 3D graphical representation including principal component F3 (15.51% of variability) was not used due to the complicated and confusing representation of the data in the plot. PCA scores of each wine were dispersed into a factor plot of principal components 1 and 2 (Figure 4). It could be observed that samples divided into 3 clusters. With a few exceptions, wines were grouped together according to varieties. The PCA scores of GV wines were projected with a positive score of F2 component. RR wines were projected in the second and fourth quadrant of the PCA plot. Finally, MT wines' projection can be observed in the third quadrant, in the zone of the negative score of F1 and F2.

Specific freatures of the elemental composition of each wine variety were evaluated by the combination of observations and projection plots of variables (Figures 3, 4). The RR wines have positive scores for F1 component, which is strongly correlated with REEs. This implies that this variety is characterised by higher average concentrations of REEs compared to MT and GV. The GV wines are distinguished by a higher average concentration of Sn while the MT winde have a higher average Mn/Cr ratio. Both MT and GV varieties are characterised by a relatively lower content of REE. Further analysis of the PCA wine projection showed that some of the wine samples were not correctly classified into a corresponding group. Two RR and one MT sample were placed into the group of GV wines and also one MT sample was incorrectly classified into the group of RR varietal wines. This was probably caused by relative similarities in the elemental composition of the tested cultivars and also by

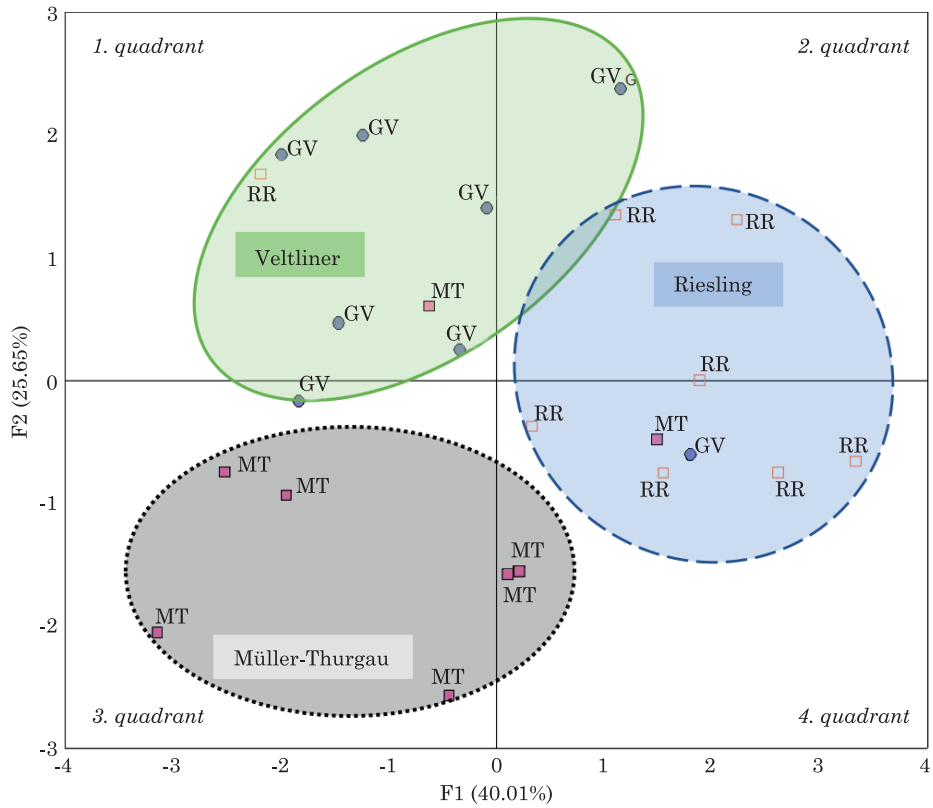

Fig. 4. Projection of the PCA score of varietal wine into a 2-D factor plane of principal components F1 and F2
■ MT – Müller Thurgau; □ RR – Rhine Riesling; ● GV – Green Veltliner

random errors in the analysis. Explanation of this result may be based on genetical connections between RR and MT cultivars. The cultivar Riesling was bred by crossbreeding Müller-Thurgau and Madeleine roayale cultivars. The effectiveness of PCA differentiation of samples into the groups of varietal wines, despite some inaccuracies, is still realatively high (83.3%).

The original dataset was used to assemble the canonical discriminant fuctions and classification functions. These fuctions were applicable for mathematical description of the characteristics of different wine varieties and for the identification of a variety of known and unknown samples. These functions were constructed by the method of canonical discriminant analysis. Two discriminant fuctions, for differentiation of wines on the basis of elemental composition, were calculated. The Rao's approximation set at a 95% significance level proved that at least one of the vectors of the means was different from another, which confirmed statistical significance of the fuctions (Table 5). Discriminant fuctions and variable contributions are presented in Table 6. Graphical visualisation of the variable - function correlations

Table 5

Rao's approximation and detail characteristics of the discriminant fuctions

Function	Eigenvalue	Per cent variability	Cumulative variability	Correlation coefficient
1	3.957	75.69%	75.69%	0.894
2	1.271	24.31%	100.0%	0.748
	Wilks' Lambda	Chi-Square	degrees of freedom	P
1	0.089	42.36	16	0.0003
2	0.440	14.35	7	0.045

95% significance level

Table 6

Discriminant fuctions presented as raw and standardised coefficients for canonical variables

Elements	Function 1		Function 2	
	raw	standardized	raw	standardized
Constant	-0.0001		-13.49	
Sn	0.104	-0.174	0.421	0.704
Gd	0.011	-0.917	0.005	0.448
Al	-0.003	1.527	-0.001	-0.446
Mn/Cr	0.0001	-0.001	-0.021	-0.664
Mo/Sn	1.253	-1.458	0.794	0.924
Tm/Yb	-51.87	0.691	52.90	0.705
Yb/Lu	2.201	-0.953	1.596	0.691
Tb	-0.094	1.742	-0.051	-0.941

is presented in Figure 5. A detailed description and characteristics of the fuctions can be found in Table 5. Standardised coefficients and a structure matrix are presented in Table 7. Beside the discriminant fuctions, another important output of this analysis is the predictive classification functions (Table 8). These fuctions can be used for classification of wine samples of uknown variety.

Discrimination of individual wine samples is graphically presented in a scatter plot (Figure 5), where almost complete discrimination of the varieties can be observed. All three clusters shows sufficient resolution of the discrimination with centroid distances > 3 (Table 9). The greatest distance between wine variety centroids is in the case of GV and MT. The nearest centroids of varietal groups are RR and MT. As mentioned above, those two cultivars are genetically connected, which can explain these relatively small distances. On the basis of discriminant fuctions, it was possible to divide the wines of known varieties into correct groups at a succes rate of 95.83% (Table 10).

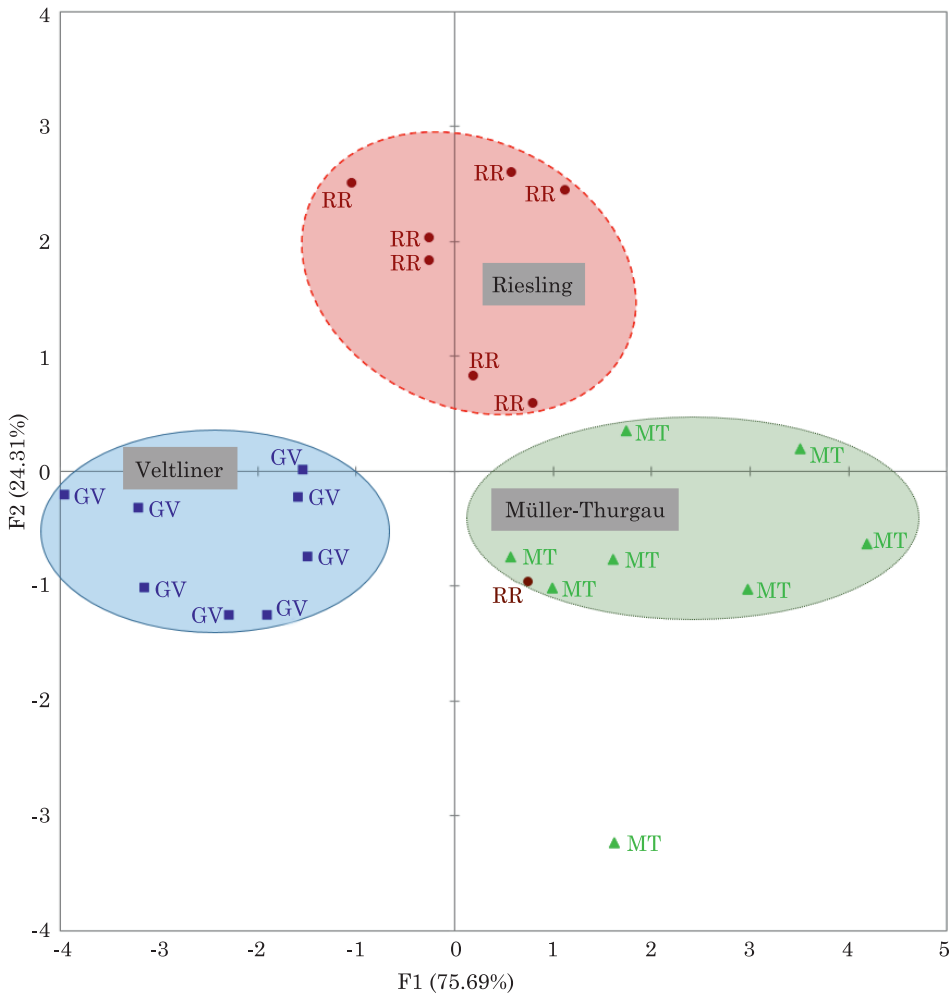

Fig. 5. Canonical discriminant analysis score plot. F1 and F2 are canonical discriminant fuctions ▲ MT – Müller Thurgau), ● RR (Rhine Riesling, ■ GV – Green Veltliner

To verify the functionality and robustness of the classification model, it was necessary to perform cross-validation test. This test was carried out by the exclusion of one specific observation from the model which acted as a unknown sample. The predictive ability of the model was then tested by the newly constructed model. Every wine sample was removed one by one and tested. The succes rate of correctly classified samples was 70.83% (Table 11). This poorer predictive capability, in comparison with the discrimination model (which was almost 100%), is due to several factors, for example an excluded sample is not classified using the discriminator which described the variety. Another factor is connected with cross-validation methodology itself. In this case, when a model is based on 24 (3x8) samples, by exlusion of one

Table 7

Structure matrix of discriminant functions

Elements	Structure matrix	
	function 1	function 2
Sn	0.257	0.170
Gd	0.063	0.487
Al	0.319	0.099
Mn/Cr	-0.235	-0.232
Mo/Sn	-0.254	0.203
Tm/Yb	0.147	0.558
Yb/Lu	-0.081	0.552
Tb	0.037	0.437

Table 8

Predictive classification functions of varietal wines

Variable	MT	RR	GV
Sn	1.964	2.75	1.589
Gd	-0.718	-0.727	-0.768
Al	0.001	0.004	0.013
Mn/Cr	-0.219	-0.268	-0.224
Mo/Sn	7.160	6.622	1.654
Tm/Yb	1047	1270	1295
Yb/Lu	62.91	62.44	53.29
Tb	1.778	1.839	2.193
Constant	-214.6	-244.6	-218.1

MT – Müller Thurgau, RR – Rhine Riesling, GV – Green Veltliner

Table 9

Distance between centroids of wine cultivar clusters on the plane of discriminant fuctions F1 and F2

Centroid	Centroid distances
RR - MT	3.026
GV - RR	3.369
GV - MT	4.545

RR - MT – distance between Rhine Riesling and Müller Thurgau cluster centroids, GV - RR – distance between Green Veltliner and Rhine Riesling cluster centroids, GV - MT – distance between Green Veltliner and Müller Thurgau cluster centroids

Table 10

Classification matrix of known samples of varietal wines – discrimination efficiency

Variety	MT	RR	GV	Total	Correct (%)
MT	8	0	0	8	100.0
RR	1	7	0	8	87.50
VZ	0	0	8	8	100.0
Total in group	9	7	8	24	95.83

MT – Müller Thurgau, RR – Rhine Riesling, GV – Green Veltliner

Table 11

Matrix of cross-validation – predictive efficiency

Variety	MT	RR	GV	Total	Correct %
MT	5	3	0	8	62.50
RR	2	6	0	8	75.00
GV	0	2	6	8	75.00
Total in group	7	11	6	24	70.83

of the observations, the mathematical model loses an important amount of source data (12.5% from the varietal group), which can deteriorate an overal integrity. A solution to this problem might be some expansion of the wine samples database.

Direct comparison of the results obtained in the present study with references was not possible because of the original nature of this work. No articles dealing with the use of non-combined elemental analysis as a tool for varietal authenticity had been published before. However, it is possible to make a comparison with studies using other analytical methods. In most cases, the discrimination is based on data obtained by analysis of organic compounds in wine by HPLC, GC or NMR (Makris et al. 2006, Nasi et al. 2008, Godelmann et al. 2013). Geana et al. (2014) used the elemental composition of wines combined with their phenolics profile for discrimination of 22 Drasagani wines from Romania. PCA analysis was based on variables Cs, Na, Zn, Ni, U, Ba, (+)-catechin, ferulic acid and resveratrol. It was possible to differentiate a Drasagani wine variety from other varieties grown in the Dragasani vineyard at a 100% success rate. Another closely related study, which employed elemental parameters (Ca, Li, Fe and Si) combined with organic parameters (shikimic acid and ethanolamine), was conducted by Charlton et al. (2010). Their aim was to classify Czech, Hungarian, Romanian and South African wines according to varieties and vintages. Data evaluation was performed by Classification and Regression Tree (CART) and Discrimination Partial Least Squares (DPLS). The accuracy of the classification determined by CART was 65% and almost 100% by DPLS. This relati-

vely high disparity in the classification accuracy shows that selection of a suitable statistical method is equally important as precision of laboratory determinations. A different approach was chosen by KUMŠTA et al. (2014). Wine anthocyanin fingerpriting was used for discrimination of Blaufrankish, Blauer Portugieser and Saint Laurent varieties of red wine. In the work of KUMŠTA et al, the dataset was composed from results of analyses of 17 wines. Principal Component Analysis was used in that case as the preselection of variables for Canonical Discriminant Analysis. Combination of PCA with CDA resulted in 100% differentiation of 17 wines of three cultivars. LAMPÍŘ (2013) has successfully used data from RP- HPLC analysis of phenolic substances in 27 Czech varietal wines for differentiation of 7 cultivars. Data were analysed by Canonical Variate Analysis (CVA) and achieved a 100% success rate of discrimination. Beside elemental analysis and chromatography, a combination of mid-infrared spectroscopy (MIR) and UV-VIS spectroscopy of phenolic wine extracts was used by EDELMANN et al. (2001) for discrimination of red wine cultivars. To remove spectral interferences from nonspecific carbohydrates and organic acids, solid phase extraction was performed. Data analysis was realised by Hierarchical Cluster Analysis and soft Independent Modeling of Class Analogy (SIMCA). The discrimination rate of 97% was achieved for 4 red cultivars with data from MIR spectroscopy. Evaluation of spectra from UV-vis spectroscopy resulted in discrimination of just 2 from the 4 tested cultivars. Compared to previously published articles, it is obvious that the system presented here ensures very similar efficiency of discrimination of varietal wines from a specific wine-growing area. It cannot be expected that this approach will be universal for varietal wines from geographically different areas due to the influence of the climate and soil characteristics. However, specific regional models for differentiation of the most popular varietis can be constructed.

CONCLUSIONS

The basic premise underlying discrimination of varietal wines with the help of elemental analysis arose from the physiological and metabolical differences between *Vitis vinifera L.* cultivars. Some of the differences were expected in the way of intake of inorganic and organic substances by the roots. This scientific hypothesis was confirmed by the elemental analysis of three tested wine varieties: Green Veltliner, Rhine Riesling and Müller -Thurgau from Moravian region in Czech Republic. Differences in elemental composition within these three varieties were determined by ANOVA. Statistically significant parameters were Tm/Yb, Al, Yb/Lu at $P < 0.05$ and Sn, Gd, Tb, Mo/Sn, Mn/Cr at $P<0.1$. These variables were further used for classification and discrimination by PCA and CDA. Principal component analysis distributed the observations into three groups according to variety with sa-

tisfactory success rate. Finally, discrimination of the wines by CDA was performed. The use of classification functions resulted in the distribution of wines into varietal groups with succes rate of 95.83%. The predictive capability of the model was finally tested by cross- validation. The test results were influenced by the smaller number of samples used to calculate the discrimination functions. The predictive success for uknown samples, determind by cross-validation, was 70.83%.

The literature review suggests that chromatographic or combined techniques focus on determination of specific organic compounds. These methods can demonstrate smaller differences between similar cultivars. They also ensure an acceptable degree of discrimination with a relatively small numbers of tested wines. It can be expected that simple elemental-based methodology can be more susceptible to the incomplete distribution of crossbred wine varieties. From the results reported herein it is obvious that the effectiveness of authenticity testing achieved by the methodology presented in this study (95.83%) is quite comparable the results found in previously published articles. The main advantage of our approach is the relative simplicity of analyses when a robust and correctly validated method for elemental analysis of wine is prepared. The suggested solution is also economical and fast. A suitable area where simple elemental analysis with ANOVA – CDA can be applied to rapid discrimination of wines is when wines are made from grapes grown on cultivars with significantly genetically different root systems, which is the main differentiation factor. The robustness of the discrimination methodology can be further increased by the expansion of the number of wine samples. Overall, the wine varietal authentification approach presented in this paper has proven to be a promising option for interregional varietal wine discrimination.

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