



Application of benchtop total-reflection X-ray fluorescence spectrometry and chemometrics in classification of origin and type of Croatian wines

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ABSTRACT

The contents of selected metals (K, Ca, Fe, Cu, Zn, Mn, Sr, Rb, Ba, Pb, Ni, Cr and V) in 70 wine samples from Continental and Adriatic part of Croatia and different types of wine (red and white) were determined by TXRF. The aim of this study was to compare the elemental composition of wines from two different regions and to determine the discriminant ability of each variable and to indicate which variables discriminate between the four categories considered.

Principal component analysis and cluster analysis showed that K, Mn, Ba and Ni can be considered as the most important characteristics to distinguish between Continental red and white wines, Rb, Ni and Ba for Continental red and Adriatic red wines while Sr is the only metal that completely distinguishes the samples of each category. Finally, linear discriminant analysis showed good recognition (100%) and prediction abilities (96.43%) using these selected elements.

Introduction

Wine is a common alcoholic beverage made from fermented grapes or other fruits. In the process of winemaking yeast consumes the sugar in the grapes and converts it to ethanol and carbon dioxide. Wine also contains a mixture of other alcohols, sugars, organic acids and inorganic components. Its composition is strongly influenced by many different factors, from the vineyard to fermentation in the cellar. The interest in the study of major, trace and ultratrace metals in wines is due to their potential toxicity and the use of mineral profiles to determine the authenticity of wines.

The major elements in wine with concentrations greater than 10 mg/L are K, Ca, Mg, and Na. Elements such as B, Mn, Al, Fe, Cu, Zn, Sr and Rb are in the trace range (0.1–10 mg/L), while elements such as Li, Sc, Ti, V, Cr, Co, Ni, As, Se, Mo, Ag, Cd, Sn, Sb, Ba, rare earths, Hg, Tl, Pb, etc. are in the ultratrace range (<0.1 mg/L) (Grindlay et al., 2011).

The elemental composition of wine depends on several factors and is directly related to the quality and organoleptic characteristics of the wine. The origin of metals in wine is twofold: endogenous and exogenous. The origin of the most common wine elements is endogenous, as

they come from the soil in which the vines grow and are transferred to the grapes. In this case, the type of soil and grapes, as well as climatic conditions, are important factors that influence the content of elements in wine. Exogenous elements refer to external contaminants resulting from human activities such as cultivation practices, winemaking protocols and environmental pollution. In particular, fertilization, pesticide use, various oenological practices and impurities are important sources of toxic and potentially toxic metals. It is known that some elements (e.g., Al, Zn, Fe, and Cu) can contribute significantly to the deterioration of the organoleptic characteristics of wine by contributing to the formation of undesirable aroma and flavor changes. It is therefore necessary to control their concentration in wine during the various stages of wine-making (Tariba, 2011). In addition, the elemental composition of wine is an important factor to consider when evaluating its health safety. The content of toxic and potentially toxic metals in wine (As, Cd, Pb, Cu, Zn, Cr, etc.) must be constantly monitored and kept below the limits set by the relevant authorities, in accordance with public health regulations. For all these reasons, the determination of metal content in wine is usually the first step in identifying problems related to impurities, potential toxicity or simply quality control (Tariba, 2011).

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The determination of mineral content in wines has been extensively studied and reported (Frías et al., 2003; Paneque et al., 2010). Due to the direct relationship between metal content and soil composition, the determination of elemental concentration in wine is often used to characterise wines according to their geographical origin. This distinction can be made on the basis of major, trace and ultra-trace elements by an appropriate combination of chemical analysis and statistical treatment (Frías et al., 2003; Paneque et al., 2010).

Various analytical techniques have been used for elemental characterization of wine, the most commonly used being inductively coupled plasma mass spectrometry (ICP-MS) (Almeida et al., 2002; Selih et al., 2014), inductively coupled plasma optical emission spectrometry (ICP-OES) (Martin et al., 2012; Selih et al., 2014), flame atomic absorption spectrometry (FAAS) (Schiavo et al., 2008) and electrothermal atomic absorption spectrometry (ETAAS) (Alkış et al., 2014). In the last decades, among the various methods, total reflection X-ray analysis fluorescence spectrometry (TXRF) has also been considered a very suitable multielemental tool for use in food analysis (Borgese et al., 2015; Klockenkämper & von Bohlen, 2015). Sample preparation methods are usually a necessary and very important step in chemical analysis, and an advantage of TXRF is the ability to analyze complex liquid samples with simpler sample treatments (i.e., direct analysis or dilution samples) that are more in line with Green Analytical Chemistry principles (Dalipi et al., 2015; Jablan et al., 2021; Pessanha et al., 2010). Moreover, in most cases, quantification by TXRF can be performed directly by internal standardization and thus the use of external calibration using matrix-matched standards is not required as in the case of ICP-MS analysis (Obhodaš et al., 2021; Selih et al., 2014).

In this work, a total of 70 Croatian wine samples from two of the most important Croatian wine-growing regions (Continental Croatia (C) and Adriatic part of Croatia (P)) (Fig. 1) were analyzed by TXRF for the content of macro and trace elements (K, Ca, Fe, Cu, Zn, Mn, Sr, Rb, Ba, Pb, Ni, Cr and V) relevant in terms of their nutritional role and importance in biological mechanisms; toxicity and influence on wine quality; organoleptic properties and the information they can provide on the geographical origin of the wine.

Continental Croatia covers a region in the northeast of Croatia, while the Adriatic part of Croatia is located in the south near Adriatic Sea,

where the climate is mainly Mediterranean. Until very recently, available data on metal composition of Croatian wines were relatively scarce and limited to only several metals such as Cu, Cd, Pb and Zn (Banović et al., 2009; Šebečić et al., 1998; Šeruga et al., 2008) or took into account relatively small number of analyzed wines, usually from only one region of Croatia (Banović et al., 2009; Fiket et al., 2011; Vitali Čepo et al., 2017). However, controlling the elemental composition of wine is essential due to their organoleptic and nutritional significance, but also in order to perform wine categorization and/or to verify the authenticity of particular products. Therefore there is an increasing number of investigations being conducted around the world with the goal of evaluating the influence of particular factors (type of wine, producer, origin) on the levels of elements. The first comprehensive study of elemental composition of wines from different winegrowing regions of Croatia was published very recently (Leder et al., 2021). The authors applied isotope ratio mass spectrometry (IRMS) and ICP-OES to investigate whether multivariate statistical methods could provide the fingerprint for geographical origin determination.

In our work, in addition to investigating the impact of geographical origin on the levels of 13 macro- and trace elements in 70 wine samples from different Croatian wine-growing regions, we also focused on the investigation of the influence of the type of wine (black or white) on the elemental composition and contribution of wine consumption to the dietary intake of analyzed minerals, primarily in terms of safety and potential health hazards. For this purpose we investigated the application of low power TXRF as green, simple, fast and more available alternative to other methods usually applied for this purpose (AAS, ICP-OES, IRMS etc.)

It is interesting to remark that most of the published work dealing with the analysis of wine samples by TXRF has been performed using large-scale equipment with high-power X-ray tubes, requiring water-cooled systems and liquid nitrogen-cooled detectors (Klockenkämper & von Bohlen, 2015; Obhodaš et al., 2021; Pessanha et al., 2010). In this contribution, we explore the potential of low-power TXRF as a more cost-effective approach. In recent years, the development and commercialization of benchtop TXRF systems, that do not require cooling media or gas consumption for operation, have been successfully employed in different fields (Coccatto et al., 2016; Jablan et al., 2021;

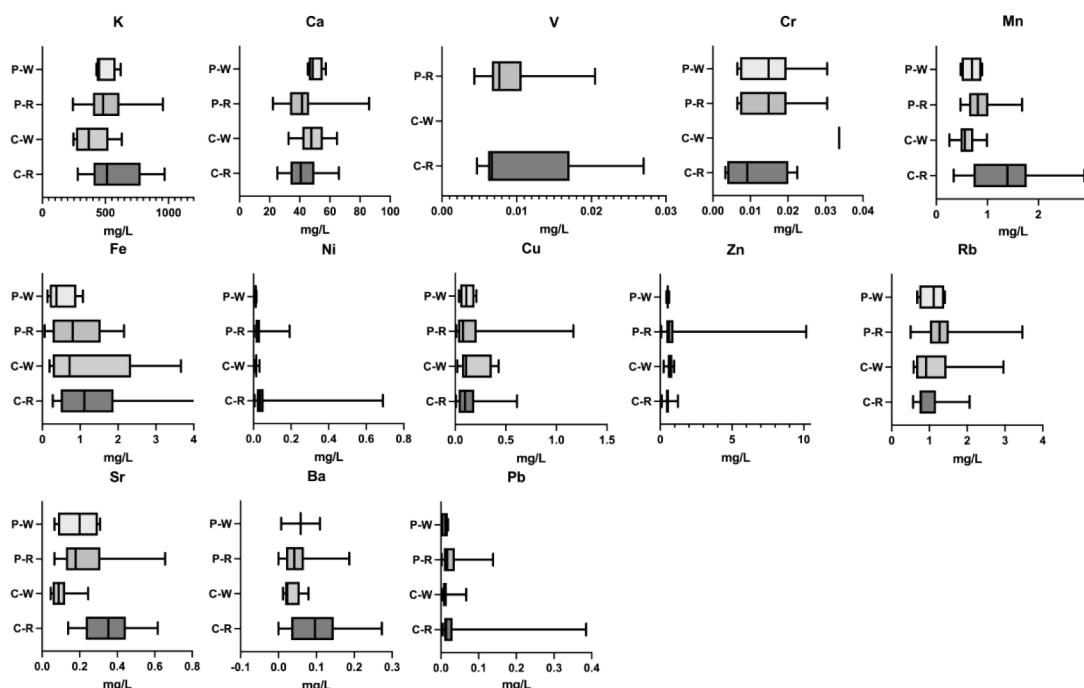


Fig. 1. Multiple Box-and-Whisker plots for the content of the elements studied in mg/L.

Machado, 2018;) but their use in the field of wine analysis have been scarcely studied (Dalipi et al., 2016).

Due to the huge and complex data set obtained in this study, some basic statistical tests and unsupervised and supervised classification methods were used to present statistically significant differences between the studied groups, to indicate if there are any patterns or trends, to explore possible wine and variable classes, or to reduce the number of variables in order to explain the data variation as much as possible.

Materials and methods

Sample collection

The wine samples were sourced from local producers in different wine growing regions in Croatia during 2014. All wines were produced in 2012 and were all commercially available in Croatia. Using the above approach, the total 70 samples analyzed were divided into 2 groups (Continental, C and Adriatic, P) and each group into 2 subgroups containing white (Continental white, C-W, $n = 10$; Adriatic white, P-W, $n = 4$), and red wines (Continental red, C-R, $n = 23$, Adriatic red, P-R, $n = 33$) produced in the same geographical area.

Sample preparation

Sample preparation for TXRF analysis was performed by measuring 990 μL of each wine sample and adding the 10 μL of a Ga solution as an internal standard at a concentration of 100 mg/L (Dalipi et al 2015). Stock solution of Ga (1000 mg/L in 5% nitric acid, TraceCERT®, standard for ICP, Sigma-Aldrich, Switzerland) was used to prepare the internal Ga standard solution. Ultrapure deionized water was obtained from a Milli-Q purification system with a minimum resistance of 18.2 M Ω and used to dilute the standard Ga stock solution (Millipore Corp., Bedford, Massachusetts, USA). The final concentration of Ga in each sample was 1 mg/L. After homogenization by stirring, 10 μL of the sample was transferred to previously cleaned and siliconized (Silicone solution, Serva, Germany) quartz glass sample carriers and dried at 50 C in a clean cabinet for later TXRF analysis. Silicone solution was used to coat all the quartz glass disc reflectors (diameter: 30 mm, thickness: 0.1 mm) to obtain a hydrophobic film to facilitate sample deposition. Three equal aliquots of each wine sample were analysed. The triplicate samples tell us about differences in sample treatment and response, but not about sample variability. Therefore, we used the average value of the triplicate samples as the central measure of the metal content of the sample. All reagents used were of analytical grade and the purity of the quartz glass reflectors was ensured in all cases before the wine samples were deposited.

Instrumentation

Measurements were performed on a bench top spectrometer S2 PICOFOX (Bruker Nano GmbH, Berlin, Germany). The TXRF spectrometer used for analysis was equipped with a molybdenum (Mo) anode X-ray tube, a multilayer monochromator (17.5 keV) and a silicon drift detector with an energy resolution of 139.17 eV (Mn-K α). The operating conditions were 50 kV and 750 μA and the acquisition time for all spectra was set to 600 s. Measurements were performed in air. The qualitative analysis and deconvolution of the spectra were performed with the included Spectra spectrometer software (Spectra Plus 5.3, Bruker AXS Microanalysis GmbH, Berlin, Germany).

Data analysis

Statistical analysis of the data was performed with PrismGraphPad 9 (GraphPad Software, Inc., San Diego, USA) and XLStat (XLStat Software, New York, USA) using the log-transformed data set of elemental concentrations. All the obtained data were subjected to chemometric

analysis methods to first verify the nature of the distribution, than compress relatively large data sets, visualize complex and high-dimensional quantitative relationships, and finally perform pattern recognition. Based on the experimental results and their chemometric evaluation, an attempt was made to evaluate the influence of the selected factors (geographical origin and/or wine type) on the content of 13 elements in 70 wine samples.

The experiments were performed in triplicate and the results obtained were expressed as means \pm standard deviation (SD) in order to describe tendency and the variation of data.

In the first step of the statistical analysis, the Kolmogorov-Smirnov test was applied to test the type of the distribution of all samples analyzed. A logarithmic transformation was performed to normalize the distribution of the element concentration data. Logarithmized data were used in all other statistical tests. Pearson's correlation coefficient was used to determine any relationship between the studied parameters as a function of concentrations ($p \leq 0.05$). The Kruskal-Wallis test followed by Dunn-Bonferroni *post-hoc* test was used for multiple comparisons between wines from different regions (Continental, C and Adriatic, P) and wine types (white, W and red, R). P values ≤ 0.05 were considered statistically significant.

In addition, principal component analysis (PCA) and cluster analysis (CA) were performed. PCA was applied to reduce the number of original variables by their linear combinations (principal components) and to discover further dependencies between the original variables. For the purpose of multivariate analysis, each of the 70 wine samples was considered as a vector with 13 variables (element concentration). Thus, a data matrix was created consisting of 13 columns for the chemical descriptors (the elements analysed) and 70 rows for the wine samples (the wine samples studied in each category). The data were prepared as log transformed data so that their values had a normal distribution.

CA by Euclidean distances and Ward's method were used as unsupervised techniques to discover the similarity between wine samples from the studied regions. Linear discriminant analysis (LDA) was used as a supervised method to discriminate wine samples between groups. A stepwise LDA using Wilk's lambda as the selection criterion and an F-statistic to determine the significance of the lambda changes are used to select variables that can be included for LDA.

Results and discussion

Mineral content

In the present work, TXRF instrumentation was used for determination of metal content of 70 wine samples. In Table S1 of [Supporting Information](#), limits of quantification (LOQs) for elements of interest in red and white wines are reported. As it is shown, LOQs are in the range of 0.007–0.269 mg/L for red wine and in the range of 0.009–0.245 mg/L for white wine, in general LOQs for white wines are slightly smaller than those from red ones. Additional information about the quality of the obtained results in terms of accuracy and precision can be found in our previous publication (Dalipi et al., 2016).

A total of 70 wines (33C and 37P) were analysed, using this proposed TXRF method, to determine the concentration of 13 elements (K, Ca, Fe, Mn, Zn, Cu, Sr, Rb, Ba, Ni, Pb, Cr, V).

The corresponding basic descriptive statistics of the metal content in the studied wine samples, considering the geographical areas (C and P) and the type of wine (R and W), is summarised in Table S2 of [Supporting Information](#).

The results obtained show a relatively high dispersion depending on the element studied, but obtained values remained generally in agreement with the literature data. The average contents of the mean values of the elements in the studied wines decreased in the order $K > Ca > Fe > Mn > Rb > Zn > Sr > Cu > Ba > Ni > Pb > Cr > V$ for the red wines and $K > Ca > Fe > Rb > Zn > Mn > Cu > Sr > Ba > Ni > Pb > V > Cr$ for the white wines. The order according to the amount of elemental content

in the wines is consistent with the trend previously reported by Fiket et al. (Fiket et al., 2011) and Kment et al. (Kment et al., 2005). The observed concentration ranges for the majority of the metals studied was in agreement with the values for Croatian wines reported in the literature by other authors (Bukovčan et al., 2008; Sebečić et al., 1998; Šeruga et al., 2008; Leder et al., 2021). A good agreement was obtained between the results of our work and the recently published work of Leder et al. (Leder et al., 2021). The only exception was the case of the low atomic number elements (K and Ca), whose concentrations were underestimated by the TXRF analysis, probably due to absorption issues from wine matrix (Dalipi et al., 2016).

Potassium content in wines is of exogenous origin, since the K concentrations in wines are directly related to the grape variety, soil, climatic conditions and temperature during fermentation and storage (Karataş et al., 2015). The values obtained in this study ranged from 241.1 mg/L to 969.3 mg/L which is in agreement with values obtained by other authors (Karataş et al., 2015). Calcium content in analyzed wines from different geographical areas varied mainly between 22 mg/L and 86 mg/L and the results were in agreement with those published by Zoecklein et al. and Pohl (Pohl, 2007; Zoecklein et al., 1995). Minor and trace elements were present in wine in significantly lower concentrations. In these terms wine can be considered as nutritional source of particular essential elements such as Mn, Fe, Cu and Zn. As presented in Table S2, their mean content in Croatian wines was 0.69–1.33 mg/L; 0.49–1.83 mg/L; 0.12–0.18 mg/L and 0.51–1.01 mg/L respectively meaning that consumption of 100 mL of wine contributes to recommended dietary allowances (RDA) with maximally 7.2%, 1.2%, 2% and 1.1% respectively. In general, these ranges are consistent with data obtained in other studies (Banović et al., 2009; Leder et al., 2021; Plotka-Wasyłka et al., 2018; Pohl, 2007; Sebečić et al., 1998; Woldemariam & Chandravanshi, 2011).

In low amounts Mn is naturally present in grapes and small variations in concentrations are usually caused by different soil compositions (Interesse et al., 1985) while higher concentrations are usually the consequence of the contamination during the different processing steps in the winery. The results obtained in this investigation were also within the ranges reported by other authors (Dragusha et al., 2017; Plotka-Wasyłka et al., 2018; Ziola-Frankowska & Frankowski, 2017). Mean concentrations ranged from 0.693 mg/l in white wines from the Adriatic region up to 1.331 mg/L in red wines from the Continental region.

Zn is a trace element that occurs in soil and can play important role in the plant growth (Carvalho et al., 1996). Zn content of wines usually increases when containers made of Zn are used during processing and maturation and also due to utilization of Zn containing pesticides.

Regarding Sr content, a relatively wide range between 0.047 mg/L to 0.655 mg/L was obtained. Our results were slightly lower compared to the work of Geana et al. and Kment et al. (Geana et al., 2013; Kment et al., 2005).

The amount of Cr in the wine samples studied varied from less than detection limit to 0.034 mg/L and for Ni from 0.003 mg/L to 0.689 mg/L. The literature data show that Cr content in wines may increase during prolonged contact of wines with materials such as stainless steel, glassware, and bottles during the ageing process (Galani-Nikolakaki et al., 2002; Lara et al., 2005). For Rb, the observed concentration range was wide from 0.257 mg/L to 2.90 mg/L and 0.50 mg/L to 3.46 mg/L, respectively.

In most cases, considering average metal concentrations, the metal content of the red wines from the Continental region was slightly higher than all others for almost all the elements studied, with the exception of Ca and Rb (Fig. 1). For most of the elements studied, the ranges of concentrations obtained in wines from two different regions overlap, and it is not readily possible, based on concentrations of analyzed elements, to identify differences between regions and between red and white wines. The exceptions are the elements Mn and Sr, which were significantly higher in red wines from the Continental region compared to all others samples analysed. In order to identify possible regional

differences and also differences between white and red wines based on metal content, statistical univariate and multivariate analyses were performed.

Significance of metals for wine quality parameters

It is of great importance to monitor the levels of metals in wines, as metals can influence organoleptic and sensory properties of wine through different mechanisms such as influencing pH or forming precipitates or unwanted aromas (Larcher & Nicolini, 2008; Volpe et al., 2009).

Potassium is the most common cause of instability because this metal is a natural constituent of grapes and its presence in wine. Potassium bitartrate has limited solubility in ethanol and its solubility progressively decreases. The formation of tartaric-potassium salts and inhibition of the degradation of mallic acid (Pohl, 2007; Zoecklein et al., 1995) and modifying the tartaric acid:malic acid ratio significantly influences pH of wines through effects on titratable acidity. Since both of the acids contribute significantly to the organoleptic properties of wine the „shift“ in their ratio can contribute to the unpleasant sourness of the wine.

The variation of Ca levels in wine is due to the content of available calcium in the soil and fertilizers applied in the vineyard (Díaz et al., 2003). One source of excess calcium is the use of calcium carbonate in deacidification rather than the recommended potassium bicarbonate (Rankine, 1989). Calcium-induced instabilities are the major cause of problems in bottled wines and are most commonly manifested through the formation of crystalline calcium L-tartrate. The occurrence of calcium L-tartrate deposits is slow and crystals usually appear months after bottling. Concentrations above 80 mg / L in white wines and 60 mg / L in red wines are considered to be at risk for the appearance of precipitates. As presented in Table 1, calcium content in analyzed wines ranged from 22.3 mg/g up to 86.0 mg/L with average values in the range from 42.7 mg/g (P-R) to 49.9 mg/L (P-W) indicating low probability of calcium-induced instability.

Minor and trace elements such as Fe, Zn, Cu, and Cr in wine reflect the content in grapes but are additionally introduced to wine through different steps of winemaking and storage. They can impact wines browning, cloudiness and astringency, influencing in that way the quality and organoleptic properties of wine. The main minor metals that cause instabilities in wine are Cu, Fe and Al while others (Sn, Pb and Ag), may be capable of causing instability in wine, but to minor extent, due to their trace amounts.

The most common is Cu instability, depending not only on Cu concentration but also on several other aspects of wine composition and storage conditions. It is limited largely to white wine and develops some time after bottling. The instability is observed as a fine haze followed by subsequent formation of yellowish mid brown deposit. The limit of 0.5 mg/L has been set as approximate „safe level“ in order to avoid copper instabilities, although they can sometimes occur in even lower levels. According to reported ranges of Cu (Table S2), Cu might be problematic

Table 1

Kruskall-Wallis study followed by Dunn-Bonferroni *post-hoc* test according to denomination of origin.

Element	p-value	Significant differences
V	0.228	
Cr	0.919	
Mn	0.005	C-R/C-W; C-W/P-R
Fe	0.141	
Ni	0.004	C-R/C-W; C-R/P-R; C-R/P-W
Cu	0.812	
Zn	0.287	
Pb	0.199	
K	0.045	C-R/C-W; C-W/P-R
Ca	0.092	
Rb	0.021	C-R/P-R
Sr	<0.0001	C-R/C-W; C-R/P-R; C-R/P-W; C-W/P-R
Ba	0.008	C-R/C-W; C-R/P-R

in terms of potential instability in certain white wines from Adriatic region.

Fe concentration in wines is mostly related to availability from the soil and the type of equipment during wine production. Fe is responsible for stability changes of old wine (Galani-Nikolaki et al., 2002; Garcia-Palacios et al., 2001) and Fe-induced instabilities occur when Fe is present in concentrations above 5 to 6 mg/L. If present at concentrations 10 mg/L or higher iron can also contribute to a formation of metallic taste of wine. Analyzed Croatian wines contained up to 8.65 mg/L of iron (Table S2), but the majority contained less than about 1.1 mg/L. Only two red wines from continental part of Croatia contained Fe levels greater than 5 mg/L making them potentially prone to iron-induced instabilities.

Nutritional and toxicological aspects of metals in Croatian wines

When consumed in moderation, wine can be a good dietary source of some essential trace elements such as Fe, Mn, Zn, Cu, Ni, and Cr. The contribution of wine consumption to the total dietary intake of particular nutritional metals is usually up to maximally 2–3 %. Particular studies indicate that contribution of beverages (particularly tea and red wine) to the intake of particular minor elements can be even higher – for example, total daily intake of Mn from particular red wine can be as high as is 0.4 mg contributing to the daily Mn intake with more than 10% (Stobbaerts et al., 1995).

On the other hand, depending on concentrations in wine, potentially toxic metals in wine can pose health hazard and their concentrations are regularly monitored and compared with maximal permissible concentrations (MPCs) (Naughton & Petrőczy, 2009) in order to establish sanitary safety of wine.

Therefore, the content of analyzed elements in this study was compared with the current MPCs set by the Croatian legislation (Croatian Regulation No. 96/96 1996) and the maximum permissible values at the international level (Office International de la Vigne et du Vin, O.I.V., standards (Organisation Internationale de la vigne et du vin, O.I.V., 2008) (Table S3).

Generally, the concentrations of metals in analyzed wines were below MPC values, with several exceptions. Zn, Ni, Pb and Cu were found in concentrations above MPCs in small number of analyzed red wines from both, Continental and Adriatic part of Croatia (Zn-one sample; Ni-four samples; Pb-one sample; Cu-one sample).

The MPC for Zn in wine has been set to 5 mg/L and observed concentrations were below 1 mg/L in majority of samples tested, which is in agreement with the values determined by Šebečić et al (Šebečić et al., 1998). The only exception was the red wine from Istria (Adriatic part), which contained elevated Zn content, far above the applicable maximum levels (10.014 mg/L).

The maximum allowed concentration of Ni in wines has been set to 0.1 mg/L according to O.I.V. (O.I.V., 2008) but 0.3 mg/L according the Croatian legislation. With the exception of four samples (two red wines from the continental region and two red wines from adriatic part of Croatia), the Ni levels detected were above the level prescribed by the O. I.V. (O.I.V., 2008), while only one sample was above the maximum permissible concentration set by Croatian legislation (Croatian Regulation No. 96/96 1996).

Lead levels were below the upper permissible limit set by the O.I.V. (0.15 mg/L) in all analyzed samples except the red wine from continental region, where Pb content was well above the MPC (0.384 mg/L).

Cu content in one red wine from Istria (1.127 mg/L) exceeded the Croatian legislation (Croatian Regulation No. 96/96 1996) and the O.I.V. (1.00 mg/L) (O.I.V., 2008) while in all other wine samples was within acceptable limits (0.009 mg/L to 0.611 mg/L). In general, this range is consistent with data obtained in other studies (Banović et al., 2009; Plotka-Wasyłka et al., 2018; Woldemariam & Chandravanshi, 2011).

Possible sources of heavy metals in wine are various - the geochemical composition of the soil, anthropogenic sources such as environmental pollution, and agricultural treatment by fertilizers,

pesticides and fungicides. Contamination can also occur at different stages of wine production. Furthermore, higher levels of heavy metals can be found in wines from vineyards located near road traffic or in industrial areas (Tariba, 2011).

Additionally, possible health risks of intake of metals through wine consumption can be investigated by the comparison of estimated intake with safety limits of intake, usually calculated based on common indices such as the provisional tolerable weekly intake (PTWI), provisional tolerable daily intake (PTDI), provisional maximal tolerable daily intake (PMTDI), permitted daily exposure (PDE), reference dose (RD) etc. This approach takes into account not only the concentration of particular metal in wine but also the average intake of wine by specific population.

An average intake of each analyzed metal/person/week was based on obtained mean values of metals in wine presented in Table S4 and taking into account the estimated average consumption of wine in Croatia which is 22.2 L per person, per year (SYRC, 2018). Estimated average intake (EAI) was compared to safety limits (SL) for the studied metals. SLs were obtained by scaling the used indices to mg of the element/person/week (average body weight 70 kg) for the sake of homogeneity. Results are presented in Table S4.

Estimated average intake of particular metals through wine consumption was comparable, although little lower in comparison to intakes assessed by other authors, for example (Gutiérrez et al., 2017.; Dalipi et al., 2015) for Spanish and Italian wines, respectively. Observed differences can be explained by significantly lower average per capita consumption of wine in Croatia in comparison to Spain (22.2 L/person/year vs. 68.7 L/person/year and 39.8 L/person/year, respectively). Results show that average consumption of Croatian wines does not significantly expose the consumers to significant levels of metals studied, in terms of toxic effects with EAI being significantly lower than SLs for particular metals.

The recent withdrawal of the PTWI for certain metals, such as lead, has made the calculation of SLs impossible and has left it necessary to find other methodologies to evaluate the risk associated with consumption of foods with significant levels of these elements. One such approach is the calculation of the target hazard quotients (THQ) where values of THQ greater than 1 present the health risk. Namely, although in majority of studies (as in ours) the amounts of the vast majority of analyzed minor and trace elements are lower than established MPCs and those wines can be considered sanitary safe, recent meta-analysis (Naughton & Petrőczy, 2009) revealed that particular wines from large number of countries included in their study exhibited THQ values significantly greater than one indicating levels of health risk, with particularly high THQ values calculated for V, Cu and Mn.

THQ values for Croatian wines calculated using the equation suggested by EPA (Dalipi et al., 2015) [1]:

$$THQ = \frac{EFR * Ed * FIR * MCs}{RfD * BWa * ATn * 1000} \quad [1]$$

where EFR is the exposure frequency to the trace element, Ed is the exposure duration (70 yrs), FIR is the food ingestion rate in grams per day for the respective food item, MCs is the concentration in wet weight of the trace element in the given food item, RfD is the oral reference dose of the trace element in µg/g/day, BWa is the reference body weight of 70 kg and ATn is the averaged exposure time (365 days*70yrs) and 1000 is the unit conversion factor. Oral reference doses are defined by the United States Environmental Protection Agency (EPA) and they are the “estimate of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime „(U.S. Environmental Protection Agency | US EPA). Indices used for the calculation of THQs are presented in the Table S5 and obtained THQ values for males and females are presented in Figure S1.

Obtained values were similar for men and women. The highest THQ values were obtained for lead in all four groups of analyzed

samples and the THQ for individual metals followed the order $Pb > Mn > Ni > Cu > Fe > Zn > Cr$ for Continental red wines; $Pb > Mn > Zn > Cu > Ni > Cr > Fe$ for Adriatic red wines; $Pb > Cu > Mn > Zn > Fe > Cr > Ni$ for Continental white wines and $Pb > Mn > Cu > Cr > Zn > Fe > Ni$ for Adriatic white wines. Obtained THQ values are significantly lower than 1 thus demonstrating that consumption of Croatian wines does not contribute significantly to the potential health hazards as consequences of lifelong intake of analyzed metals. Obtained THQs are a little lower in comparison to THQs obtained for consumption of wine in Spain and Italy, but again can be explained by significantly lower per capita wine consumption in Croatia (Dalipi et al., 2015; Gutiérrez et al., 2017).

Univariate analysis

The variation between samples within a group was very pronounced, indicating a significant influence of grape variety and geographical location on the elemental composition of the wine.

In the first step of the statistical analysis, the original data set was found to deviate from the normal distribution except for Cr (summarised in Table S6 of Appendix). Normal probability plots for Mn, as a representative example, are shown in Fig. S2. All obtained data showed less deviation from normal distribution after log transformation. Thus, the log-transformed data set was subjected to further analysis.

Correlation analysis

Pearson correlation coefficients between concentrations of metals in wines were examined for log-transformed data. The correlation matrix obtained is shown in Table S7 of Appendix. The highest positive correlation coefficients were found for Cr-Ba (0.889), Sr-Ba (0.658), Mn-Sr (0.631), Mn-Ba (0.611), Cr-Pb (0.557), Cr-Ca (0.508), V-Cr (0.504), Mn-K (0.472), Ni-Ba (0.447), Cu-Pb (0.447), Cu-Zn (0.434), Zn-Pb (0.432), and for Pb-Ca (0.408) with $p \leq 0.05$. Moreover, Ca, Ba and Ni content were positively correlated with all elements. Cr and Pb were positively correlated with all elements except Mn, K was positively correlated with all elements except Fe, Cu was positively correlated with all elements except V and Pb was positively correlated with all elements except Mn. No significant correlations were observed between the other variables.

Significant correlations between elements indicate the same source of the elements, but do not provide information about differences between wines from different regions or different types of wine (white or red). A Kruskal-Wallis followed by Dunn-Bonferroni *post-hoc* test was performed to determine the discriminant capacity of each variable and to indicate which variables discriminate among the four categories considered. The results are presented in Table 1. and show that Mn, Ni, K, Rb, Sr and Ba were the most discriminating variables. Thus, K, Mn, Ba and Ni can be considered as the most important characteristics to discriminate between continental red and white wines, and Rb, Ni and Ba can be considered to discriminate between the group of continental red and Adriatic red wines. Sr is the only metal that completely distinguishes the samples of each category.

Multivariate analysis

The metal profile of the wines was used as a chemical descriptor for classification by geographical origin and/or wine type. For the multivariate analysis, only the variables that showed some differences between the categories were used, in our case Mn, Ni, K, Rb, Sr and Ba.

Principal component analysis

The unsupervised pattern recognition method (PCA) was applied to identify the main factors controlling data variability. PCA allows visualise the information of the dataset in a few principal components (PC) preserving the maximum possible variability within this set.

Three main components were selected, which explained 59.2% of the total variability, where the first component (PC1) contributed 30.81% of

the total variance, the second component (PC2) contributed 16.18%, and the third component (PC3) contributed 12.17%. According to the loadings of the variables for the PCs, the most significant PC1 was strongly associated with the value of the variables Mn, K, Ni, and Sr, which were distributed in the negative range of the first component (PC1). The Rb and Ba had the highest positive coefficients in the positive area of the second component (PC2). The loading plot is related to the variables and indicates the similarities and correlations between the elements. Extracted correlation matrix and corresponding loading plot, as visualization of variables onto a two-factor plane (PC1 vs PC2) are presented in Fig. 2a. From the obtained values of the correlation coefficients between the factors and variables from the correlation matrix, three groups of elements can be distinguished. Thus, a close relationship was found between the concentrations of (a) K and Ni, (b) Mn and Sr, and (c) Rb and Ba. It can also be seen that there is a general relationship between K, Ni, Mn and Sr. In addition, three significant factors related to the sources of the elements in the wine samples studied were extracted. The first factor was determined with negative loadings for all elements, showing a high negative loading of Mn and Sr (-0.814 and -0.821, respectively) and a lower negative loading of K and Ni (-0.672 and -0.625, respectively). Rb (0.802) has a significant positive loadings in the second factor and corresponds positively with Ba (0.495) with lower positive loading. The third factor consists only of Ni (-0.398) with negative loading. In general, the negative loadings for all elements in the first factors indicate that they come from different sources. In particular, the concentrations of Ba, Mn, Rb, and Sr are the most informative indicators of the composition of the soil in a given geographic region. In addition, the K content is an important variable related to the ripeness of the grapes and is therefore useful for distinguishing wine types. It is assumed that the Ni concentration of the samples is probably due to a specific source of contamination.

The PCA score (Fig. 2b) for all the wine samples studied shows the projection of the cases as a function of type of wine and geographical origin into the coordinate system defined by the main factors 1 and 2, in order to determine which elements best characterise the type wines in terms of origin.

A rough clustering of wines by different geographical origins and wine types (red and white) can be observed. On PC1, the C-W wines were in positive part (on the right side of the graph), and they were roughly separated from the C-R wines (on the left side of the graph). So in this case, the PC1 is related to the type of wine. In the case of the Adriatic red and white wines, these two groups are not completely separated, probably due to the small number of samples analysed specifically for P-W. All red wines were on the positive side of PC2 as well as on the negative side of PC1. The results for red wines show that the two groups of red wines (Continental and Adriatic) can be partially separated. Moreover, the red wines from the Adriatic region showed higher values of PC2 than the red wines from the Continental region. Elements such as K, Ni, Mn and Sr showed a strong negative correlation with PC1. Elements K and Ni showed a positive correlation with PC2, while elements Mn and Sr showed a negative correlation. Moreover, the elements Rb and Ba could be assigned to the red wines from the Adriatic part of Croatia. The PC-based model derived from the original data set on the metal content of the wines studied gave a fairly satisfactory separation in the case of C-R, C-W and P-R wine. In the case of P-W wine, more samples should be included in further work.

Cluster analysis

Cluster analysis is an unsupervised method that allows different classes of geographical origins and wine types to be distinguished according to chemical compositions. The objects are grouped into clusters according to their similarity. Cluster analysis was applied to the standardized data, Euclidean distance was used to calculate sample similarities, and Ward's hierarchical agglomerative procedure was used to form clusters. The obtained results are presented as a dendrogram in Fig. 3. In general, a multivariate cluster analysis of the variables showed

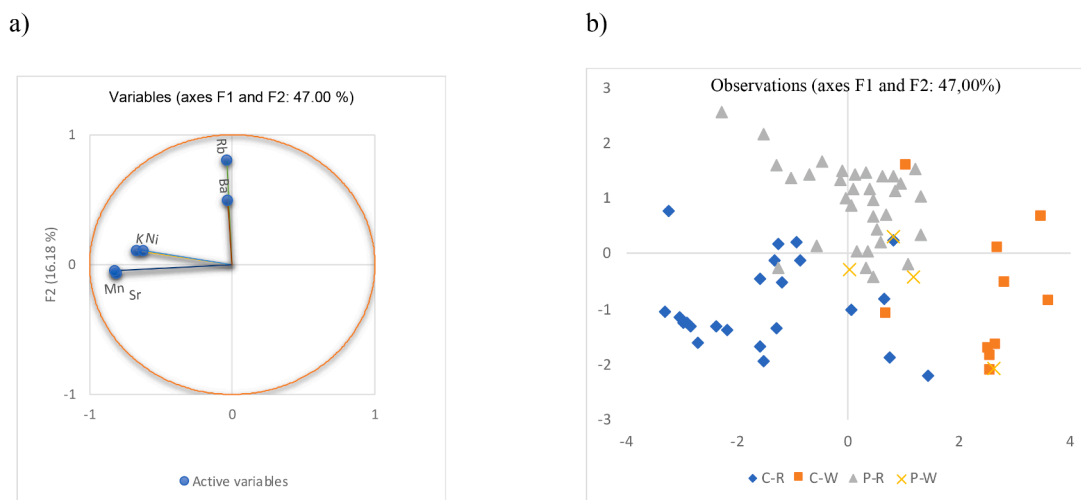


Fig. 2. Principal component analysis (PCA). a) Loading plot of elements data in wine samples. b) Scores of the wine samples on the first two PCs.

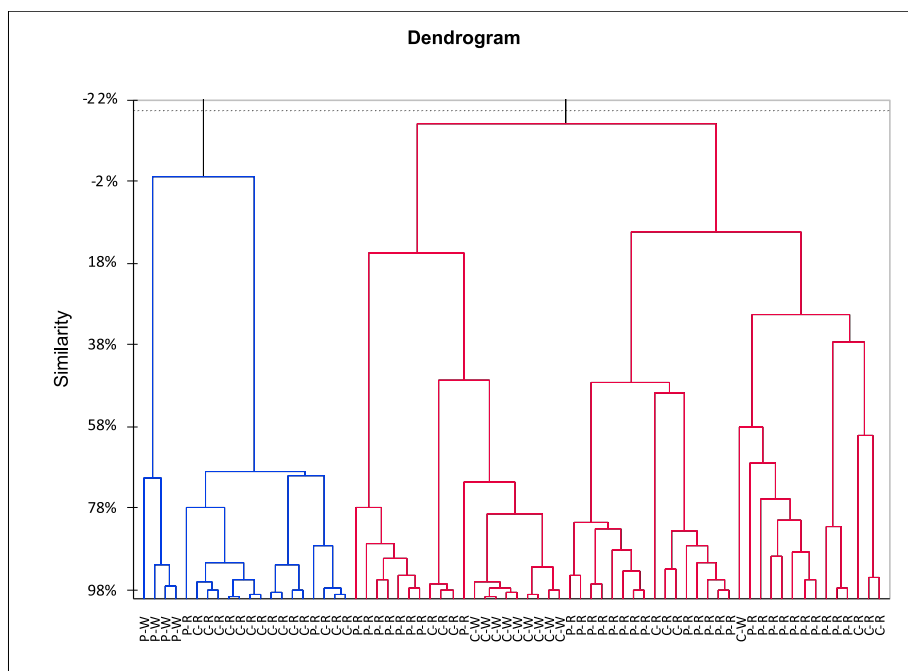


Fig. 3. Dendrogram obtained by hierarchical cluster analysis based on the Euclidean distance between samples for the metals determined by TXRF. For the abbreviations of the samples' names, see Table S2.

the presence of two main clusters corresponding to the mineral composition in the analysed wine samples. The first cluster is formed by two subclusters, one of which consists of P-W samples and the second of C-R samples with two samples as exceptions. In the second cluster, four subclusters can be observed. The first one consists of C-R samples, the second one mainly of C-W samples, and the third and fourth ones mainly of P-R samples. Accordingly, this observation suggests that the variables used have sufficient explanatory power to identify the geographical origin and type of wine.

Linear discriminant analysis

In order to evaluate possibility of classifying wine samples into these four groups, a common supervised pattern recognition technique such as linear discriminant analysis (LDA) was used. This technique is a widely used parametric method for classification purposes, which assumes an *a priori* knowledge of the number of classes and the membership of the

samples. The classification was performed according to the denominations of origin: C-R, C-W, P-R and P-W. As mentioned above, the variables selected were Mn, K, Ni, Sr, Rb and Ba. The stepwise forward selection approach was used to search for subset of variables most useful for discriminating between categories, and variables were selected according to Wilk's lambda criterion. Two statistically significant discriminant functions were obtained, in which Sr in the first function, Ni and Rb in the second one are the most important variables. The plot of the first and second functions is shown in Fig. 4, where differentiation among the analysed samples can be observed. According to function 1, a good differentiation is obtained between C-R and C-W wines, and according to function 2, the P-R category can be distinguished. The obtained results are presented in Table 2. The recognition ability was 100 % for each of the four categories. Leave-one-out cross validation was used to validate these results. The classifications obtained are shown in Table 2. The predictive ability for C-W and P-W was 100 %, for C-R was

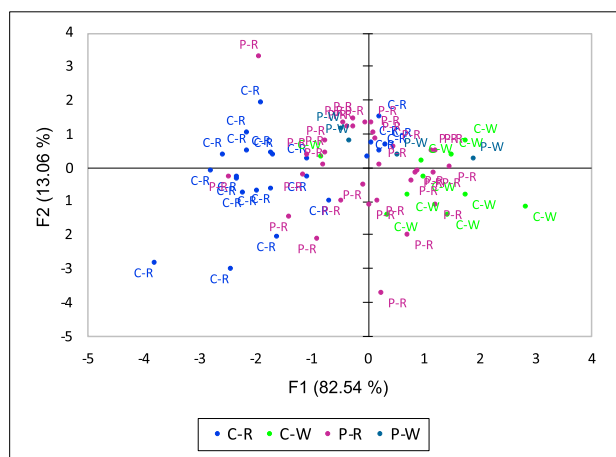


Fig. 4. Discriminant scatter plot of wine samples.

Table 2

Classification of the wine samples in the four categories (C-R; C-W; P-R; P-W) using Sr, Ni and Rb and LDA technique and Leave-one-out cross validation technique.

Recognition ability (%)	Prediction ability (%)	
C-R	100	95.6
C-W	100	100
P-R	100	90.1
P-W	100	100
Total	100	96.43

95.6% and for P-R 90.1% respectively. The data for sensitivity and specificity (Table S8) in conjunction with the LDA model for PCs show good results with sensitivity and specificity above 70%, except in the case of P-W, which was expected due to the small number of samples.

Conclusions

In this paper, thirteen elements were used to characterize different wine samples from two different geographical regions of Croatia and the type of wines. The concentrations of metals in the analyzed wines were below the MPC values, with some exceptions in a small number of analyzed red wines from both Continental and Adriatic parts of Croatia (Zn-one sample; Ni-four samples; Pb-one sample; Cu-one sample.) The results of the estimated average intake of certain metals from wine consumption show that the average consumption of Croatian wines does not expose consumers to significant amounts of the studied metals in terms of toxic effects. The THQ values obtained show that the consumption of Croatian wines does not significantly contribute to the potential health risks resulting from the lifetime intake of the metals analyzed. Univariate analysis showed that K, Mn, Ba and Ni can be considered as the most important characteristics to distinguish between Continental red wines and white wines, Rb, Ni and Ba can be considered to distinguish between the group of Continental red and Adriatic red wines while Sr is the only metal that completely distinguishes the samples of each category. PCA and CA showed that the variables used have sufficient explanatory power to group them according to the geographical origin and the type of wine. In the case of PCA, Mn, K, Ni and Sr show to be the most important ones differentiating by type of wine, while Rb and Ba are the most important variables related to origin. Finally, LDA showed good detection and prediction abilities using these selected elements. The predictive ability for C-W and P-W was 100%, for C-R 95.6% and for P-R 90.1% respectively. This study suggests that the metals are very good indicators of the origin and type of wine and can be used as criteria for distinguishing wines from Croatian wine-growing

regions. However, the absence of wines from P-R should be considered as a limitation of this study and in future works the number of analysed samples should be improved.

CRedit authorship contribution statement

D. Vitali Čepo: Conceptualization, Writing – original draft, Writing – review & editing. **M. Karoglan:** . **L. Borgese:** Methodology, Formal analysis, Investigation. **L.E. Depero:** Methodology. **E. Margui:** Methodology, Writing – review & editing. **J. Jablan:** Conceptualization, Writing – original draft, Formal analysis, Investigation, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.fochx.2022.100209>.

References

- Alkış, İ. M., Öz, S., Atakol, A., Yılmaz, N., Anlı, R. E., & Atakol, O. (2014). Investigation of heavy metal concentrations in some Turkish wines. *Journal of Food Composition and Analysis*, 33(1), 105–110.
- Almeida, C. M. R., Vasconcelos, M. T. S. D., Barbaste, M., & Medina, B. (2002). ICP-MS multi-element analysis of wine samples—A comparative study of the methodologies used in two laboratories. *Analytical and Bioanalytical Chemistry*, 374(2), 314–322. <https://doi.org/10.1007/s00216-002-1467-8>
- Banović, M., Kirin, J., Čurko, N., & Kovacević Ganić, K. (2009). Influence of vintage on Cu, Fe, Zn and Pb content in some Croatian red wines. *Czech Journal of Food Sciences*, 27(Special Issue 1), S401–S403.
- Borgese, L., Bilo, F., Dalipi, R., Bontempi, E., & Depero, L. E. (2015). Total reflection X-ray fluorescence as a tool for food screening. *Spectrochimica Acta Part B: Atomic Spectroscopy*, 113, 1–15. <https://doi.org/10.1016/j.sab.2015.08.001>
- Bukovčan, R., Kubanović, V., Gašparec-Skočić, L., & Banović, M. (2008). Validation of ICP-OES method for wine macro and microelements determination. In *Proceedings of the Joint 4th Central European Congress on Food and 6th Croatian Congress of Food Technologists, Biotechnologists and Nutritionists* (p. 169).
- Cocatto, A., Vekemans, B., Vincze, L., Moens, L., & Vandenabeele, P. (2016). Pigment particles analysis with a total reflection X-ray fluorescence spectrometer: Study of influence of instrumental parameters. *Applied Physics A*, 122(12), 1051.
- Dalipi, R., Borgese, L., Zacco, A., Tsuji, K., Sangiorgi, E., Piro, R., ... Depero, L. E. (2015). Determination of trace elements in Italian wines by means of total reflection X-ray fluorescence spectroscopy. *International Journal of Environmental Analytical Chemistry*, 95(13), 1208–1218.
- Dalipi, R., Margui, E., Borgese, L., Bilo, F., & Depero, L. E. (2016). Analytical performance of benchtop total reflection X-ray fluorescence instrumentation for multielemental analysis of wine samples. *Spectrochimica Acta Part B: Atomic Spectroscopy*, 120, 37–43. <https://doi.org/10.1016/j.sab.2016.04.00110.1016/j.foodchem.2016.09.022>
- Díaz, C., Conde, J. E., Estévez, D., Pérez Olivero, S. J., & Pérez Trujillo, J. P. (2003). Application of multivariate analysis and artificial neural networks for the differentiation of red wines from the Canary Islands according to the island of origin. *Journal of Agricultural and Food Chemistry*, 51(15), 4303–4307. <https://doi.org/10.1021/jf0343581>

- Fiket, Ž., Mikac, N., & Kniewald, G. (2011). Arsenic and other trace elements in wines of eastern Croatia. *Food Chemistry*, 126, 941–947. <https://doi.org/10.1016/j.foodchem.2010.11.091>
- Frías, S., Conde, J. E., Rodríguez-Bencomo, J. J., García-Montelongo, F., & Pérez-Trujillo, J. P. (2003). Classification of commercial wines from the Canary Islands (Spain) by chemometric techniques using metallic contents. *Talanta*, 59(2), 335–344. [https://doi.org/10.1016/S0048-9697\(01\)00912-3](https://doi.org/10.1016/S0048-9697(01)00912-3)
- Galani-Nikolakaki, S., Kallithrakis-Kontos, N., & Katsanos, A. A. (2002). Trace element analysis of Cretan wines and wine products. *The Science of the Total Environment*, 285(1–3), 155–163. [https://doi.org/10.1016/S0048-9697\(01\)00912-3](https://doi.org/10.1016/S0048-9697(01)00912-3)
- García-Palacios, A., Hoffman, H. G., Kwong See, S., Tsai, A., & Botella, C. (2001). Redefining Therapeutic Success with Virtual Reality Exposure Therapy. *CyberPsychology & Behavior*, 4(3), 341–348. <https://doi.org/10.1089/109493101300210231>
- Grindlay, G., Mora, J., Gras, L., & Loos-Vollebregt, M. (2011). Atomic Spectrometry Methods for Wine Analysis: A Critical Evaluation and Discussion of Recent Applications. *Analytica Chimica Acta*, 691, 18–32. <https://doi.org/10.1016/j.aca.2011.02.050>
- Jablan, J., Besalu, E., Žarak, M., Dumić, J., & Marguí, E. (2021). Analytical potential of total reflection X-ray fluorescence spectrometry for simultaneous determination of iron, copper and zinc in human blood serum and plasma. *Talanta*, 233, Article 122553. <https://doi.org/10.1016/j.talanta.2021.122553>
- Karataş, D., Aydın, F., Aydın, I., & Karataş, H. (2015). Elemental composition of red wines in Southeast Turkey. *Czech Journal of Food Sciences*, 33 (2015)(No. 3), 228–236. <https://doi.org/10.17221/58/2015-CJFS>
- Klockenkämper, R., & von Bohlen, A. (2015). Total-Reflection X-Ray Fluorescence Analysis and Related Methods: Second Edition. In *Total-Reflection X-Ray Fluorescence Analysis and Related Methods: Second Edition*. Doi: 10.1002/9781118985953.
- Kment, P., Mihaljević, M., Ettler, V., Šebek, O., Strnad, L., & Rohlová, L. (2005). Differentiation of Czech wines using multielement composition – A comparison with vineyard soil. *Food Chemistry*, 91(1), 157–165. <https://doi.org/10.1016/j.foodchem.2004.06.010>
- Larcher, R., & Nicolini, G. (2008). Elements and inorganic anions in winemaking: Analysis and applications. *Hypphenated Techniques in Grape and Wine Chemistry*, 289–333.
- Leder, R., Petric, I., Jusup, J., & Banović, M. (2021). Geographical Discrimination of Croatian Wines by Stable Isotope Ratios and Multielemental Composition Analysis. *Frontiers in Nutrition*, 8, Article 625613. <https://doi.org/10.3389/fnut.2021.625613>
- Machado, I. (2018). A green analytical method for the determination of Cu, Fe, Mn, and Zn in wheat flour using total reflection X-ray fluorescence. 5.
- Martin, A. E., Watling, J., & Lee, G. (2012). The multi-element determination and regional discrimination of Australian wines. *Food Chemistry*, 133(3), 1081–1089. <https://doi.org/10.1016/j.foodchem.2012.02.013>
- Naughton, D., & Petróczi, A. (2009). Correction: Heavy metal ions in wines: Meta-analysis of target hazard quotients reveal health risks. *Chemistry Central Journal*, 3, 6. <https://doi.org/10.1186/1752-153X-3-6>
- Obhodas, J., Valković, V., Vinković, A., Sudac, D., Čanadija, I., Pensa, T., Fiket, Ž., Turyanskaya, A., Bretschneider, T., Wilhelmer, C., Gunchin, G., Kregsamer, P., Wobrauschek, P., & Strelh, C. (2021). X-ray Fluorescence Techniques for Element Abundance Analysis in Wine. *ACS Omega*, 6(35), 22643–22654. <https://doi.org/10.1021/acsomega.1c02731>
- Organisation Internationale de la vigne et du vin. oiv.int. Retrieved April 28, 2021, from <http://www.oiv.int/fr/>.
- Paneque, P., Alvarez-Sotomayor, M. T., Clavijo, A., & Gómez, I. A. (2010). Metal content in southern Spain wines and their classification according to origin and ageing. *Microchemical Journal*, 94(2), 175–179. <https://doi.org/10.1016/j.microc.2009.10.017>
- Pessanha, S., Carvalho, M. L., Becker, M., & von Bohlen, A. (2010). Quantitative determination on heavy metals in different stages of wine production by Total Reflection X-ray Fluorescence and Energy Dispersive X-ray Fluorescence: Comparison on two vineyards. *Spectrochimica Acta Part B: Atomic Spectroscopy*, 65(6), 504–507. <https://doi.org/10.1016/j.sab.2010.04.003>
- Plotka-Wasyłka, J., Frankowski, M., Simeonov, V., Polkowska, Ż., & Namieśnik, J. (2018). Determination of Metals Content in Wine Samples by Inductively Coupled Plasma-Mass Spectrometry. *Molecules*, 23(11). <https://doi.org/10.3390/molecules23112886>
- Pohl, P. (2007). What do metals tell us about wine? *TrAC Trends in Analytical Chemistry*, 26(9), 941–949. <https://doi.org/10.1016/j.trac.2007.07.005>
- Rankine, B. C. (1989). *Making Good Wine: A Manual of Winemaking Practice for Australia and New Zealand*. Sun Books.
- Schiavo, D., Neira, J. Y., & Nóbrega, J. A. (2008). Direct determination of Cd, Cu and Pb in wines and grape juices by thermospray flame furnace atomic absorption spectrometry. *Talanta*, 76(5), 1113–1118. <https://doi.org/10.1016/j.talanta.2008.05.010>
- Šebecić, B., Pavišić-Strache, D., & Vedralina-Dragojević, I. (1998). Trace elements in wine from Croatia. *Deutsche Lebensmittel-Rundschau*, 94(10), 341.
- Selih, V. S., Sala, M., & Drgan, V. (2014). Multi-element analysis of wines by ICP-MS and ICP-OES and their classification according to geographical origin in Slovenia. *Food Chemistry*, 153, 414–423. <https://doi.org/10.1016/j.foodchem.2013.12.081>
- Šeruga, M., Nemet, I., & Laslavić, B. (2008). Heavy metals content of some Croatian wines. *Deutsche Lebensmittel-Rundschau*, 104(11/12), 46–54.
- Stobbaerts, R., Robberecht, H., & Deelstra, H. (1995). Daily Dietary Intake of Manganese by Several Population Groups in Belgium: Preliminary Reports. *Journal of Trace Elements in Medicine and Biology*, 9(1), 44–48. [https://doi.org/10.1016/S0946-672X\(11\)80008-8](https://doi.org/10.1016/S0946-672X(11)80008-8)
- Tariba, B. (2011). Metals in Wine—Impact on Wine Quality and Health Outcomes. *Biological Trace Element Research*, 144, 143–156. <https://doi.org/10.1007/s12011-011-9052-7>
- Vitali Čepo, D., Pelajić, M., Vinković Vrček, I., Krivohlavek, A., Žuntar, I., & Marko, K. (2017). Differences in the levels of pesticides, metals, sulphites and ochratoxin A between organically and conventionally produced wines. *Food Chemistry*, 246. <https://doi.org/10.1016/j.foodchem.2017.10.133>
- Volpe, M. G., La Cara, F., Volpe, F., De Mattia, A., Serino, V., Petitto, F., ... Di Stasio, M. (2009). Heavy metal uptake in the enological food chain. *Food Chemistry*, 117(3), 553–560. <https://doi.org/10.1016/j.foodchem.2009.04.033>
- Woldemariam, D. M., & Chandravanshi, B. S. (2011). Concentration levels of essential and non-essential elements in selected Ethiopian wines. *Bulletin of the Chemical Society of Ethiopia*, 25(2), Article 2. <https://doi.org/10.4314/bcse.v25i2.65852>
- Zoecklein, B., Fugelsang, K. C., Gump, B. H., & Nury, F. S. (1995). *Wine Analysis and Production*. Springer.