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ELEMENTAL ANALYSIS OF CZECH WINES INCLUDING WINES FROM ORGANIC PRODUCTION

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ABSTRACT

Ensuring the safety of wine as worldwide major beverage commodity is of paramount importance for many control institutions and research groups. This study delves into the elemental composition of Czech wines, with a particular focus on wines from organic production, to comprehensively assess their safety in terms of elemental contaminants. The investigation aims to shed light on potential risks associated with trace elements and heavy metals in wines and to compare these risks between conventionally produced and organically produced wines. The work further focuses on major nutritionally important elements in wine. The aim of this article was screening of elemental composition (Mg, Ca, Cr, Zn, Pb, Cd, Co, Mn, Fe, Cu, Ni, Al, P) of Czech white wines (Moravia region) of different varieties and vintages. Total of 45 samples of different vintage and varieties were analyzed. 35 samples were conventionally produced, 10 samples were produced in the organic production regime. Elemental composition comparison of organic and conventional wines was evaluated. Inductively coupled plasma optical emission spectrometry (ICP-OES) was used for the analysis of wine. Analysis of variance (ANOVA) and principal component analysis (PCA) were used for the data evaluation. Results of this study extended the knowledge about the elemental composition of Czech wines and presents the comparison with wines from different important wine regions. Results showed that Czech wines are not deviating fundamentally from wines from Europe, Australia, and South America in terms of concentration of Mg, Ca, K, P, Zn, Pb, Cd, Co, Mn, Fe, Cu, Ni and Al. Important fact, connected to food safety, is that all tested wines complied with national and European legislation and with OIV limits. Statistically significant differences were found in case of Zn, Ni, Mn, Al, Cd and Mg on significancy level 0.05. Except magnesium, higher concentrations of these metals were found in samples of conventional wines. A more in-depth analysis has attributed these differences to application of synthetic pesticides, for example dithiocarbamate mancozeb.

Keywords: wine, organic wine, elemental analysis, ICP-OES, ANOVA, dithiocarbamate

INTRODUCTION

Wine production in the Czech Republic has a rich and longstanding tradition dating back to the 2nd century. Throughout history, the country has developed distinct wine regions, with Bohemia and Moravia being the two main areas of focus. These regions are further divided into smaller sub-regions, each with its own unique characteristics suitable for growing different grape varieties. The annual wine production in the Czech Republic ranges between 0.7 and 1.1 million hectoliters. While the volume of wine production in the Czech Republic cannot be directly compared to other countries with a long tradition in wine production, Czech wines are recognized abroad and sought after by customers in the domestic market due to their quality **(Bublíková, 2019)**. Achieving high-quality wine production relies on various factors that contribute to the final product. These factors include the composition of the soil, the region's climate, the cultivation of quality grapes, optimizing the manufacturing process, and careful considerations given to transportation and storage. Each of these factors plays a crucial role in shaping the chemical composition of the wine, which in turn influences its sensory and nutritional properties.

Wine is a complex matrix consisting of both organic and inorganic components. While the focus is often on organic components, the significance of inorganic components in wine should not be overlooked. Although the content of inorganic components is generally lower than that of organic components, they play a pivotal role in determining the authenticity, safety, and nutritional properties of the wine **(Pořízka, Diviš, Štursa V., 2018; Jackson, 2014; Pohl, 2007; Galani-Nikolakaki, Kallithrakas-Kontos, and Katsanos, 2002; Brescia et al., 2002; Moreno et al., 2004; Martin, Watling, and Lee, 2012; Šelih, Šala, and Drgan, 2014, Pořízka et al., 2021)**. Elements present in wine can originate from two main sources: endogenous and exogenous. Endogenous sources are primarily associated with the soil characteristics, climatic conditions, specific wine cultivars, and the age of the vines. These factors contribute to the unique mineral composition found in wines from different regions and vineyards. However, the elemental composition of wine can be significantly influenced by endogenous sources of elements, mainly due to the application of various fertilizers and pesticides in the vineyard. For example, the content of copper, lead, zinc, manganese, or cadmium in wine can be increased through these practices **(Vrščaj et al., 2022)**.

Vines are currently grown in the Czech Republic under the regime defined by the Regulation of the European Council (EC). 1308/2013. The method of wine production is then further covered in European Commission Regulation (EC) No. 606/2009 on oenological practices. This regime is considered in the Czech Republic as conventional production of grapes and wine. In recent years, there has been a notable trend in the wine industry towards organic grape production. This movement stems from a growing desire for sustainable agricultural practices with minimal impact on the environment. Organic grape and wine production systems prioritize reducing mineral fertilizers and agrochemical pesticides to achieve sustainable agriculture. Organic viticulture adheres to European regulations (EC) No. 203/2012, allowing the use of authorized plant protection agents. Among mineral fungicides, only copper and sulfur agents are permitted for controlling fungal diseases **(Fragoulis et al., 2009)**. Comparative studies examining the elemental composition of wines from conventional and organic farming have revealed significant differences, highlighting the impact of agricultural practices on the final product **(Vrček et al., 2011; Čepo et al., 2018; Tobolková et al., 2014; Pořízka et al., 2021)**. These studies contribute to our understanding of how different approaches to viticulture can shape the elemental profile of wines, ultimately influencing their characteristics and potential health benefits. The analysis of wine's elemental composition is primarily performed using advanced techniques such as inductively coupled plasma mass spectrometry (ICP-MS) **(Almeida et al., 2002; Grindlay et al., 2009; Quétel et al., 2001**) and inductively coupled plasma optical emission spectrometry (ICP-OES) (**Silva et al., 2007; Grindlay et al., 2008**). Researchers also employ other methods such as graphite furnace atomic absorption spectrometry or flame atomic absorption spectrometry **(Fereira et al., 2008; Kristl, Veber, and Slekovec, 2002)**. Less commonly used techniques include X-ray fluorescence spectrometry **(Gruber et al., 2006; Carvalho et al., 1996**) and hydride generation atomic fluorescence spectrometry (**Segura, Madrid, and Cámara, 1999**), which have been applied in specific cases. There is currently no actual scientific publication that would be devoted in detail to the elemental composition of Czech wines with an emphasis on organic production. This article aims to investigate the elemental composition of Czech white wines from the Moravia region, encompassing different varieties and vintages. A total of 45 wine samples, representing various combinations of vintage and grape varieties, were carefully selected for analysis. Within this set, ten samples were sourced from organic wine production. By comparing the elemental composition of organic and conventional wines, this study seeks to shed light on potential differences and their implications. To carry out this analysis, inductively coupled plasma optical emission spectrometry (ICP-OES) was employed as the analytical technique. The obtained data were then subjected to analysis of variance (ANOVA) and principal component analysis (PCA) to assess patterns and relationships among the elements present in the wines.

MATERIAL AND METHODS

Samples and sample preparation

A total of 45 wine samples were collected for this study. The sample set consisted of 10 exclusively organic samples sourced from the 2021 vintage, while the remaining 35 samples represented conventional wines from both the 2020 and 2021 vintages. To ensure a comprehensive investigation, a diverse range of grape varieties was meticulously selected. Organic wine included cultivars ′Pinot Gris′, ′Manzoni Bianco′, ′Gewürztraminer′, ′Gryllus′, ′Sauvignon′, ′Malverina′, ′Hibernal′, ′Johanniter′, ′Rhine Riesling′. Conventional wines were represented by cultivars ´Gewurztraminer´, ´Pinot Gris´, ´Sauvignon´, ´Hibernal´, ′Chardonnay′, ′Müller Thurgau′, ′Neuburger′, ′Green Veltliner′, and ′Pálava′. Before the analysis, the wine samples underwent filtration using 0.45 µm nylon syringe filters. The filtered wines were then carefully transferred into plastic test tubes to preserve their integrity for subsequent laboratory investigations.

Determination of elemental composition

Elemental analysis of wine was performed by ICP-OES Horiba Jobin Yvonne Ultima 2 (Horiba Scientific, France) with measurement conditions of 15 rpm of peristaltic pump; RF power 1300 W, argon plasma gas flow 14 L/min, auxiliary gas flow 0.15 L/min, sheath gas 0.7 L/min (K, Na, Mg, Ca) and 0.2 L/min (Cr, Zn, Pb, Cd, Co, Mn, Fe, Cu, Ni, Al, P). Instrument was calibrated by standards made of individual 1 g/L stock solutions (Analytika Praha, Czech Republic). Calibration was prepared by the standard addition method.

Statistical analysis

Data analysis and statistical evaluation were conducted using Microsoft Excel (Microsoft, USA) and XL-stat (Addinsoft, France). Various statistical approaches were employed to process the results. Prior to the main data analysis, preliminary checks were performed to identify outliers and assess the distribution of the data. The Grubbs test for outliers did not detect any significant outliers, and the data exhibited a normal Gaussian distribution.

Analysis of variance (ANOVA) was utilized to assess parameters that displayed statistically significant differences between the groups of organic and conventional wines. P-values resulting from the ANOVA analysis are reported to quantify the significance of these differences.

Principal component analysis (PCA) based on Pearson correlation was employed to achieve multivariate characterization of the samples and identify specific associations between observations (wine samples) and original variables (elemental composition).

RESULTS AND DISCUSSION

Among the tested wines, the most abundant elements were potassium (431 ± 78 mg/L), calcium (56.5 ± 6.1 mg/L), magnesium (50.5 ± 6 mg/L), and phosphorus (68.5 ± 21 mg/L). No statistically significant differences were observed between organic and conventional wines for these elements, except for magnesium (p = 0.0302). Conventional wines exhibited significantly higher magnesium content compared to organic wines.

In a study conducted by **Kment et al. (2005)**, multielement analysis of 31 Czech wines from Bohemian regions, including Prague, Karlštejn, Mělník, Roudnice, Žernoseky, and Most, revealed a potassium concentration range of 493-3056 mg/L, calcium range of 47.7-210 mg/L, magnesium range of 48.9-108 mg/L, and phosphorus range of 0.26-47.3 mg/L. **Koreňovská and Suhaj (2005)** reported higher concentrations of calcium and magnesium in Slovakian wines from six different regions compared to the tested Czech samples. Similarly, **Drava and Minganti (2019)** investigated the mineral composition of Italian wines and obtained results consistent with the findings of the present study.

Two studies on the elemental composition of Croatian wines have addressed the differences between organic and conventional wines. **Čepo et al. (2018)** found lower concentrations of potassium, magnesium, and calcium in tested white wines from eleven Croatian regions compared to the Czech wines in this study. When comparing organic and conventional wines, they discovered that organic wines from nine out of eleven locations exhibited significantly lower magnesium concentrations. In contrast, **Vrček et al. (2011)** reported comparable levels of magnesium and calcium to the tested Czech wines, as well as higher potassium levels in Croatian organic and conventional wines. They found no significant differences in the potassium, magnesium, and calcium content between Croatian organic and conventional wines.

Overall, the concentration of potassium, calcium, magnesium, and phosphorus in wine can vary considerably, and no general trend related to viticulture systems has been established **(Sauvage et al., 2002).**

Table 1 Summary of the elemental composition of wines

		P	*Mg*	*Ca*	*K*	*Al*	*Mn*	*Fe*
Organic	**Min**	45.7	39.2	47.8	282	0.59	0.47	0.57
	Max	96.8	56.8	70.3	684	2.40	1.37	5.85
	Mean	61.2	46.9	55.2	415	1.38	0.74	1.67
	SD	15.9	5.3	6.4	128	0.54	0.30	1.65
Conventional	**Min**	19.6	28.2	41.6	300	0.43	0.63	0.44
	Max	147	61.4	72.9	539	3.30	1.63	3.23
	Mean	70.6	51.5	56.9	431	2.04	0.99	1.21
	SD	22.0	5.8	6.0	59.3	0.67	0.20	0.58
All wines	**Min**	19.6	28.2	41.6	283	0.43	0.47	0.44
	Max	147.2	61.4	72.9	684	3.30	1.63	5.85
	Mean	68.5	50.5	56.5	431	1.90	0.93	1.31
	SD	21.0	6.0	6.1	77.8	0.70	0.25	0.92
ANOVA P value		**0.2139**	**0.0302**	**0.4273**	**0.99**	**0.0062**	**0.0032**	**0.1697**
		Cd	***Co***	***Cu***	***Ni***	***Pb***	***Cr***	***Zn***
Organic	**Min**	0.34	0.005	0.04	0.019	0.002	0.039	0.320
	Max	0.42	0.006	0.17	0.021	0.002	0.058	0.921
	Mean	0.37	0.005	0.08	0.019	0.002	0.045	0.557
	SD	0.02	0.0003	0.05	0.001	0.0001	0.005	0.2230
Conventional	**Min**	0.28	0.004	0.01	0.019	0.002	0.036	0.242
	Max	0.75	0.006	0.30	0.023	0.002	0.064	1.172
	Mean	0.49	0.005	0.10	0.021	0.002	0.047	0.861
	SD	0.12	0.0003	0.07	0.001	0.00004	0.004	0.1724
All wines	**Min**	0.28	0.004	0.01	0.019	0.002	0.036	0.242
	Max	0.75	0.006	0.30	0.023	0.002	0.064	1.172
	Mean	0.46	0.005	0.10	0.020	0.002	0.047	0.794
	SD	0.12	0.0003	0.07	0.001	0.0001	0.004	0.2225
ANOVA P value		**0.0062**	**0.5486**	**0.4867**	**< 0.0001**	**0.0706**	**0.1309**	**< 0.0001**

Note: Concentrations are provided in mg/L, * µg/L, SD is sample standard deviation, ANOVA α = 0.05

The present study also investigated the content of microelements in the wines. Significant differences in elemental composition between the tested organic and conventional wines were observed for zinc (Zn), nickel (Ni), manganese (Mn), aluminum (Al), and cadmium (Cd), as indicated by the probability values presented in Table 1. The most notable difference was found in the residual concentration of zinc (<0.0001), with higher concentrations observed in conventional wines. This phenomenon can be attributed to the application of the dithiocarbamate fungicide mancozeb, which contains zinc and manganese bound to its organic structure. Such fungicides are not permitted in organic viticulture, which could explain the differences between the Czech organic and conventional wine samples. This claim is supported by the higher manganese levels observed in conventional samples ($p = 0.0032$) and by the findings of **La Pera et al. (2008)**, who studied the influence of mancozeb fungicide application on the elemental composition of Italian red wines. They found that wines made from grapes treated with mancozeb exhibited higher concentrations of zinc and manganese. Furthermore, the application of these fungicides was also associated with higher cadmium levels in grapes and wine, which aligns with the significantly higher concentration of cadmium observed in conventional samples ($p = 0.0062$). The cadmium levels in Czech wines (0.46 ± 0.12 µg/L) were comparable to those reported in Croatian wines (**Vrček et al., 2011; Čepo et al., 2018**). Substantially higher amounts of cadmium were found in wines from northern and central Italy collected in 2018 (**Drava and Miganti, 2019**) and in wines from Turkey (**Alkis et al., 2014**). Nickel was present in significantly higher concentrations in conventional samples ($p < 0.0001$), which is consistent with the results of **Vrček et al. (2011)** who reported similar levels of nickel.

Regarding the remaining analyzed elements, no differences were observed between production systems, but they were examined in terms of potential toxicity and their influence on wine sensory properties. The aluminum content in the tested Czech wines ranged from 0.43 to 3.3 mg/L, with a statistically significant difference between organic and conventional wines ($p = 0.0062$). These concentrations were below the limit set by the International Organisation of Vine and Wine (OIV). Similar aluminum content in wine has been reported in Australian (**McKinnon and Scollary, 1997**), French (**Jos et al., 2004**), Hungarian (**Muranyi and Papp, 1998**), and Spanish wines (**Moreno et al., 2007; Alvarey et al., 2007; Jos et al., 2004**). **Šperková and Suchánek (2005)** reported lower concentrations of aluminum in Czech wines from the Bohemian region, indicating significant variations in elemental composition between the Moravian and Bohemian wine regions of the Czech Republic. Lower aluminum concentrations compared to Czech wines were also found in samples from Croatia (**Vrček et al., 2011; Čepo et al., 2018**). In terms of the difference between organic and conventional wines, Czech organic wines exhibited an average lower concentration of aluminum than conventional wines (1.38 ± 0.54 mg/L and 2.04 ± 0.67 mg/L, respectively). This phenomenon was also observed by Croatian researchers **Vrček et al. (2011)** and **Čepo et al. (2018)**, who reported approximately two times higher concentrations of aluminum in conventional Croatian wines compared to organic samples.

Regarding the remaining analyzed elements, no differences were observed between production systems, but they were examined in terms of potential health risks and their influence on wine sensory properties. The concentrations of Pb and Cr in the tested Czech wines were 0.002 ± 0.0001 mg/L and 0.047 ± 0.004 mg/L, respectively. These results are comparable to those reported for wines from Australia **(McKinnon and Scollary, 1997**), France (**Jos et al., 2004; Kořeňovská and Suhaj, 2005**), Germany (**Thiel, Danzer, and Fresenius, 1997; Kořeňovská and Suhaj, 2005**), Italy (**Kořeňovská and Suhaj, 2005**), Spain (**Hernandez et al., 1996; Frias et al., 2003**), and Croatia (**Vrček et al., 2011; Čepo et al., 2018**). **Šperková and Suchánek (2005)** reported a higher concentration of lead in wines from the Bohemian wine region of the Czech Republic, with an average value of 0.030 mg/L. The data from this study did not reveal any significant difference between organic and conventional samples in terms of Pb and Cr content, as both elements were present in concentrations below the limits set by legislation.

The analysis of copper (Cu), iron (Fe), and cobalt (Co) was also conducted, and the results are presented in Table 1. No significant differences were found between the organic and conventional wines (Table 1). Copper concentrations in the tested wines ranged from 0.01 to 0.3 mg/L, which is similar to the findings of studies analyzing wines from Argentina (**Lara et al., 2005**), Australia **(McKinnon and Scollary, 1997**), France (**Jos et al., 2004**), Hungary (**Muranyi and Papp, 1998**), and the Czech Republic (**Šperková and Suchánek, 2005**). There is inconsistency in the evaluation of the effects of viticulture methods on copper levels in wine. Vrček et al. (2011) found higher concentrations of copper in organic Croatian wines, while **Kořeňovská and Suhaj (2012)** reported higher concentrations of copper in conventional Slovakian wines, with some samples exceeding the 1 mg/L limit. **Garcia-Esparza et al. (2006)** did not find any significant differences between organic and conventional Italian wines, which is consistent with the present study. Iron was present in the tested wines in a wide range of concentrations, from 0.44 to 5.85 mg/L, which is consistent with the findings of many researchers (**McKinnon and Scollary, 1997; Kment et al., 2005; Hernadez et al., 1996; Frias et al., 2001; Thiel, Danzer, and Fresenius, 1997**).

For the visual characterization of the elemental composition of wine samples, principal component analysis (PCA) was performed, and the results are presented in Figure 1. The PCA biplot (Figure 1) was constructed from the principal components F1 and F2, which together accounted for 39.4% of the variability. A closer examination of the biplot reveals a clear separation of organic wine observations from conventional counterparts. Almost all organic wines are projected in the biplot area with negative scores for component F1. Component F1 had the highest factor loadings from the variables Zn (0.72), Ni (0.65), and Mn (0.59), which contributed the most to the multivariate differentiation and supported the findings of the ANOVA. These three elements were found to have higher concentrations in conventional wines compared to organic wines.

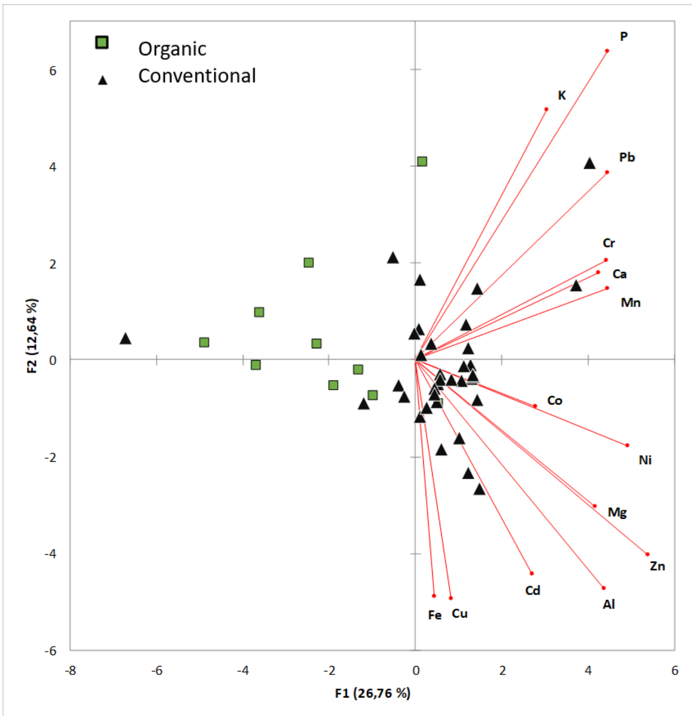

Figure 1 Principal component analysis biplot of variables F1 and F2 with projection of observations and original variables

The overall assessment of the content of nutritionally interesting elements in the tested Czech wines was finally evaluated by comparing the presented results with global nutritional tables managed by the Institute of Food Research (IFR) and the British Nutrition Foundation (BNF) (McCance and Widdowson, 2015). By comparison, it can be stated that Czech wines do not differ significantly in the concentration of P, Fe, Cu, and Mn. The comparison further revealed slightly lower amounts of K, Ca, and Mg in Czech wines compared to the results published in global nutritional tables.

CONCLUSION

The results of this study significantly contribute to the existing knowledge on the elemental composition of Czech wines, as well as provide valuable insights through comparisons with wines from diverse and renowned wine regions worldwide. By examining the concentrations of essential elements, including magnesium (Mg), calcium (Ca), potassium (K), phosphorus (P), zinc (Zn), lead (Pb), cadmium (Cd), cobalt (Co), manganese (Mn), iron (Fe), copper (Cu), nickel (Ni), and aluminum (Al), we gain a comprehensive understanding of the elemental profile of Czech wines.

In terms of elemental composition, the results revealed that Czech wines align closely with wines from other prominent wine regions. This finding highlights the consistency and reliability of the wine production process, as well as the influence of geographical and climatic factors that contribute to the elemental characteristics of wines. The concentrations of Mg, Ca, K, P, Zn, Pb, Cd, Co, Mn, Fe, Cu, Ni, and Al in Czech wines are within the ranges reported for wines from Europe, Australia, and South America, underscoring the general similarities in elemental profiles across different wine regions.

One critical aspect of this study involved comparing wines produced according to organic viticulture principles with wines lacking organic certification. Through rigorous statistical analysis, statistically significant differences in the concentrations of Zn, Ni, Mn, Al, Cd, and Mg between these two categories of wines was observed. These findings proved the impact of viticultural practices, particularly the use of synthetic pesticides, on the elemental composition of wines. Notably, the application of the dithiocarbamate mancozeb, a fungicide commonly utilized in conventional viticulture, emerged as a probable source of elevated Zn, Mn, and Cd levels in conventional wines. This finding aligns with previous research conducted by scientists investigating the influence of mancozeb fungicides on elemental composition.

The results underscore the direct link between viticultural practices and the elemental composition of wines. Organic viticulture, which prohibits the use of synthetic pesticides, exhibited lower concentrations of Zn, Mn, and Cd compared to conventional wines. These findings validated initial scientific hypothesis, highlighting the importance of sustainable viticultural practices in producing wines with distinct elemental profiles.

Importantly, despite the observed differences in elemental composition between organic and conventional wines, it is crucial to note that all wines analyzed in this study complied with national and European legislation, as well as the limits set by the OIV. These regulations ensure consumer safety and confirm that the elemental variations observed do not compromise the overall quality or safety of the wines. General conclusion can be made, that both organic and conventional methods of growing vines lead to the production of a wine that is harmless to the

Overall, this study provides valuable insights into the elemental composition of Czech wines, offers a comparative analysis with wines from various international regions, and reinforces the importance of organic viticulture in influencing the elemental characteristics of wines. The findings contribute to the ongoing exploration of viticulture practices and their impact on wine quality, supporting the continuous improvement of sustainable wine production methods.

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