Fast-SEC coupled to ICP-MS for selected metal determination and its stability over time in polish wine

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Abstract
Metal ions contribute to the wine browning (as reaction catalysts), turbidity (in complexes), and astringency (due to interaction with tannins). Being aware that bioavailability and metabolism of metal ions in the human body are strictly related with their concentration and the form they occur it is important to monitor not only the total content of metals but also their speciation. Thus, we can obtain more information about their potential toxicity to human health. Present research shows the application of fast size exclusion chromatography (Fast-SEC) coupled to ICP-MS for the evaluation of the distribution of elements in different sized complexes in Polish wine. The study was focused on the selected ten elements (Al, B, Ba, Cu, Fe, Mn, Ni, Pb, Sr, Zn). Additional information of this work was the evaluation of the metal complex stability over time. Based on the obtained results, it can be seen that metal complexes present in the studied wine samples are stable even 10 months after the wine bottle opening.

Introduction
Analysis of Polish wines are rarely addressed topic in the literature. This is probably due to the fact that wine production in Poland is still a niche, aimed mainly at enriching the ecotourism offer of a given agrotourism farm. Polish wine is a product coming from small wineries, established due to strong motivation and passion of founders. Unfavorable conditions for vines cultivation forced winemakers to look for more resistant hybrid species of grapes, these are mostly: Regent, Rondo (red wines) and Solaris (white wines) [1].
Climate condition, winemaking technology and the grape cultivars used for its production cause that each wine has its own specific characteristic which is related with the wine composition and thus its organoleptic and aesthetic properties [1].

Metals stand as an important constituent of wines influencing their organoleptic properties and conservation [2, 3]. They influence the pH and ionic balance which in turn supports the cellular metabolism of yeast. Since specific metallic ions have important role in redox reactions, their presence influences wine browning, turbidity, and astringency [3]. Metals in wine come from primary (endogenous) sources of natural origin like soil, in which vines are grown and secondary sources (exogenous). Exogenous sources result from external contamination like: environmental pollution of a specific region where the vineyard is located, viticulture practice—the use of fertilizers rich in Ca, Cu, or K, the use of pesticides or fungicides being a potential source of Cd, Cu, Mn, or Pb; or due to wine making technology and long contact of acidic wine with aluminum, brass, and stainless steel parts of the equipment used for wine production [4, 5]. The amount of metal varies during the vinification process. Concentration of most of metals present in wine is decreasing with each step of vinification process due to yeast consumption or precipitation with the use of tartarate [5]. However, if the maceration process is carried out and is extended too much or the wine has prolonged contact with the metal parts of the equipment the concentration of some metal ions such as Cr, Cu, Fe, and Zn may increase [5].

Monitoring of the content and forms of metals present in wines is important for both wine production and human health. Due to stable wine consumption after a significant boom in early 2000s, it is becoming one of the source of metal ions in a human diet; it contributes not only to the intake of metals that are essential for human health (i.e., Cu, Fe, and Zn) but also those which can cause adverse effects (i.e., Al, Pb) [3, 6]. However, their toxicity, bioavailability, and metabolism of metals in the human body is related to their chemical form and the concentration level at which they occur. Knowledge about metals pathways and their transport is crucial. Thus, in addition to monitoring the total content of metals in wine, metal-binding ligands and complexes tracking their speciation is of high interest [2].

Metals in wine can potentially occur as dissolved free ions and as complexes with various organic substances constituting wine matrix: low molecular weight compounds such as organic acids, amino acids and high molecular weight ones such as polysaccharides and polyphenols [7].

Analytical methods used for the metal complex analysis in wines and beverages usually involves extraction techniques like solid phase extraction or liquid–liquid extraction [8]. Herein, we describe the application of a fast size exclusion chromatography (Fast-SEC) ICP-MS methodology for evaluation of elements (Al, B, Ba, Cu, Fe, Mn, Ni, Pb, Sr, Zn) distribution in different sized complexes in wine originating from Polish wineries (located in different parts of Poland). An important part of the study was the assessment of the evaluation of distribution of different sized metal complexes over time.

Results and discussion

All ten measured analytes were determined in the studied wine samples. Detailed results together with the standard deviation as well as RSD are given in Table 1. The most abundant elements were: B (2.9–5.2 mg/dm$^3$ for the white wine, 3.6–5.6 mg/dm$^3$ for the red wine); Mn (0.8–2.9 mg/dm$^3$ for the white wine, 0.6–2.3 mg/dm$^3$ for the red wine); Zn (0.4–1.1 mg/dm$^3$ for the white wine, 0.07–1.4 mg/dm$^3$ for the red wine); and Fe (0.5–4.2 mg/dm$^3$ for the white wine, 0.6–2.5 mg/dm$^3$ for the red wine). The least abundant was Ni (0.003–0.1 mg/dm$^3$ for the white wine, 0.03–0.4 mg/dm$^3$ for the red wine) and Pb (0.008–0.1 mg/dm$^3$ for the white wine, 0.3–1.7 mg/dm$^3$ for the red wine). It needs to be mentioned that Pb content in examined red wines is higher than the acceptance limits established by The International Organization of Vine and Wine, which is 0.15 mg/dm$^3$ [9]. Higher content of the selected elements observed in red wines than white wines fits in the trends described in the literature [10].

It is difficult to observe any uniform tendency among the studied wines.

In the next stage, we assessed metal complexes size distribution in wine with the use of Fast-SEC ICP-MS. SEC with element selective detection by ICP-MS has been extensively used for similar applications including Pb speciation in wine [11], metal carbohydrate complexes in fruit and vegetable [12], Ni speciation in Sebertia acuminata [13], and metal complexes in cocoa [14]. However, due to the poor separation efficiency of classical SEC columns, this chromatographic technique has been considered unable to provide speciation data and used solely for the estimation of the molecular weight of the species present [15]. The recent introduction of SEC materials, having the solvent stability and mechanical strength which allow their use in Ultra-High-Performance Liquid Chromatography (UPLC) mode, provided significant improvements in separation efficiency and 3–fourfold shortening of the analysis time [16, 17]. In a given study, the species were separated within 15 min producing sharp peaks (2 peaks for B, Ba, Pb, and Sr; 1 peak for the rest of compounds). The separation efficiency was compared to HILIC which is considered to have much better resolving power [18]. A care has been taken to assure that the eluent is compatible with both detections techniques which required. Fast-SEC ICP-MS allowed the observation of relatively high molecular weight (10 kDa) species which
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cannot be analyzed by HILIC ICP-MS. Peak widths and peak shapes are repeatable - 3% RSD. Moreover, in HILIC...
Thus, size calibration and complete validation should be done to create robust and reliable method for speciation analysis in wine.

The additional information obtained in this research was the evaluation of the distribution of elements in different sized complexes over time. Hence, the stability of metal–organic matter complexes formed in wine was checked on wine from freshly opened bottle and then 10 months after the wine bottle opening. The retention time, number of peaks, and in most cases even intensities of peaks responsible for the compounds associated with the monitored metal ions did not change. Based on the mentioned observation, it can be assumed that metal complexes formed in wine are stable regardless the chemical changes occurring in wine during its ageing. However, different analytical techniques like ESI–MS, which allows metal complex identification should be used to be sure that the metal complexes analyzed are not a different complex of the same species.

Materials and methods

Reagents

The reagents used for digestions, dilutions, and the preparation of HPLC mobile phases were: ammonium acetate (≥ 98% for molecular biology, Sigma-Aldrich St. Louis, USA), nitric acid (V) (70%, Fisher Chemical, Loughborough, UK), acetonitrile (≥ 99.9%, Sigma-Aldrich St. Louis, USA) hydrogen peroxide (30%, Sigma-Aldrich St. Louis, USA), hydrochloric acid (37%, Fluka, Steinheim, Germany). Standard solutions (1000 ppm, SPC SCIENCE) of Al, B, Ba, Cu, Fe, Mn, Ni, Pb, Sr, Zn were used for the preparation of calibration curves and as internal standards (Sc and Rh). Methanol (Sigma-Aldrich St. Louis, USA) was used for column cleaning. Milli-Q® Type 1 Ultrapure Water Systems (Millipore, Bedford, MA) deionized water was used throughout.

Instrumentation

MiniSpin centrifuge (Eppendorf AG, German) and DigiPrep MS system (SCP Science, Quebec, Canada) were used. Two chromatographic systems: Agilent 1200 Series (Tokyo, Japan) and Dionex Ultimate 3000 RS (Thermo Scientific, UHPLC, Germany) were used. The ICP-MS spectrometer was ICP-MS 7500 (Agilent Technologies, Tokyo, Japan) equipped with an integrated autosampler (I-AS).

Wine samples

For the analysis, 13 bottles of wines from different regions of Poland were purchased in the local market. The wine bottles were protected from light and were kept in consistent temperature (20 °C) and (4 °C) after opening. The bottles of wine were opened directly before the analysis. Red wine (six bottles), white wine (seven bottles) were analyzed. Specific characteristic of all the wine samples is gathered in Table 2.

Total metal analysis

The method used for the elemental analysis was based on the method described in the previous studies [21–23]. In brief, for the total metal analysis 25 cm³ of wine was evaporated to dryness (80 °C through 7 h) and then digested with a mixture of nitric acid and hydrogen peroxide in ratio 3:1 in DigiPREP MS system for 2 h at 65 °C. The digests were cooled down to the room temperature. After an appropriate dilution and the addition of internal standards (Sc and Rh), the total content of metals of interest, including: Al, B, Ba, Cu, Fe, Mn, Ni, Sr, Pb, Zn was measured by inductively coupled plasma mass spectrometry. The experimental
Fig. 2  Cu (II) and Zn (II) complex size distribution over time in red wines
parameters, such as inductively coupled plasma power, torch position, and voltage applied to extraction and focusing lenses, were optimized daily. Hydrogen was used as a reaction gas to reduce spectral interferences. Analytical blanks were run in parallel. External calibration using 12-point calibration curves for metals of interest was used.

Fig. 3  Ba (II) and Sr (II) complex size distribution over time in analyzed wines
Speciation analysis

The selected wine samples were diluted (1:1 for the white wine and 1:2 for the red wine) with the mobile phase. For the separation of metal species with the SEC column (Acquity UPLC BEH 125 SEC 1.7 μm, 4.6 mm × 150 mm and guard column Acquity UPLC Protein BEH SEC guard column 125 Å, 1.7 μm, 4.6 mm × 30 mm 1 K—80 K, 1/ pk), the mobile phase of 10 mM ammonium acetate (pH 6.5) and ACN was used as mobile phases with a flow rate 0.1 cm³/min at gradient 0–2.5 min 80% B; 2.5–22.5 min 50% B; 22.5–25 min 50% B; 25–26 min 80% B; 26–35 min 80% B. Injection volume was 2 mm³.

The separated species were detected by ICP-MS on the basis of their metal-specific signal; the conditions were the same as those used for the total metal analysis.

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References

1. Dobrowolska-Iwanek J, Gąstoł M, Krośniak M, Jancik M, Zagrodzki P (2014) S Afr J Enol Vitic 35:1
2. Płotka-Wasylka J, Rutkowska M, Cieślik B, Tyburcy A, Namieśnik J (2017) J Anal Meth Chem 23:1
3. Tariba B (2011) Biol Trace Element Res 144:143
4. Dumitriu GD, Teodosiu C, Morosanu I, Jitar O, Cotea VV (2019) Bio Web Conf 15:02024
5. Fabjanowicz M, Płotka-Wasylka J (2021) Trends Food Sci Tech 112:382
6. Acuti D, Mazzoli V, Grazzini L, Rinaldi R (2020) British Food J 122:2655
7. Latorre M, Herbello-Hermelo P, Peña-Farfal C, Neira Y, Bermejo-Barrera P, Moreira-Piñeiro A (2019) Talanta 195:558
8. Fabjanowicz M, Płotka-Wasylka J (2021) Trends Food Sci Tech 112:382