

Rapid Determination of Trace Elements in Macedonian Grape Brandies for Their Characterization and Safety Evaluation

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Abstract A graphite furnace atomic absorption spectrometry (GFAAS) was used for determination of trace elements (Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) in “rakija” samples, a grape brandy traditionally produced in Republic of Macedonia by distillation of grape pomace or wine, for characterization and safety evaluation. Cd, Pb, Cr, and Ni were determined directly, while Cu, Fe, Mn, and Zn were quantified after appropriate dilution. The calibration curves of all elements were linear with correlation coefficients (R^2) ranging from 0.9995 to 0.9998. The accuracy of the method was checked with a standard addition method showing good repeatability and reproducibility (relative standard deviation (RSD) <10 %). Relationship between several metal concentrations (Cu, Fe, Mn, Zn) in brandies and distillation system (homemade/industrial) on one side and aging mode (oak barrels/oak chips) on the other side were demonstrated. Two homemade brandies showed Cu, Fe, and Zn concentrations higher than industrial distillates and thus were found to be not safe for consumption because of Cu and Zn over the maximum allowed values. For the industrially produced brandies, Mn was identified to be a suitable marker related to aging with oak chips regardless variety, while Cu a marker for the influence of oak chip type.

Principal component analysis applied on the content of elements clearly showed a good separation in terms of distillation and aging method.

Keywords Rakija (grape brandy) · Aging · Oak · Trace elemental analysis · GFAAS

Introduction

Trace element concentration in distillates produced around the world (brandy, cognac, rum, and whisky) is a significant parameter with a positive or negative effect on the quality of the final product (Green et al. 1997; Ibanez et al. 2008; Rodriguez et al. 2010; Ivanova-Petropulos et al. 2013; Rodríguez-Solana et al. 2014). From nutritional and toxicological points of view, elements are classified into essential and non-essential. Elements such as Ca, Cr, Co, K, Mg, Mn, Na, Se, and Zn are considered as essential elements for the human organism, while As, Cd, and Pb are harmful elements because they are not chemically or biologically degradable (Ivanova-Petropulos et al. 2013; Rodríguez-Solana et al. 2014).

The sources of metals in alcoholic beverages are multiple including raw materials, vessels used during fermentation, distillation equipment, added substances during brewing, bottling process, aging/storage, and adulteration (Rodriguez et al. 2010; Rodríguez-Solana et al. 2014; Ivanova-Petropulos et al. 2015). Therefore, information about the content of metals in brandies is of great importance for producers and consumers for their health protection against toxic elements, as well as for the government authorities in order to guarantee the quality of the own traditional product.

Sensitive techniques, such as flame atomic absorption spectrometry (FAAS), graphite furnace atomic absorption spectrometry (GFAAS), atomic fluorescence spectrometry

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(AFS), inductively coupled plasma–optical emission spectrometry (ICP-OES), inductively coupled plasma–mass spectrometry (ICP-MS), and potentiometric and voltammetric methods, are required for accurate determination of metals in various samples (Mena et al. 1997; Onianwa et al. 1999; Karadjova et al. 2002; Ibanez et al. 2008; Rodriguez et al. 2010; Ivanova-Petropulos et al. 2013; Ly et al. 2013; Froes-Silva et al. 2015; Seeger et al. 2015). Analysis is performed directly on undiluted samples, after a water dilution, or after digestion in open/closed vessel systems with HNO₃, HCl, H₂O₂, or their mixtures. Obviously, direct analysis is preferred due to the risk of contamination/losses of analytes during mineralization. Reviews on sources, effects, concentrations, and determination methods of metals and the need of their monitoring in alcoholic beverages were published by Ibanez et al. (2008) and Szymczycha-Madeja et al. (2015).

GFAAS is a common technique for quantitative analysis of metals in various samples, including brandies too (Cameán et al. 2001; Bonić et al. 2013). This technique allows direct determination of a very small volumes or masses of samples, with high sensitivity and high S/N ratios, primarily because there are no flame gases to dilute the atoms that are analyzed. GFAAS is not suitable for fast multielement analysis but offers high sensitivity and selectivity for determination of very low levels of metals.

“Rakija” (grape brandy) is a traditional alcoholic drink in the Republic of Macedonia and other Balkan countries, which is produced by distillation of grape pomace or wine either traditionally (homemade) or industrially. The annual production of grape brandy from various grape varieties in 2010 was 130,620 h L (SSO 2011). Although Macedonian regulations control several parameters associated to the quality of alcoholic beverages such as methanol, aldehydes, esters, alcohols, total acidity, extract, and furfural (Official Gazette of Republic of Macedonia 2010) and metals such as Cu, Fe, Zn, Pb, and Sn (Official Gazette of SRM 1980), large quantities of brandies produced under domestic conditions are sold on the market without any quality control. The concentration of metals in beverages produced in various countries has been the subject of many studies (Mena et al. 1997; Capote et al. 1999; Cvetković et al. 2002; Soufleros et al. 2004; Jurado et al. 2007; Barciela et al. 2008; Ivanova-Petropulos et al. 2013; Bonić et al. 2013; Ly et al. 2013; Rodríguez-Solana et al. 2014; Froes-Silva et al. 2015), but to the best of our knowledge, such data are not readily available for brandy samples from Republic of Macedonia.

Therefore, the aims of the present work were (1) to report a simple and fast method based on GFAAS technique for direct determination of trace elements (Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn) in brandies and (2) to study the effect of various technologies for grape brandy production (home-produced brandies using copper still units and stored in stainless steel tank, and industrially produced using stainless steel

distillation apparatus, as well as brandies aged in oak barrels and aged in presence of oak chips) on the metal content. In addition, GFAAS method was optimized and validated.

Material and Methods

Chemicals and Reagents

Monoelemental stock solutions of 1000 µg mL⁻¹ containing Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn and absolute ethanol for analysis were purchased from Merck (Darmstadt, Germany). The working aqueous standards for GFAAS were prepared by suitable dilution of the stock solutions. Ultrapure water (18.2-MΩ cm resistivity) obtained in laboratory with the Millipore equipment (Bedford, USA) was used for dilutions. Matrix modifiers 10 % NH₄H₂PO₄ and 1 % MgNO₃ (Perkin Elmer Pure, Shelton, USA) were used to compensate for matrix effects in GFAAS. Certified reference material, CRM 1643e trace elements in water (National Institute of Standards and Technology (NIST), Canada), was purchased from LGC Promochem (Wesel, Germany).

Grape Brandies

Three kinds of brandies (eight samples in total), produced with different technologies, were subjected to analysis, as follows: three brandies (B1, B2, B3) homemade and stored in stainless steel tanks and five brandies produced by industrial distillation of which two (B4, B5) aged in French oak barrels and three (B6, B7, B8) aged with different oak chips. In fact, aging of rakija in oak wood barrels or in the presence of oak chips in the tanks is traditionally used in Macedonia before the consumption in order to improve the intensity and complexity of the flavor and aroma of the brandy. Therefore, we decided to compare the element content of brandy samples aged with and without oak. All brandies were produced in 2014 from wines of Vranec variety, except brandy B5, which was produced from Muscat grapes (Muscat Temjanika and Muscat Ottonel). The industrial distillation occurred in Elenov winery, Demir Kapija, Macedonia, using stainless steel and copper unit (1000-L capacity; Cadalpe, Italy). Table 1 summarizes the available information about the analyzed brandies.

Brandies contained from 47 to 62 % (v/v) alcohol and only the middle distillation fraction was retained. After distillation, brandies B1, B2, and B3 were stored in stainless still tanks of 1000 L, while B4 and B5 brandies were aged in French oak barrels (225 L).

A brandy of industrial origin with 56 % (v/v) alcohol was divided into three lots (B6, B7, and B8), kept in 10-L stainless steel tanks. Each of them contained French oak chips, light, medium, and heavy (French Oak, Erbslöh, Geisenheim,

Table 1 Description of the analyzed Macedonian brandies

| Brandy | Production | Aging | | Wine variety | Content of alcohol (% v/v) |
|--------|-------------------------|-----------------|---|--------------|----------------------------|
| | | Period (months) | Storage/aging | | |
| B1 | Domestic distillation | 8 | Stainless still tank | Vranec | 48 |
| B2 | Domestic distillation | 8 | Stainless still tank | Vranec | 56 |
| B3 | Domestic distillation | 8 | Stainless still tank | Vranec | 47 |
| B4 | Industrial distillation | 8 | French oak barrel | Vranec | 62 |
| B5 | Industrial distillation | 8 | French oak barrel | Muscat | 62 |
| B6 | Industrial distillation | 8 | Stainless still tank, French light-roasted oak chips | Vranec | 56 |
| B7 | Industrial distillation | 8 | Stainless still tank, French medium-roasted oak chips | Vranec | 56 |
| B8 | Industrial distillation | 8 | Stainless still tank, French dark-roasted oak chips | Vranec | 56 |

Germany), toasted at various temperatures (120–160, ~200, and ~250 °C, respectively; Table 1), added in a dose of 2 g L⁻¹.

All brandies were aged for 8 months at constant temperature (10–12 °C) in a cellar. For analysis, brandy samples were collected in glass bottles of 100 mL.

Operation of GFAAS

A graphite furnace atomic absorption spectrometer Perkin Elmer model PinAAcle 900T (Norwalk, CT, USA) was used for determination of Cd, Pb, Cr, and Ni in brandy samples without dilution, while Cu, Fe, Mn, and Zn were quantified after appropriate dilution. Sample aliquots of 20 µL were directly injected into the graphite tube, and then, a volume of 5 µL of chemical modifier was added. Matrix modifiers were used according to the recommendation of the instrument manufacturer. The operating conditions in GFAAS are presented in Table 2.

Statistical Analysis

XLSTAT software, version 7.5.2, Addinsoft (Paris, France), was employed for statistical analyses. One-way analysis of variance (ANOVA) was applied to the results of metal concentration to identify significant differences ($p < 0.05$) among samples. Principal component analysis (PCA) was used for classification and separation of samples and to find whether the pattern of elemental composition could reflect the type of distillation device and aging approach. PCA is an unsupervised multivariate method based on the linear transformation of variables into a set of linearly uncorrelated variables, namely, principal components (Miller and Miller 2000). The results of PCA are a set of loading vectors and score vectors. The loading vectors represent the principal components and reflect the individual contribution of a variable. The score vectors represent the projection of each sample on orthogonal basis and highlight the strong influence of the first principal components on data variability. PCA was used with good results

for classification and characterization of traditional alcoholic beverages, such as Orujo de Galicia (Ivanova-Petropulos et al. 2013; Rodríguez-Solana et al. 2014; Lachenmeier et al. 2005), Brazilian ready-to-drink beverages (Froes-Silva et al. 2015), beers, and other spirit drinks (Picinelli Lobo et al. 2005; Lachenmeier 2007).

Results and Discussion

Validation of the GFAAS Method

The GFAAS method was validated for the determination of elements in alcoholic beverages in terms of matrix effect, limit of detection, accuracy, and precision (Table 3). The matrix effect was assessed from the ratio of calibration curves drawn for alcoholic (50 % v/v)/aqueous standards. Limits of detection (3σ criterion) were calculated using residual standard error ($s_{y/x}$ and parameters of the calibration curves). Accuracy was checked by analyzing CRM 1643e in both aqueous and alcoholic (50 % v/v) solution and using in each case the calibration established with aqueous standards.

Table 3 presents the data about parameters of the calibration curves, matrix effects, and limits of detection. The presence of 50 % (v/v) ethanol in samples had no significant influence on calibration sensitivity since the matrix effect was in the range of 0.97–1.08 compared to aqueous solution. Although the limits of detection in alcoholic matrix were slightly higher, the capability of the GFAAS method for metal determination in beverages was not diminished. Results obtained in the analysis of CRM 1643e are presented in Table 4.

According to the data in Table 4, GFAAS method using aqueous standards for calibration provides reliable results for metal concentrations in beverages. The t test revealed no significant difference in the analysis of CRM in aqueous/ethanol matrix against certified values for $n = 3$ and 95 % confidence level ($t_{\text{calc, water}}$ in the range of 0.15–4.19 < $t_{\text{tab}} = 4.30$; t_{calc} ,

Table 2 Operating conditions in GFAAS for metal determination in brandies

| | Cd | Pb | Cr | Ni | Cu | Fe | Mn | Zn |
|--|---|---|---|---------------------|---|---|---|---|
| Signal processing | Peak area | | | | | | | |
| Read time | 5 s | | | | | | | |
| Sample volume | 20 μL | | | | | | | |
| Background correction | Transversally heated graphite furnace atomizer and longitudinal Zeeman effect | | | | | | | |
| Wavelength (nm) | 228.80 | 283.31 | 357.87 | 232.00 | 324.75 | 248.33 | 279.48 | 213.86 |
| EDL current (mA) | 230 | 400 | | | | | | |
| HCL current (mA) | | | 25 | 25 | 15 | 20 | 20 | 15 |
| Calibration range ($\mu\text{g L}^{-1}$) | 0–5 (seven points) | 0–20 (seven points) | 0–20 (seven points) | 0–20 (seven points) | 0–20 (seven points) | 0–20 (seven points) | 0–20 (seven points) | 0–10 (seven points) |
| Matrix modifier | 1 % $\text{NH}_4\text{H}_2\text{PO}_4$ + 0.06 % $\text{Mg}(\text{NO}_3)_2$ (5 μL) | 1 % $\text{NH}_4\text{H}_2\text{PO}_4$ + 0.06 % $\text{Mg}(\text{NO}_3)_2$ (5 μL) | 0.3 % $\text{Mg}(\text{NO}_3)_2$ (5 μL) | – | 0.1 % Pd + 0.06 % $\text{Mg}(\text{NO}_3)_2$ (5 μL) | 0.3 % $\text{Mg}(\text{NO}_3)_2$ (5 μL) | 0.1 % Pd + 0.06 % $\text{Mg}(\text{NO}_3)_2$ (5 μL) | 0.1 % $\text{Mg}(\text{NO}_3)_2$ (5 μL) |
| Furnace program | | | | | | | | |
| Drying | | | | | | | | |
| Temperature ($^{\circ}\text{C}$) | 110 | 110 | 110 | 110 | 110 | 110 | 110 | 110 |
| Ramp (s) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Hold (s) | 40 | 40 | 30 | 30 | 30 | 30 | 30 | 30 |
| Ar (mL min^{-1}) | 250 | 250 | 250 | 250 | 250 | 250 | 250 | 250 |
| Drying | | | | | | | | |
| Temperature ($^{\circ}\text{C}$) | 130 | 130 | 130 | 130 | 130 | 130 | 130 | 130 |
| Ramp (s) | 15 | 15 | 15 | 15 | 15 | 15 | 15 | 15 |
| Hold (s) | 40 | 40 | 30 | 30 | 30 | 30 | 30 | 30 |
| Ar (mL min^{-1}) | 250 | 250 | 250 | 250 | 250 | 250 | 250 | 250 |
| Pyrolysis | | | | | | | | |
| Temperature ($^{\circ}\text{C}$) | 500 | 850 | 1500 | 1100 | 1200 | 1400 | 1300 | 700 |
| Ramp (s) | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |
| Hold (s) | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 |
| Ar (mL min^{-1}) | 250 | 250 | 250 | 250 | 250 | 250 | 250 | 250 |
| Atomization | | | | | | | | |
| Temperature | 1500 | 1600 | 2300 | 2300 | 2000 | 2100 | 2300 | 1800 |
| Ramp | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Hold | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 |
| Ar | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cleaning | | | | | | | | |
| Temperature ($^{\circ}\text{C}$) | 2450 | 2450 | 2450 | 2450 | 2450 | 2450 | 2450 | 2450 |
| Ramp (s) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Hold (s) | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Ar (mL min^{-1}) | 250 | 250 | 250 | 250 | 250 | 250 | 250 | 250 |

Table 3 Calibration parameters, matrix effects, and detection limits in GFAAS using aqueous standards or standards prepared in ethanol

| Element | Matrix ^a | Slope | Intercept | Correlation coefficient (<i>r</i>) | Matrix effect ^b | Residuals (<i>s_{y/x}</i>) ^c | LOD ($\mu\text{g L}^{-1}$) ^d |
|---------|---------------------|---------|-----------------------|--------------------------------------|----------------------------|---|---|
| Cd | Water | 0.0242 | 0.00099 | 0.9996 | 1.04 | 8.4×10^{-6} | 0.12 |
| | Ethanol | 0.0252 | -0.00113 | 0.9996 | | 1.9×10^{-5} | 0.27 |
| Cu | Water | 0.00166 | 1.1×10^{-5} | 0.9996 | 1.08 | 5.6×10^{-7} | 0.60 |
| | Ethanol | 0.00180 | -6.4×10^{-4} | 0.9996 | | 8.1×10^{-7} | 1.00 |
| Ni | Water | 0.0037 | -5.1×10^{-4} | 0.9997 | 1.05 | 2.2×10^{-6} | 0.67 |
| | Ethanol | 0.0039 | -0.00137 | 0.9995 | | 4.5×10^{-6} | 1.00 |
| Cr | Water | 0.01077 | 9.8×10^{-4} | 0.9997 | 1.00 | 1.8×10^{-5} | 0.43 |
| | Ethanol | 0.01078 | 3.0×10^{-4} | 0.9998 | | 1.6×10^{-5} | 0.43 |
| Pb | Water | 0.00253 | -2.4×10^{-4} | 0.9997 | 0.97 | 8.1×10^{-7} | 0.57 |
| | Ethanol | 0.00245 | -4.3×10^{-4} | 0.9997 | | 8.6×10^{-7} | 0.68 |
| Fe | Water | 0.01099 | -0.00327 | 0.9995 | 0.98 | 3.5×10^{-5} | 1.00 |
| | Ethanol | 0.01083 | -0.00353 | 0.9996 | | 2.9×10^{-5} | 1.00 |
| Mn | Water | 0.00894 | -5.2×10^{-4} | 0.9998 | 0.99 | 2.4×10^{-6} | 0.29 |
| | Ethanol | 0.00884 | -3.1×10^{-4} | 0.9996 | | 4.9×10^{-6} | 0.37 |
| Zn | Water | 0.03179 | -0.00142 | 0.9996 | 1.00 | 5.7×10^{-5} | 0.36 |
| | Ethanol | 0.03170 | -0.00254 | 0.9995 | | 8.1×10^{-5} | 0.46 |

^a Fifty-percent (v/v) ethanol^b Ratio of ethanol/water calibration slopes^c Calculated for $n = 7$ calibration standards^d Three-sigma criterion, calculated from residual standard deviation and parameters of the calibration curve

ethanol in the range $0.39-2.87 < t_{\text{tab}} = 4.30$). In the same time, no significant difference was found between results obtained for aqueous matrix and ethanol matrix ($n_1 + n_2 = 6$; 95 % confidence level; $t_{\text{calc, water/ethanol}}$ in the range of $0.01-2.61 < t_{\text{tab}} = 2.78$), confirming that the method is accurate and appropriate for analysis of metals in brandies without previous sample pretreatment.

In addition, the accuracy and precision were checked using a standard addition method (Table 5). One brandy sample (B1) was spiked with two appropriate volumes of the mono-elemental standard solution containing Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn, the first standard addition (STD-I) with concentration of $10 \mu\text{g/L}$ and the second standard addition

(STD-II) with concentration of $100 \mu\text{g/L}$. Satisfactory results for the recovery were obtained (range 92.7–109 %), confirming that the method is accurate and convenient for quantitative analysis of elements in brandy samples.

Additionally, to confirm the accuracy of the method, repeatability and reproducibility were checked. Repeatability describes the precision of within-run replicates, and reproducibility describes the precision of between-run replicates (Miller and Miller 2000). Precision of the method was defined as a relative standard deviation (RSD) calculated as a percentage using the standard deviation divided by the mean of replicated samples (Ivanova-Petropulos et al. 2016). Thus, repeatability was checked with ten replicated measurements on

Table 4 Results ($\mu\text{g L}^{-1}$) for the analysis of CRM 1643e by GFAAS and calibration with aqueous standards

| Element | Certified | Aqueous matrix | | 50 % (v/v) ethanol matrix | |
|---------|-----------------|--------------------|-----------------------|---------------------------|-----------------------|
| | | Found ^a | Recovery ^a | Found ^a | Recovery ^a |
| Mn | 38.9 ± 0.45 | 38.4 ± 1.22 | 98 ± 3 | 39.4 ± 4.39 | 101 ± 11 |
| Fe | 98.1 ± 1.4 | 97.7 ± 11.2 | 100 ± 11 | 100 ± 13.7 | 102 ± 14 |
| Cu | 22.7 ± 0.31 | 23.1 ± 1.00 | 101 ± 4 | 22.1 ± 1.30 | 97 ± 6 |
| Zn | 78.5 ± 2.20 | 76.6 ± 2.0 | 98 ± 3 | 77.7 ± 3.7 | 99 ± 5 |
| Pb | 19.6 ± 0.21 | 19.4 ± 0.87 | 99 ± 4 | 19.1 ± 1.31 | 97 ± 7 |
| Pb | 19.6 ± 0.21 | 19.4 ± 0.87 | 99 ± 4 | 19.1 ± 1.31 | 97 ± 7 |
| Cr | 20.4 ± 0.24 | 20.3 ± 1.14 | 100 ± 6 | 21.5 ± 2.76 | 105 ± 15 |
| Ni | 62.4 ± 0.69 | 61.2 ± 1.63 | 98 ± 3 | 61.2 ± 3.55 | 98 ± 6 |

^a Mean \pm uncertainty ($n = 3$; 95 % confidence level)

Table 5 Results of Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn analyses for checking the accuracy and precision of the method by standard addition method

| Elements | The content of standard addition in brandy B1 (6 repetitions) | | | |
|----------|---|------------------|---------------------|------------------|
| | STD-I (10 µg/L) | | STD-II (100 µg/L) | |
| | Found concentration | Recovery percent | Found concentration | Recovery percent |
| Mn | 37.8 ± 0.28 | 96.4 ± 0.73 | 120 ± 1.41 | 93.8 ± 1.1 |
| Fe | 38.8 ± 1.20 | 95.0 ± 3.01 | 131 ± 8.49 | 105 ± 6.53 |
| Cu | 6127 ± 4.24 | 99.9 ± 0.07 | 6187 ± 10.6 | 99.3 ± 0.17 |
| Zn | 31.3 ± 0.99 | 92.7 ± 3.00 | 118 ± 1.41 | 96.7 ± 1.15 |
| Cd | 10.3 ± 0.46 | 97.1 ± 4.48 | 98 ± 0.64 | 98.2 ± 0.63 |
| Pb | 17.9 ± 0.85 | 96.1 ± 4.71 | 108 ± 2.83 | 101 ± 2.62 |
| Cr | 10.5 ± 0.88 | 109 ± 8.63 | 97.3 ± 1.63 | 98.3 ± 1.62 |
| Ni | 12.2 ± 0.85 | 96.7 ± 7.07 | 105 ± 9.55 | 96.6 ± 9.36 |

B1 sample performed within 1 day. The RSDs for each element were satisfactory, ranging from 0.11 % for Cu to 9.96 % for Cr (Table 6). Reproducibility was also checked with replicated sample analyses in three different days (3 replicates × 3 injections × 3 days), and the RSD for each element was calculated (Table 6). The RSD values ranged from 0.13 % for Cu to 9.80 % for Cr.

Metal Content in Brandies

Table 7 summarizes the content of metals in homemade (B1, B2, B3) and industrially distilled brandies aged in oak barrels (B4, B5) or in the presence of oak chips toasted at different temperatures (B6, B7, B8).

Copper was the dominant element in all analyzed brandies, ranging between 2010 and 6120 µg L⁻¹ with two exceptions (B2 71,200 µg L⁻¹ and B3 17,300 µg L⁻¹), followed by zinc (ranging between 18 and 175 µg L⁻¹, with exception of B2 (3160 µg L⁻¹)), iron (range 11–841 µg L⁻¹), and manganese (range 29–118 µg L⁻¹). In general, Cu, Fe, and Zn were present in a significantly higher amount ($p > 0.05$) in homemade

Table 6 Results for repeatability and reproducibility of Cd, Cr, Cu, Fe, Mn, Ni, Pb, and Zn (µg L⁻¹) in brandy

| Sample | Mn | Fe | Cu | Zn | Cd | Pb | Cr | Ni |
|-----------|--|------|------|-------|------|------|------|------|
| B1 brandy | Repeatability (10 replicates in 1 day) | | | | | | | |
| <x> | 28.6 | 30.3 | 6120 | 22.6 | 0.25 | 7.93 | 0.15 | 1.92 |
| SD | 2.08 | 2.08 | 7.00 | 2.08 | 0.02 | 0.32 | 0.02 | 0.16 |
| RSD (%) | 7.26 | 6.86 | 0.11 | 9.18 | 8.22 | 4.05 | 9.96 | 8.39 |
| B1 brandy | Reproducibility (3 replicates × 3 injections × 3 days) | | | | | | | |
| <x> | 28.1 | 30.7 | 6124 | 21.83 | 0.26 | 7.70 | 0.14 | 1.93 |
| SD | 1.28 | 1.65 | 7.94 | 2.02 | 0.02 | 0.35 | 0.01 | 0.15 |
| RSD (%) | 4.56 | 5.35 | 0.13 | 9.26 | 5.95 | 4.50 | 9.80 | 7.90 |

<x> average value, SD standard deviation, RSD relative standard deviation

brandies B1–B3 (average values in µg L⁻¹ 31,500 Cu; 370 Fe; and 1120 Zn) compared to industrially distilled brandies aged in French oak barrels (average values in µg L⁻¹ 2130 Cu, 68 Fe, and 30 Zn) or aged in the presence of chips (average values in µg L⁻¹ 4400 Cu, 214 Fe, and 24 Zn). According to the Macedonian legislation that sets the allowable limit of Cu at 10 mg L⁻¹ (Official Gazette of SRM 1980), the threshold was surpassed twofold and more than sevenfold in the homemade brandies B2 and B3. The high concentration of Cu in distilled beverages could come from several sources, such as distillation equipment (Soufleros et al. 2004; Adam et al. 2002), galvanized metal fermentation drums (Reilly 1972), metallic storage containers (Guerrero et al. 1996), CuSO₄ applied in the vineyards, or water employed in the dilution of spirit (Ibanez et al. 2008). Since rakija of homemade origin is obtained in Cu “alembic,” the distillation facility represents the main source of Cu. As mentioned by Soufleros et al. (2004), decreasing of the Cu levels in distillates could be achieved by a careful cleaning of alembics after each distillation process. Copper acts as a catalyst, favoring the formation of volatile aroma compounds that improve the brandy quality. However, high concentration of Cu has a negative influence on the flavor, taste, and color as well on consumers’ health due to the catalytic development of carcinogenic ethyl carbamate (Almeida Neves et al. 2007; Szymczycha-Madeja et al. 2015). Therefore, the content of Cu has to be controlled for brandies put on the market and sale of illicit produced brandies has to be prevented in order to protect the consumers’ health and licensed producers.

The content of Cu in industrial distillates was lower than in homemade distillates and did not surpass the maximum allowed limit set in the Macedonian legislation. The lower Cu loading is the results of using industrial distillation facilities made of stainless steel, automation, and temperature control in the distillation process. Reaich (1998) has shown that the temperature in the distillate affects the Cu content in whiskey.

Table 7 Concentration ($\mu\text{g L}^{-1}$) of elements in Macedonian brandies determined by GFAAS (mean \pm uncertainty for 95 % confidence level; $n = 3$)

| Sample ^a | Mn | Fe | Cu | Zn | Cd | Pb | Cr | Ni |
|----------------------------|----------------|--------------|-------------------|-----------------|------|--------------|---------------|---------------|
| B1 | 29 \pm 3 | 30 \pm 5 a | 6,120 \pm 156 | 23 \pm 2 a, b | <0.3 | 8 \pm 1 a | <0.4 | 2.0 \pm 0.3 |
| B2 | <0.4 | 841 \pm 35 | 71,200 \pm 1290 | 3160 \pm 110 | <0.3 | 9 \pm 1 a | 2.0 \pm 0.3 | <1 |
| B3 | 49 \pm 4 | 241 \pm 25 | 17,300 \pm 470 | 175 \pm 19 | <0.3 | 12 \pm 2 a | 21 \pm 2 | 7 \pm 1 |
| B4 | 38 \pm 3 a | 62 \pm 6 | 2,010 \pm 87 a | 33 \pm 5 | <0.3 | <0.7 | <0.4 | <1 |
| B5 | 36 \pm 3 a | 74 \pm 7 | 2,250 \pm 95 a | 27 \pm 4 a | <0.3 | <0.7 | <0.4 | <1 |
| B6 | 118 \pm 10 b | 37 \pm 5 a | 3,450 \pm 119 | 18 \pm 2 b | <0.3 | <0.7 | <0.4 | <1 |
| B7 | 87 \pm 7 | 11 \pm 2 | 4,360 \pm 134 | <0.5 | <0.3 | <0.7 | <0.4 | <1 |
| B8 | 110 \pm 11 b | 594 \pm 35 | 5,400 \pm 114 | 48 \pm 6 | <0.3 | <0.7 | 12 \pm 2 | 5 \pm 1 |
| Maximum limit ^b | – | 10,000 | 10,000 | 500 | – | 500 | – | – |

Same letters in the column indicate that the results are not significantly different between each other ($p > 0.05$)

^a Description of samples is presented in Table 1

^b Official Gazette of SRM 1980

Copper concentrations in the analyzed Macedonian brandies were similar to those reported for other brandies (Barciela et al. 2008; Szymczycha-Madeja et al. 2015), such as Orujo de Galicia aged in oak (Rodríguez-Solana et al. 2014), Šljivovica plum brandies of different ages and origin (Bonić et al. 2013), and Venezuelan spirituous beverages (Hernández-Caraballoa et al. 2003), but much higher than in aniseed spirits (Jurado et al. 2007). In these studies, Cu concentration was higher in the beverages without certified brand origin, which was in accordance to our results.

Lead, like Cu, could be a common contaminant in brandies, mostly derived from the lead-welding repairs of the distillation stills (Green et al. 1997), water used for dilution of distillates, or fertilizers used for vine plant treatments (Soufleros et al. 2004). In our study, the content of Pb was below the detection limit of GFAAS ($1.2 \mu\text{g L}^{-1}$) for the brandies produced in industrial distillation units (B4–B8; Table 4). The level of Pb was in the range of 8–12 $\mu\text{g L}^{-1}$ in the domestic-produced brandies (B1–B3; Table 4) also, far below the maximum allowed concentration ($500 \mu\text{g L}^{-1}$) according to the Macedonian regulations (Official Gazette of SRM 1980). Obtained results were in agreement with those reported in brandy samples ($ND 313 \mu\text{g L}^{-1}$) by Szymczycha-Madeja et al. (2015), in spirits ($<6 \mu\text{g L}^{-1}$) found by Jurado et al. (2007), or in spirits aged in oak (1.12 to $34.4 \mu\text{g L}^{-1}$) reported by Rodríguez-Solana et al. (2014).

Zinc and Fe were found in all analyzed brandies at concentration levels below the maximum allowable limits set in the Macedonian legislation, as given in Table 5. The results obtained in this work were much lower than those reported by Bonić et al. (2013) in Šljivovica plum brandies (80 – $750 \mu\text{g L}^{-1}$ Zn and 670 – $2290 \mu\text{g L}^{-1}$ Fe) and Venezuelan spirituous beverages (Cocuy; 120 – $970 \mu\text{g L}^{-1}$ Cu and 260 – $360 \mu\text{g L}^{-1}$ Fe; Hernández-Caraballoa et al. 2003).

The concentrations of Cr and Ni were below detection limits in GFAAS ($0.4 \mu\text{g L}^{-1}$ Cr and $1 \mu\text{g L}^{-1}$ Ni) in brandies B4–B7 industrially produced. Chromium and Ni could be quantified only in an industrially produced brandy aged with heavy-roasted oak chips and two homemade brandies (Table 4). However, these two elements are not regulated. The content of Cd was below the detection limit in GFAAS ($0.3 \mu\text{g L}^{-1}$) in all samples. Under such circumstances, Cd, Cr, Pb, and Ni were found as not useful parameters in characterizing the Macedonian brandies. The results obtained for these elements agree with the literature data (Onianwa et al. 1999; Bonić et al. 2013; Rodríguez-Solana et al. 2014).

In order to improve their sensory properties, such as intensity and complexity of the flavor and aroma, brandy samples were subjected to aging in inox barrels (without addition of any supplements) and aging in oak barrels and aging in presence of oak chips, in order to see whether the metal concentration will be modified. Thus, the influence of aging on elements content in industrially processed distillates was checked. According to the results in Table 7, brandies aged in oak barrels (B4, B5) presented lower amount of Cu (mean $2130 \mu\text{g L}^{-1}$), Fe (mean $68 \mu\text{g L}^{-1}$), and Mn (mean $37 \mu\text{g L}^{-1}$) than brandies aged with oak chips (B6–B8; mean values in $\mu\text{g L}^{-1}$ Cu 4400, Fe 214, Mn 105). As regards the influence of toasted oak chips on brandy aging, it was observed that the use of heavy toasted variety (B8) resulted in an increase of Fe, Cu, and Zn without surpassing the allowed limits according to the Macedonian legislation (Table 5). Among the elements under study, Mn could be a suitable marker related to aging with oak chips regardless variety as its concentration was significantly higher in these brandies. Copper could be also a marker related to oak chip variety as Cu content in brandies increased with the oak-toasting degree.

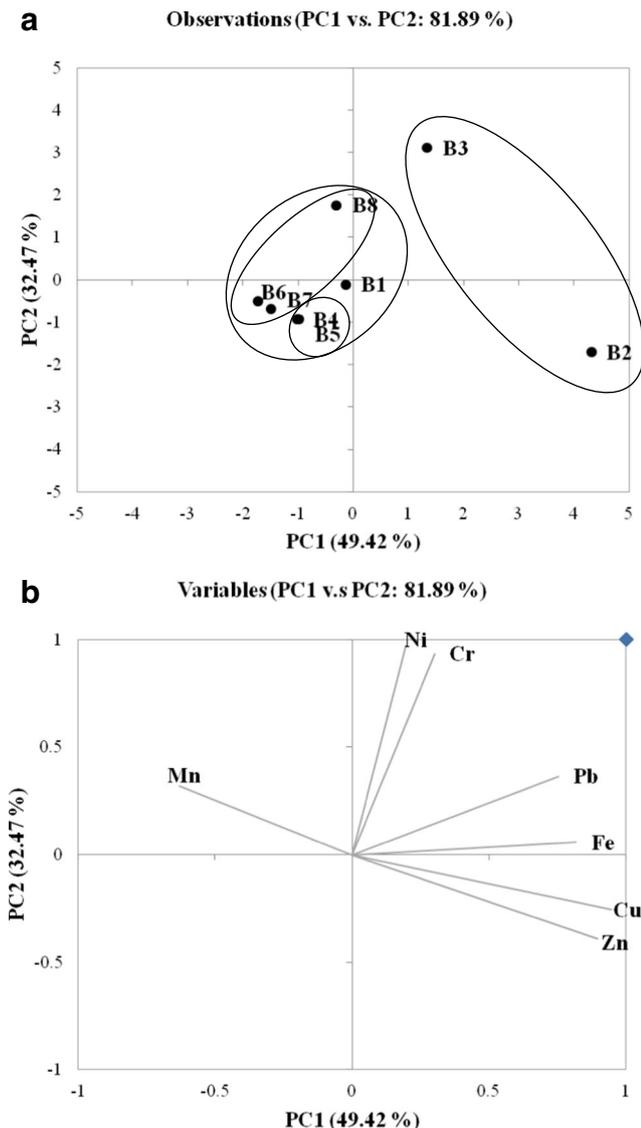


Fig. 1 **a** Eigenvector projection of Macedonian grape brandy samples in the space defined for the two first principal components. **b** PCA loadings of elements in grape brandies

Pattern Recognition of Brandies Using PCA

PCA performed on the concentrations of all elements, except Cd, in the brandies, revealed that the first two PCs explained 81.89 % of the variability. The first PC (49.42 %) was mainly associated with Cu (0.946), Zn (0.896), and Fe (0.816) and thereby, to brandy origin and distillation system. This was sustained by higher concentrations of Cu, Fe, and Zn in homemade distillates, consistent with the metal origin in the material of the distillation vessel rather than fermentation container Reilly (1972). The second PC linked to Ni (0.973) and Cr (0.933) explained 32.47 % of variability and was a pattern of brandy aging with oak chips. The first two PCs allowed a clear discrimination between brandies (Fig. 1).

Thus, brandies B4 and B5 aged in oak barrels were very closely located because of the similar contents of Cu, Zn, Fe, and Mn. Brandies aged in the presence of oak chips (B6–B8) were grouped separately based on the similar Mn contents. Brandy B1 joined this group based on the comparable Zn, Fe, and Mn contents, even though the Cu content was higher but did not surpass the allowed limit given in the Macedonian legislation. Moreover, PCA provided a clear separation of the homemade brandies, which did not correspond from the qualitative point of view in terms of Cu (B2, B3) and Zn (B2).

Conclusion

Present study demonstrates the usefulness of an integrated approach based on a fast chemical metal analysis in combination with unsupervised chemometric technique (PCA) for the characterization and safety evaluation of alcoholic drinks within a study on Macedonian grape brandies. Concentrations of Cu, Mn, Fe, and Zn were suitable parameters to establish pattern recognition for home/industrial distillation process, aging method, and type of oak chips, while Cd, Pb, Cr, and Ni, usually found to be lower than the detection limit of the GFAAS method, were not taken into consideration. The results showed that the distillation process influences the mineral content of grape brandies. Brandies produced in domestic conditions presented high Cu and Zn contents, much over the maximum allowed levels, which may pose a concern for consumer's health. Brandies produced in industrial distillation units were found to be safe for consumption as the determined metals were below the maximum allowable concentrations. Manganese and Cu could be suitable markers for aging of industrial brandies with oak chips. PCA analysis was a useful chemometric tool to establish pattern recognition by providing a good separation of the industrial brandies in terms of aging mode. Based on PCA, it was also possible to discriminate the homemade brandies which did not comply in terms of Cu and Zn contents with the requirements in the Macedonian legislation. The element chemical analyses of Macedonian rakija is compulsory to support the brand development from local grape varieties in order to sustain the product quality and acknowledgment of this traditional alcoholic beverage.

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Compliance with Ethical Standards

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Conflict of Interest Violeta Ivanova-Petropulos declares that she has no conflict of interest. Biljana Balabanova declares that she has no conflict of interest. Elena Bogeva declares that she has no conflict of interest. Tiberiu Frentiu declares that he has no conflict of interest. Michaela Ponta declares that she has no conflict of interest. Marin Senila declares that he has no conflict of interest. Rubin Gulaboski declares that he has no conflict of interest. Florin Dan Irimescu declares that he has no conflict of interest.

Ethical Approval This article does not contain any studies with animals.

Informed Consent It was obtained from all individual participants included in the study.

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