Research Article

Biljana Arsić*, Danijela Kostić, Jelena Mrmošanin, Ivana Zlatanović, Snežana Marković, Stefan Petrović, Vesna Stankov-Jovanović, Sonja Janković, Ivana Rašić Mišić, Milan Mitić, Aleksandra Pavlović, Snežana Tošić, Aneta Georgijev, Gordana Stojanović

Quality control analyses of selected honey samples from Serbia based on their mineral and flavonoid profiles, and the invertase activity

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Abstract: Nowadays, climate changes induce deviations in the composition of honey varieties characteristic of a specific geographic region. Therefore, according to Codex Alimentarius for honey, the slight variations in values of determined parameters cannot be strictly regarded as a sign of its adulteration. Here, modified methods are presented for preparing honey samples for inductively coupled plasma-optical emission spectroscopy (ICP-OES) analysis and the high-performance liquid chromatography (HPLC) method to use them as fast and reliable methods in the food industry. ICP-OES analysis suggested that all analysed honey samples are natural, and HPLC-DAD (diode-arrray detection) analysis on the flavonoids showed that one sample is probably not natural. Invertase activity showed an overlap with this HPLC-DAD finding but suggested more samples as possibly adulterated, which can be neglected due to possible thermal treatment of those natural samples, causing a decrease in invertase activity. Therefore, results obtained from the analyses of investigated honey samples based on the mineral content, number, and quantity of flavonoids, together with the invertase activity, indicated that the combination of analyses could be a reliable tool for determining the quality of honey samples.

Keywords: elements, flavonoids, invertase, honey, quality

*** Corresponding author: Biljana Arsić,** Department of Chemistry, Faculty of Sciences and Mathematics, University of Niš, Višegradska 33, 18000 Niš, Republic of Serbia, e-mail: biljana.arsic@pmf.edu.rs
Danijela Kostić, Jelena Mrmošanin, Ivana Zlatanović, Stefan Petrović, Vesna Stankov-Jovanović, Sonja Janković, Ivana Rašić Mišić, Milan Mitić, Aleksandra Pavlović, Snežana Tošić, Gordana Stojanović: Department of Chemistry, Faculty of Sciences and Mathematics, University of Niš, Višegradska 33, 18000 Niš, Republic of Serbia
Snežana Marković, Aneta Georgijev: Timomed d.o.o. Knjaževac, Kej Veljka Vlahovića 7a, 19350 Knjaževac, Republic of Serbia

1 Introduction

Honey is a product of honeybees (*Apis mellifera*) containing sugars and water and small quantities of minerals, fatty acids, amino acids, vitamins, and proteins [1]. Botanical and geographical origins and purity are important factors affecting price [2].

The content of present minerals in honey and honey products was investigated extensively using different methods for sample preparation and different instrumental techniques, such as inductively coupled plasma-mass spectrometry (ICP-MS) [3] and inductively coupled plasma-optical emission spectroscopy (ICP-OES) [4].

Invertase activity is one of the critical parameters for estimating honey quality and freshness [5]. Bogdanov et al. [6] suggest that honey should have more than 10 IN (invertase number) and more than 4 for honey samples with a low enzymatic activity. Due to its susceptibility to storage and heating, it has been shown as a better freshness indicator than the diastase activity [6].

Flavonoids are present in tiny quantities in honey but significantly contribute to its nutritional and health properties [7]. Publications are available on flavonoids as a marker of the geographical [4] or biological origin [8] of honey or both [9].

Honey samples of different geographical origins can be distinguished using mineral content and chemometrics, and the quality can also be estimated. Still, studies have yet to compare the potential of mineral and flavonoid profiles with invertase activity on the same samples to determine the quality of honey samples.

This work aimed to estimate the quality of honey samples from the Republic of Serbia using their mineral and flavonoid profiles and the invertase activity. Some of the existing methods for preparing samples for ICP-OES were improved here. Clearly, the matrix effect was considered, and correction factors were applied to express quantities

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of determined flavonoids, which was mainly not applied in most of the published honey analyses. However, the matrix effect consideration has often been reported in analyses of other food varieties, such as milk and oil [10,11]. The previously published methods for preparing samples for ICP-OES were improved for easy, fast, and reliable work in the food industry.

2 Methods

2.1 Samples

Six honey samples (**1**–**6**) were obtained from "Timomed d.o.o." Knjazevac. Samples **1**, **2**, and **6** are blossom honey samples. Sample **3** is blossom honey from Subotica, sample **4** is meadow honey from a beekeeper from Negotin, and sample **5** is acacia honey from Knjaževac.

2.2 Chemicals

30% Hydrogen peroxide (Fluka, Switzerland) and ~65% nitric acid (Merck, Germany) were used for sample preparation for ICP-OES analysis. In performed analyses, ultra-pure (0.05 µS/cm) deionized water (MicroMed high purity water system, TKA Wasseraufbereitungssysteme GmbH) was used.

Quercetin hydrate ≥95%, rutin hydrate ≥94%, and (+)-catechin hydrate 98% were purchased from Sigma Aldrich (USA). Caffeic acid 99.5% was obtained from Dr. Ehrenstorfer GmbH (Germany). Gallic acid monohydrate, 98%, was provided by J&K Scientific GmbH (Germany). All the other chemicals used were analytical-grade reagent chemicals.

2.3 Physicochemical analyses of honey samples

All standard procedures used are described in detail in the Supplementary Material.

2.4 Determination of the content of the investigated elements in selected honey samples

2.4.1 Preparation of honey samples for the analysis of elements using ICP-OES

All volumetric flasks for preparing honey samples were cleaned with 5% HNO_3 and deionized water. Until recording, all prepared honey samples were stored in plastic containers. Honey samples **1**–**6** were prepared for ICP-OES analysis using four methods, details of which are provided below.

2.4.1.1 Method I

Method I is a modification of the already published method for preparing honey samples from the Canary Islands for atomic absorption spectrophotometry (AAS) [12]. Previously thermally treated porcelain crucibles in the electric oven at 500°C with steady mass were stored up until the experiment in the desiccators and were used for the preparation of honey samples for ICP-OES. Around 2.5 g of honey was weighed into prepared porcelain crucibles, and then they were put first into the electric dryer at 30°C for 1 h, then the temperature was raised to 60°C, and the samples were kept at that temperature for 2 h. The porcelain crucibles with the sample were transferred from the electric dryer to the electric oven, where the temperature was set to 350°C and kept for 8 min, then gradually cooled to 100°C for 2 h 20 min. The samples were kept at 100°C for 30 min. The temperature was increased to 150°C for the next 30 min, then 200°C for the next 30 min, and 250°C for the next 30 min. The oven was then switched off with the samples inside overnight. The following day, the oven's temperature before rising was 250°C and reached 350°C after around 75 min. The crucibles were kept for 2 h at 350°C, then increased to 500°C for the next hour and kept for 6 h. The oven was switched off, and the crucibles were left inside overnight. The following day, when the oven reached 500°C, the pots were kept at that temperature for 2 h 30 min. Then, the mineralized residues were dissolved in 2 mL conc. HNO_3 and diluted to 25 mL with deionized water.

2.4.1.2 Method II

Method II is a modification of the previously published method for the preparation of samples [13]. The heating plate was used, and the sand bath was placed on it. When the temperature reached 115°C, the heating was decreased, and the porcelain crucibles with honey samples (around 2.5 g) were placed into the sand bath and left for 3.5 h. Then, 3 mL conc. HNO_3 was added to each porcelain crucible and left to dissolve and evaporate in the sand bath. Afterwards, the crucibles were transferred into the oven and left at 450°C for 30 min, then increased to 500°C and left for 8 h; then the oven was switched off, and the pots were left inside overnight and for the next 2 days. They were again for 1 h at 500°C and then placed in the desiccator. After cooling, 2 mL conc. HNO_3 was added and then

Table 1: The ICP-OES parameters of the selected analytical lines for each element (wavelength, plasma view mode, correlation coefficient (*r*), LOD, LOQ, and linear range)

Element	λ (nm)	Plasma view mode	*r*	LOD (ppm)	LOQ (ppm)	Linear range (ppm)
Al	396.152	Axial	0.99953	0.00136	0.00453	0–100
As	189.042	Axial	0.99990	0.00415	0.01384	0–5
B	249.773	Axial	0.99999	0.00084	0.00279	0–25
Ca	393.366	Radial	0.99991	0.00014	0.00048	0–100
Cd	226.502	Axial	1	0.00016	0.00055	0–5
Co	228.616	Axial	1	0.00017	0.00006	0–5
Cr	284.325	Axial	0.99997	0.00058	0.00193	0–5
Cu	324.754	Axial	0.99998	0.00062	0.00206	0–50
Fe	259.940	Axial	0.99999	0.00042	0.00140	0–50
Hg	194.227	Axial	0.99999	0.00123	0.00409	0–5
K	766.490	Radial	0.99942	0.04477	0.14924	0–100
Mg	279.553	Radial	0.99999	0.00059	0.00196	0–100
Mn	257.610	Axial	0.99999	0.00010	0.000318	0–50
Na	589.592	Radial	1	0.00052	0.00173	0–100
Ni	231.604	Axial	0.99995	0.00037	0.00125	0–50
P	213.618	Radial	0.99993	0.00459	0.01531	0–100
Pb	220.353	Axial	0.99991	0.00178	0.00593	0–5
Se	196.090	Axial	0.99990	0.00545	0.01817	0–50
Si	251.611	Axial	1	0.00157	0.00524	0–100
V	311.071	Axial	1	0.00049	0.00162	0–25
Zn	202.548	Axial	1	0.00010	0.00032	0–50

evaporated to dryness on the sand bath. They were again placed in the oven at 500°C and left for 5 h. Then, the oven was switched off, and the pots were left in the oven. About 2 mL conc. HNO_3 was poured into the dry residue, and then deionized water was added to the mark into the volumetric flask (50 mL).

2.4.1.3 Method III

Method III is a modification of the earlier published method [14]. The investigated samples of honey (around 2.5 g) in Erlenmeyer flasks were dissolved into 36 mL demineralized water, then 0.16 mL conc. HNO_3 was added, and then 3.6 mL hydrogen peroxide (30%). They were placed in the ultrasound bath. After 30 min, some lost colouration, and some had a pale yellow colour. The samples were left there for 5 h 30 min. Afterwards, the solutions were filtered through blue-collar into the volumetric flask (50 mL) and filled with deionized water up to the mark.

2.4.1.4 Method IV

Method IV is a modification of the previously published method [12]. Around 2.5 g of honey was dissolved with 5 mL conc. HNO_3 and 1 mL H_2O_2 (30%) was heated on the hot plate until dissolution, left to cool, and then deionized water was added to the mark in the volumetric flask (25 mL).

2.4.2 ICP-OES analysis

Honey samples were analysed on an iCAP 6000 ICP-OES (Thermo Scientific, Cambridge, UK). The iTEVA operating software was used for this iCAP type of instrument.

The following operating conditions were used: the RF power generator for axial and radial plasma view mode 1,150 W, flush pump rate 100 rpm, analysis pump rate 50 rpm, coolant gas flow 12 L/min, auxiliary and nebulizer gas flow 0.5 L/min.

The following standards were used for the calibration curves: multielement standard solution III (Ca, K, Mg, and Na) and IV (Al, As, Ba, Be, B, Cd, Cr, Co, Cu, Fe, Pb, Mn, Ni, Se, Tl, V, and Zn), as well as individual standard solution of Si, P, and Hg (Fluka Analytical).

Concentrations were read at the wavelength with the best calibration curve, satisfying the criteria of no spectral interferences and with a high signal/background ratio. Wavelengths for each element were chosen based on selection criteria, and the characteristics of calibration curves

(r, limit of detection [LOD], and limit of quantification [LOQ]) are provided in Table 1.

2.5 Determination of organic acids and flavonoids in selected honey samples

2.5.1 Extraction of organic acids and flavonoids

The extraction of organic acids and flavonoids was done following the previously published procedure [15] with slight modifications. Around 3 g of honey samples were mixed with 4.5 mL of deionized water acidified with concentrated HCl to achieve pH 2.0, put in the ultrasound bath until the liquefication, and then added 30 µL hesperetin solution in methanol (217 µg/mL) which is used as internal standard. Then, these samples were transferred to the previously prepared (6 mL of the mixture made from methanol and deionized water (1:1, v/v) STRATA-X SPE cartridges (60 mg, 3 mL) (Phenomenex Inc., Torrance, CA, USA). Afterwards, the cartridges were treated with 2 mL acidified water and 5 mL ultrapure water. Then, the samples were dried for 13 min using a vacuum. The phenolic fraction remained on the cartridges and was eluted with 2 mL of methyl alcohol and acetonitrile (2:1, v/v) mixture. Phenolic fractions were then diluted twice with 10 mM sulfuric acid. Obtained solutions were transferred into glass bottles and left in the fridge. A blank probe comprised 4.5 mL of acidified water and 30 µL of hesperetin in methanol.

2.5.2 HPLC-DAD analysis of honey organic acids and flavonoids

An Agilent Technologies 1100 Series chromatograph containing a degasser, a binary pump, a thermostated column (Zorbax Eclipse XDB-C18, 4.6 × 150 mm, 5 µm), and an ultraviolet-visible (UV/Vis) detector were used for the analysis of flavonoids. The wavelength range was 180–400 nm, and the detection wavelengths used were 254, 280, and 350 nm. The mobile-phase components were the same as those used by Bertoncelj et al. [15]. The mobile-phase gradient was as follows: 0–5 min: 10% B; 5–50 min: 10–60% B; 50–52 min: 60–80% B; 52–60 min: 80% B; 60–70 min: 80–10% B; 70–80 min: 10% B. The mobile phase flow was 0.5 mL/min, the column was thermostated at 25°C, and the injected volume was 20 µL.

Calibration samples (matrix-based standard) were prepared by the extraction from 1.5 g of selected honey sample **3**, which had previously been spiked with hesperetin as the internal standard (0.03–1.2 mg/kg honey). The calibration samples were then treated according to the procedures described above. The calibration curve was made for the internal standard, hesperetin: Area = 207.26 × concentration + 57.859, R^2 = 0.9713. There are numerous proposed approaches for calculating LOD and LOQ [16]. LOD was calculated in this study as LOD = $3.3\sigma/S$, and LOQ value as LOQ = $10\sigma/S$. Here, σ is the standard deviation of the intercept of the chosen curve, and S is the slope of the calibration curve.

Organic acids and flavonoids were identified by comparing their retention times and spectral characteristics with the reference compounds (Figure S1) using the ChemStation. The quantification for flavonoids was performed based on the calibration curve of the internal standard, hesperetin, similar to the previous study [15], and the results were expressed as the mg hesperetin equivalents per kg of the honey sample (mg/kg).

2.6 Determination of the invertase activity in the selected honey samples

Determination of the invertase activity of selected honey samples was done following the previously published method [17]. To prepare the phosphate buffer solution (0.1 M; pH = 6.0), 11.66 g of KH_2PO_4 and 2.56 g of $Na_2HPO_4 \times 2H_2O$ were dissolved in water and diluted to 1 L. The substrate was a *p*-nitrophenyl-α-D-glucopyranoside (*p*NPG) solution (0.02 M). It was prepared by dissolving 6.0252 g of *p*NPG in a buffer solution and made up to 1 L. The solution of *p*NPG was dissolved by heating, but not above 60°C, and cooled after the solution was complete. Reaction-terminating solution (3 M, pH = 9.5) was made by dissolving 363.42 g of tris-(hydroxymethyl) aminomethane in water and diluting to 1 L. pH-value of 9.5 was adjusted with 3 M HCl solution.

A mass of honey (5.0 g) was quantitatively transferred into a volumetric flask (25 mL), rinsed with buffer solution, and filled to the mark. A volume of 5 mL of substrate solution was placed in a test tube in the water bath at 40°C for 5 min before adding the honey solution. A 0.5 mL of honey solution (starting time) was added. The contents were hand-mixed vigorously and incubated at 40°C. After exactly 20 min, 0.5 mL of the reaction-terminating solution was added and hand-mixed again (sample solution). 5 mL of substrate solution at 40°C was incubated for the blank. After 5 min, 0.5 mL of reaction-terminating solution was added, the tube was closed, mixed well, and then 0.5 mL of honey solution was added. A separate blank for each tested honey was prepared. The solutions were cooled to room temperature as quickly as possible, and the absorbances of the sample solutions and

the blank were measured in 1 cm cells at 400 nm. The readings were taken after about 15 min.

The absorbance of the blank was subtracted from that of the sample solution (ΔA). Therefore, the honey invertase activity (IA) can be calculated from the absorbance measured at 400 nm and is expressed in units/kg (U/kg): IA (U/kg) = 6 × 0.05 × 0.05298 × 104 × ΔA = 158.94 × ΔA, where 6 is a factor for the mL of sample solution used (total volume), 0.05 converts reaction time from 20 min to 1 min, 104 converts the amount of honey taken (0.1 g in 0.5 mL) to 1 kg, and 0.05298 = 7.37/139.11 is a conversion factor for µg into µM per mL. Invertase activity can also be expressed as: IN = (U/kg)/7.345.

2.7 Statistical analysis

The statistical differences in results obtained using ICP-OES with four preparation methods for each element and each investigated honey were checked using ANOVA from XLSTAT 2022 (Addinsoft, New York, USA). The tolerance level was set to 0.05, and the confidence interval was 95%. A pairwise comparison was performed using the Tukey test. Statistically significant differences between values were labelled with different letters in the results tables.

3 Results and discussion

All selected honey samples (**1**–**6**) mainly satisfy all prerequisites according to international regulatory standards of the International Honey Commission [6] determined at the laboratory at the company "Timomed d.o.o." Knjaževac. In analysed honey samples, moisture content ranges from 18.0 to 19.8% (allowed <20%), acidity ranges from 13 to 57 meq/kg (allowed <50 meq/kg), reducing sugars content is in the range 74.07–81.3 g/100 g (allowed >60 g/100 g), sucrose is in the range 1.2–4.7 g/100 g (allowed <5 g/100 g), diastase activity goes from 5 to 29.4 (not less than 8 Schade units and in the case of honey samples with a low natural enzyme content not less than 3 Schade Units), and hydroxymethylfurfural is in the range 0–54 mg/kg (allowed <40 mg/kg, and in areas with tropical temperatures <80 mg/kg) (Table S2). Values that deviate to a small extent from the recommended values can be a sign of thermal treatment [18,19] or a consequence of increased temperatures due to climate changes. Therefore, additional analyses are necessary for a fair estimation of the quality of honey samples.

3.1 Contents of the selected elements determined by ICP-OES

The selection of the sample preparation method is one of the most important steps in the analytical procedure that influences the validity of the obtained results. A preparation method's efficiency depends on the element's nature, matrix content, and decomposition conditions [20]. The content of selected elements shows different values depending on the preparation methods used (Tables 2–4).

The content of investigated elements in samples of the individual beekeepers (**1**–**6**) was compared, and toxic elements concentrations were checked against European regulations.

Values for elements obtained by ICP-OES (Tables 2–4) using different preparation methods show, in most cases, statistically different results using ANOVA.

Previous investigations show that some methods for the preparation of samples are better for the determination of particular elements [21]. The values found in honey samples from the Slovakia market show similar values for potassium (406.07–930.78 mg/kg) to most of the investigated samples in this study, higher than obtained values for calcium (293.92–320.03 mg/kg), in most cases higher than those for magnesium (56.56–64.19 mg/kg), and significantly higher values than found values for sodium (43.90–49.77 mg/kg) [22]. In Australian/Queensland honey samples, the values also vary for potassium (202–4,600 mg/kg), magnesium (6–190 mg/kg), sodium (3.7–382.7 mg/kg), phosphorus (12–920 mg/kg), and calcium (21–270 mg/kg) [1]. Compared to honey samples previously analysed in Serbia, values obtained for potassium are lower, and the same is true for magnesium and sodium. Calcium concentrations are like those found in the investigated honey samples [23]. Honey samples from Romania show a high range of potassium concentrations with a mean value of 190.43 mg/kg; the same is true for magnesium (mean 13.19 mg/kg), sodium (9.78 mg/kg), and calcium (31.73 mg/kg) [2]. A high range of concentrations of macroelements is also present in investigated honey samples from Serbia. The calcium level in investigated honey samples from Greece was similar to or lower than in this study [24].

In honey samples from the Slovakia market, selenium was only detected in sunflower honey; manganese was in similar concentrations (0.45–1.49 mg/kg) to found values, and iron was found in the concentration range 3.88–5.20 mg/kg; zinc (1.11–2.09 mg/kg) and copper (1.46–1.58 mg/kg) were in similar concentrations to most of the analysed honey samples [22]. Previously reported manganese concentrations in honey samples from Serbia show higher values than those found in this study, and the same statement is valid also for nickel, zinc, copper, iron, and chromium. Cobalt concentrations are similar

Table 2: The content of essential macroelements (K, Mg, Na, P and Ca) (mean ± SD [mg/kg]) in selected honey samples (**1**–**6**) prepared with four different methods for ICP-OES analysis

Sample	Method	K	Mg	Na	P	Ca
1	I	262.3 ± 0.4^{a}	11.68 ± 0.03^{b}	0.809 ± 0.003^{b}	33.8 ± 0.2^{b}	72.7 ± 0.5^{c}
2		473 ± 3^{a}	14.6 ± 0.1^{b}	0.528 ± 0.001^{b}	56.4 ± 0.2^{b}	165.7 ± 0.8^{a}
3		21.4 ± 0.5^{d}	2.58 ± 0.02^{d}	0.418 ± 0.004^{c}	4.50 ± 0.04^{d}	21.87 ± 0.05^{d}
4		745 ± 1^{a}	33.7 ± 0.2^{a}	0.611 ± 0.003^{b}	115.9 ± 0.3^{a}	148 ± 1^{a}
5		466 ± 1^{a}	12.9 ± 0.1^{c}	0.661 ± 0.003^{b}	64.3 ± 0.2^{b}	48.9 ± 0.5^{c}
6		244.7 ± 0.8^{a}	7.36 ± 0.06^{b}	0.503 ± 0.002^{b}	20.28 ± 0.03^{b}	55.3 ± 0.1^{b}
1	II	201 ± 2^{c}	11.2 ± 0.1^{c}	0.171 ± 0.004^{d}	34.8 ± 0.1^{a}	103.2 ± 0.7^{a}
2		389 ± 1^{c}	14.05 ± 0.07^{c}	0.287 ± 0.004^{d}	58.5 ± 0.2^{a}	59.9 ± 0.5^{c}
3		769 ± 3^{a}	49.6 ± 0.5^{a}	0.935 ± 0.008^{b}	130.3 ± 0.4^{a}	232 ± 2^{a}
4		41.5 ± 0.1^{d}	8.28 ± 0.06^{b}	0.112 ± 0.004^{d}	26.22 ± 0.08^{d}	48.0 ± 0.2^{d}
5		260 ± 2^{d}	13.8 ± 0.1^{b}	0.23 ± 0.01^{d}	67.2 ± 0.2^{a}	51.8 ± 0.1^{b}
6		203.4 ± 0.8^{c}	7.05 ± 0.09^{b}	0.209 ± 0.002^{d}	21.83 ± 0.08^{a}	62.4 ± 0.6^{a}
1	III	264 ± 6^{a}	10.6 ± 0.3^{d}	0.524 ± 0.006^{c}	26.4 ± 0.2^{c}	49 ± 1^{d}
2		442 ± 2^{b}	12.55 ± 0.09^{d}	0.43 ± 0.01^{c}	44.8 ± 0.1^{c}	37.6 ± 0.2^{d}
3		364 ± 2^{b}	31.7 ± 0.1^{c}	0.373 ± 0.004^{d}	52.06 ± 0.06^{b}	121.2 ± 0.9^{c}
4		664 ± 9^{b}	34.0 ± 0.5^{a}	0.575 ± 0.002^{c}	100.2 ± 0.6^{b}	105.9 ± 0.9^{c}
5		358 ± 6^{b}	11.3 ± 0.1^{d}	0.550 ± 0.004^{c}	50.5 ± 0.2^{c}	27.4 ± 0.2^{d}
6		219 ± 4^{b}	5.6 ± 0.1^{c}	0.46 ± 0.01^{c}	14.9 ± 0.1^{c}	32.6 ± 0.5^{c}
1	IV	222 ± 1^{b}	18.38 ± 0.04^{a}	3.52 ± 0.04^{a}	22.1 ± 0.2^{d}	79.6 ± 0.7^{b}
2		393 ± 5^{c}	22.8 ± 0.2^{a}	4.421 ± 0.003^{a}	38.8 ± 0.3^{d}	81 ± 1^{b}
3		334 ± 3^{c}	38.2 ± 0.2^{b}	3.68 ± 0.02^{a}	44.9 ± 0.3^{c}	147 ± 1^{b}
4		566 ± 3^{c}	34.2 ± 0.2^{a}	3.12 ± 0.02^{a}	85.8 ± 0.2^{c}	110.5 ± 0.1^{b}
5		299 ± 3^{c}	21.7 ± 0.2^{a}	4.74 ± 0.01^{a}	41.39 ± 0.09^{d}	75.1 ± 0.4^{a}
6		190 ± 4^{d}	13.6 ± 0.2^{a}	3.60 ± 0.02^{a}	12.80 ± 0.06^{d}	63.4 ± 0.6^{a}

or higher depending on the samples and the method used to prepare the samples for ICP-OES analysis [23]. Determined concentrations of manganese, vanadium, and zinc in honey samples from Romania are similar to the found values in analysed samples, lower for nickel, copper, and cobalt than found and higher for chromium [2]. Some other honey samples from Romania show manganese values similar to those found in analysed honey samples, and zinc and copper values were similar to ours or lower [2]. Greek honey samples show similar values for copper, iron, manganese, and silicon, although there are significant differences in nickel and zinc levels [24].

Most of our investigated honey samples show higher contamination with lead than previously published [23]. The investigated samples in this study show lower contamination with arsenic and cadmium than those previously investigated from Serbia [23]. Aluminium, lead, arsenic, and cadmium concentrations are higher than those in Romanian honey samples [2]. Obtained results for lead were similar to those found in honey samples from Greece, where, like in this study, some values exceeded the new recommended value for lead (0.1 mg/kg) but were less than the previously used limit value (1 mg/kg) [24]. Aluminium values in investigated Greek honey samples [24] were like found values, and arsenic was similar to or lower than in this study. Commission Regulation (EU) 2023/915 gave maximum levels for lead (0.1 mg/kg) for honey, but no values were provided for the maximum allowed contents of cadmium, mercury, and arsenic in honey [23]. Hopefully, these values will be defined in the future.

Performed ICP-OES analysis on the investigated honey samples suggests that all investigated honey samples are natural based on the content of elements, excluding potentially toxic and toxic elements. Since the level of toxic elements is essential due to the safety consumption of honey, their determination is mandatory according to Codex Alimentarius for honey.

3.2 Organic acids and flavonoids in honey samples

The organic acids were detected based on the available external standards, comparing the retention times and UV/Vis spectra recorded under the same conditions. Free gallic acid and *trans*-ferulic acid were not detected in investigated honey samples, syringic acid was detected in samples **2** and **4**, tartaric acid was found in all analysed

Table 3: The content of essential trace elements (Mn, Ni, Zn, B, Cu, Fe, Co, Cr, and Si) (mean ± SD [mg/kg]) in selected honey samples (n.d. – not detected) prepared with four different methods for ICP-OES analysis

Sample	Method	Mn	Ni	Zn	B	Cu	Fe	Co	Cr	Si
1	I	0.619 ± 0.003^{b}	1.224 ± 0.009^{b}	0.791 ± 0.008^{c}	4.79 ± 0.06^{a}	1.135 ± 0.009^{b}	4.76 ± 0.03^{b}	n.d.^{c}	n.d.^{c}	18.65 ± 0.07^{b}
2		0.790 ± 0.001^{a}	1.06 ± 0.05^{b}	3.30 ± 0.01^{a}	4.20 ± 0.03^{a}	1.73 ± 0.02^{b}	3.54 ± 0.01^{b}	n.d.^{c}	n.d.^{c}	23.65 ± 0.09^{a}
3		0.056 ± 0.001^{d}	0.081 ± 0.002^{b}	0.0000 ± 0.0000^{d}	1.11 ± 0.02^{d}	1.072 ± 0.004^{b}	1.634 ± 0.002^{d}	n.d.^{b}	n.d.^{b}	5.70 ± 0.01^{d}
4		0.630 ± 0.004^{a}	0.937 ± 0.005^{b}	1.074 ± 0.005^{c}	8.63 ± 0.05^{b}	2.23 ± 0.01^{b}	4.823 ± 0.007^{c}	0.028 ± 0.001^{b}	n.d.^{b}	23.30 ± 0.08^{a}
5		0.485 ± 0.003^{b}	0.853 ± 0.006^{b}	0.482 ± 0.003^{d}	4.43 ± 0.02^{a}	5.48 ± 0.02^{a}	3.73 ± 0.01^{b}	0.008 ± 0.001^{b}	n.d.^{b}	11.59 ± 0.01^{b}
6		0.294 ± 0.001^{b}	0.520 ± 0.001^{b}	1.083 ± 0.005^{d}	1.94 ± 0.02^{a}	0.912 ± 0.005^{c}	4.010 ± 0.006^{b}	n.d.^{b}	n.d.	22.6 ± 0.1^{a}
1	II	0.756 ± 0.006^{a}	10.42 ± 0.03^{a}	1.381 ± 0.002^{a}	0.20 ± 0.01^{d}	3.15 ± 0.02^{a}	31.2884 ± 0.0000^{a}	0.023 ± 0.001^{b}	0.68 ± 0.01^{a}	19.2 ± 0.1^{a}
2		0.665 ± 0.004^{d}	3.16 ± 0.03^{a}	1.057 ± 0.006^{d}	1.3 ± 0.1^{c}	3.57 ± 0.03^{a}	17.16 ± 0.08^{a}	0.131 ± 0.004^{a}	0.72 ± 0.02^{a}	15.8 ± 0.2^{b}
3		0.555 ± 0.004^{a}	1.15 ± 0.01^{a}	1.84 ± 0.01^{a}	10.48 ± 0.09^{a}	3.81 ± 0.03^{a}	12.77 ± 0.05^{a}	0.064 ± 0.002^{a}	0.49 ± 0.03^{a}	19.9 ± 0.2^{a}
4		0.3491 ± 0.0000^{d}	4.226 ± 0.002^{a}	0.632 ± 0.004^{d}	n.d.^{d}	3.28 ± 0.01^{a}	28.13 ± 0.06^{a}	0.221 ± 0.006^{a}	0.66 ± 0.01^{a}	16.8 ± 0.1^{b}
5		0.589 ± 0.004^{a}	3.76 ± 0.02^{a}	0.607 ± 0.004^{c}	1.54 ± 0.04^{c}	3.73 ± 0.04^{b}	19.55 ± 0.13^{a}	0.065 ± 0.004^{a}	0.67 ± 0.01^{a}	20.14 ± 0.08^{a}
6		0.496 ± 0.002^{a}	16.1 ± 0.1^{a}	1.72 ± 0.02^{a}	n.d.^{b}	2.532 ± 0.004^{a}	40.3 ± 0.1^{a}	0.0698 ± 0.0000^{a}	0.46 ± 0.02	17.00 ± 0.04^{b}
1	III	0.496 ± 0.002^{c}	0.288 ± 0.004^{c}	1.16 ± 0.01^{b}	0.54 ± 0.05^{c}	0.70 ± 0.01^{c}	2.32 ± 0.03^{d}	0.031 ± 0.002^{a}	0.040 ± 0.003^{b}	5.80 ± 0.03^{c}
2		0.684 ± 0.004^{b}	0.157 ± 0.002^{c}	1.892 ± 0.004^{b}	0.86 ± 0.03^{d}	0.700 ± 0.004^{c}	1.35 ± 0.02^{c}	0.020 ± 0.002^{b}	n.d.^{b}	5.67 ± 0.03^{c}
3		0.144 ± 0.002^{c}	0.059 ± 0.006^{c}	1.021 ± 0.004^{b}	5.58 ± 0.05^{c}	0.456 ± 0.006^{c}	1.75 ± 0.02^{c}	n.d.^{b}	n.d.^{b}	18.6 ± 0.2^{b}
4		0.492 ± 0.008^{c}	0.106 ± 0.004^{d}	1.61 ± 0.02^{a}	4.03 ± 0.09^{c}	0.78 ± 0.02^{c}	1.54 ± 0.05^{d}	0.007 ± 0.001^{d}	n.d.^{b}	11.8 ± 0.2^{d}
5		0.435 ± 0.002^{d}	0.103 ± 0.002^{c}	1.034 ± 0.004^{a}	0.282 ± 0.006^{d}	0.500 ± 0.008^{c}	1.89 ± 0.03^{d}	n.d.^{c}	n.d.^{b}	2.60 ± 0.05^{c}
6		0.220 ± 0.002^{d}	0.052 ± 0.002^{c}	1.67 ± 0.01^{b}	n.d.^{b}	1.00 ± 0.02^{b}	1.59 ± 0.04^{d}	n.d.^{b}	0.256 ± 0.002	1.75 ± 0.06^{d}
1	IV	0.476 ± 0.003^{d}	0.023 ± 0.002^{d}	0.800 ± 0.007^{c}	1.29 ± 0.06^{b}	0.229 ± 0.008^{d}	3.33 ± 0.01^{c}	0.033 ± 0.002^{a}	n.d.^{c}	4.9 ± 0.1^{d}
2		0.685 ± 0.006^{c}	0.049 ± 0.001^{d}	1.194 ± 0.009^{c}	1.94 ± 0.02^{b}	0.199 ± 0.008^{d}	3.45 ± 0.04^{b}	0.023 ± 0.001^{b}	n.d.^{c}	5.43 ± 0.02^{c}
3		0.209 ± 0.003^{b}	0.066 ± 0.002^{bc}	0.983 ± 0.009^{c}	6.28 ± 0.01^{b}	0.201 ± 0.006^{d}	3.90 ± 0.02^{b}	n.d.^{b}	n.d.^{b}	17.56 ± 0.07^{c}
4		0.512 ± 0.002^{b}	0.127 ± 0.007^{c}	1.558 ± 0.004^{b}	19.38 ± 0.05^{a}	0.518 ± 0.005^{d}	4.96 ± 0.04^{b}	0.0164 ± 0.0005^{c}	n.d.^{b}	14.2 ± 0.1^{c}
5		0.473 ± 0.002^{c}	0.052 ± 0.002^{d}	0.872 ± 0.006^{b}	1.96 ± 0.05^{b}	0.235 ± 0.003^{d}	2.262 ± 0.008^{c}	n.d.^{c}	n.d.^{b}	2.48 ± 0.05^{c}
6		0.260 ± 0.002^{c}	0.0214 ± 0.0000^{c}	1.13 ± 0.01^{c}	0.003 ± 0.001^{b}	0.087 ± 0.001^{d}	2.88 ± 0.05^{c}	n.d.^{b}	n.d.	2.20 ± 0.02^{c}

Table 4: Toxic and potentially toxic elements (Al, Pb, As, and Cd) concentrations (mean ± SD [mg/kg]) in selected honey samples prepared with four different methods for ICP-OES analysis

Sample	Method	Al	Pb	As	Cd
1	I	4.27 ± 0.02^{b}	0.111 ± 0.007^{a}	n.d.^{a}	n.d.^{a}
2		5.47 ± 0.02^{b}	0.19 ± 0.01^{a}	n.d.^{a}	n.d.^{a}
3		1.881 ± 0.006^{b}	0.039 ± 0.004^{d}	n.d.^{a}	n.d.^{b}
4		3.62 ± 0.02^{c}	0.111 ± 0.004^{b}	n.d.^{b}	0.0025 ± 0.0005^{ab}
5		3.61 ± 0.03^{b}	0.194 ± 0.008^{a}	n.d.^{a}	n.d.^{a}
6		5.40 ± 0.02^{b}	0.049 ± 0.003^{a}	n.d.^{a}	n.d.^{b}
1	II	6.88 ± 0.03^{a}	0.038 ± 0.006^{b}	n.d.^{a}	n.d.^{a}
2		5.76 ± 0.03^{a}	0.071 ± 0.003^{b}	n.d.^{a}	n.d.^{a}
3		5.04 ± 0.03^{a}	0.21 ± 0.02^{b}	n.d.^{a}	n.d.^{b}
4		4.04 ± 0.03^{b}	0.111 ± 0.007^{b}	0.027 ± 0.005^{a}	n.d.^{b}
5		8.66 ± 0.02^{a}	0.089 ± 0.008^{c}	n.d.^{a}	n.d.^{a}
6		6.51 ± 0.05^{a}	0.25 ± 0.01^{a}	n.d.^{a}	n.d.^{b}
1	III	0.863 ± 0.006^{d}	0.109 ± 0.003^{a}	n.d.^{a}	n.d.^{a}
2		0.67 ± 0.01^{d}	0.152 ± 0.007^{b}	n.d.^{a}	n.d.^{a}
3		0.93 ± 0.01^{d}	0.43 ± 0.03^{a}	n.d.^{a}	n.d.^{b}
4		0.945 ± 0.008^{d}	0.093 ± 0.006^{b}	n.d.^{b}	n.d.^{a}
5		1.06 ± 0.01^{c}	0.137 ± 0.006^{b}	n.d.^{a}	n.d.^{a}
6		0.51 ± 0.01^{c}	0.13 ± 0.01^{a}	n.d.^{a}	n.d.^{b}
1	IV	0.947 ± 0.003^{c}	0.104 ± 0.008^{a}	n.d.^{a}	n.d.^{a}
2		0.87 ± 0.01^{c}	0.120 ± 0.003^{b}	n.d.^{a}	n.d.^{a}
3		1.641 ± 0.005^{c}	0.16 ± 0.01^{c}	n.d.^{a}	0.0045 ± 0.0005^{a}
4		4.29 ± 0.02^{a}	0.295 ± 0.006^{a}	n.d.^{b}	0.0060 ± 0.0000^{a}
5		0.71 ± 0.01^{d}	0.072 ± 0.002^{c}	n.d.^{a}	n.d.^{a}
6		0.422 ± 0.009^{d}	0.070 ± 0.005^{a}	n.d.^{a}	0.0010 ± 0.0000^{a}

samples, caffeic acid was found in samples **1**, **2**, and **4**–**6**, and rosmarinic acid was present in samples **3**–**6**.

Flavonoids were identified based on external standards. Because honey is a complex mixture, it was decided to quantify the flavonoids based on the internal standard hesperetin (Table 5). Similarly to the previous study [15], the influence of the matrix on the determination of flavonoids was investigated with the addition of the hesperetin as the internal standard into each sample and a comparison of the value of the added hesperetin and values obtained based on the calibration curve. The highest matrix effect was observed in the sample of honey **2**. In most cases, the calculated value of the hesperetin ranged from 1.99 to 2.09 mg/kg (added 2.17 mg/kg). The correction factor was, therefore, applied to express the concentrations of found flavonoids.

Table 5: Spectrum and content of flavonoids (mg/kg) in selected honey samples (**1**–**6**) determined using HPLC-DAD

Flavonoids/samples	Concentrations (mg/kg)					
	1	**2**	**3**	**4**	**5**	**6**
Chrysin	0.066	0.400	0.665	0.075	0.164	0.082
Myricetin	0.257	0.714	0.176	0.210	0.142	0.303
Pinocembrin	—	1.334	1.369	0.525	0.817	0.648
Rutin	—	0.073		—	—	—
Myricetin derivative 1	—	—	0.180	—	—	—
Myricetin derivative 2	—	—	0.099	—	—	—

"—" The flavonoid is not detected or its content is below the limit of quantification.

Concentrations of the flavonoids were in the range found previously in Slovenian honey samples [15] for chrysin (0.066–0.665 mg/kg) and pinocembrin (0.525–1.369 mg/kg), higher for myricetin (0.142–0.714 mg/kg) and its derivatives. Rutin, quercetin, and luteolin were detected in most of the investigated samples but not quantified since the values of LOD and LOQ are equal to 0.003 and 0.01 mg/kg, respectively. Rutin was quantified in one investigated sample (0.073 mg/kg). Among all analysed samples, sample **1** shows a very narrow spectrum of flavonoids and low concentrations of these biologically important compounds. Flavonoids in honey may originate from flower nectar, propolis, and pollen [26]. It is well known that natural honey contains high quantities of flavonoids from propolis: flavones (chrysin, galangin, and techtocrysin) and flavanones (pinocembrin and pinobanksin) [27]. Flavonoids from propolis are unique and they are very expensive and cannot be obtained easily from the extracts of plants. Therefore, their presence in honey samples is a sign of the

natural origin of the sample. Based on this, sample **1** is adulterated honey because it lacks pinocembrin and has a very low value for the content of chrysin. The rest of the honey samples investigated are probably natural. Similar quantities and the spectrum of flavonoids were found in honey samples from Montenegro [28], Romania [29], and China [9]. Chromatograms of investigated honey samples (**1**–**6**) are presented in Figure S2.

3.3 Invertase activity in selected honey samples

Invertase activity was determined spectrophotometrically, and all values show that the investigated honey samples exhibited low enzymatic activity (Table S2). Samples **1**–**3** are adulterated or thermally treated because they have invertase activity less than 4 IN [6]. For samples **4**–**6**, it can be said that they are honey samples with low enzymatic activities [6].

The invertase activity of the investigated honey samples with INs of more than 4 was similar to those found in Croatia [30], Spain [31], and the Czech Republic [32].

4 Conclusions

Four honey sample preparation methods preceding ICP-OES analysis were used, and the selected elements were quantified. Performed ICP-OES analysis on the investigated honey samples suggests that all investigated honey samples are natural based on the contents of macroelements and essential trace elements. Flavonoid analysis with HPLC-DAD revealed that one sample is probably adulterated and that the rest of the investigated samples are natural based on the spectrum of flavonoids and their contents. The analysis based on the mineral content, number and quantity of flavonoids, and the invertase activity can be a reliable "forensic tool" for determining the quality of honey samples.

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References

[1] Hungerford NL, Tinggi U, Tan BLL, Farrell M, Fletcher MT. Mineral and trace element analysis of Australian/Queensland Apis mellifera honey. Int J Env Res Public Health. 2020;17(17):6304.

[2] Voica C, Iordache AM, Ionete RE. Multielemental characterization of honey using inductively coupled plasma mass spectrometry fused with chemometrics. J Mass Spectrom. 2020;55(7):e4512.

[3] Silva B, Gonzaga LV, Maltez HF, Samochvalov KB, Fett R, Costa ACO. Elemental profiling by ICP-MS as a tool for geographical discrimination: The case of bracatinga honeydew honey. J Food Compost Anal. 2021;96:103727.

[4] Tomczyk M, Tarapatskyy M, Dzugan M. The influence of geographical origin on honey composition studied by Polish and Slovak honey. Czech J Food Sci. 2019;37:232–8.

[5] Julika WN, Ajit A, Ismail N, Aqilah N, Naila A, Sulaiman AZ. Sugar profile and enzymatic analysis of stingless bee honey collected from local market in Malaysia. IOP Conf Ser Mater Sci Eng. 2020;736:062001.

[6] Bogdanov S, Lüllmann C, Martin P, von der Ohe W, Russmann H, Vorwohl G, et al. Honey quality and international regulatory standards: review by the International Honey Commission. Bee World. 1999;80(2):61–9.

[7] Pyrzynska K, Biesaga M. Analysis of phenolic acids and flavonoids in honey. Trends Anal Chem. 2009;28(7):893–902.
[8] Akbari E, Baigbabaei A, Shahidi M. Determination of the floral origin of honey based on its phenolic profile and physicochemical properties coupled with chemometrics. Int J Food Prop. 2020;23(1):506–19.
[9] Cheung Y, Meenu M, Yu X, Xu B. Phenolic acids and flavonoids profiles of commercial honey from different floral sources and geographical sources. Int J Food Prop. 2019;22(1):290–308.
[10] Rejczak T, Tuzimski T. QuEChERS-based extraction with dispersive solid phase extraction clean-up using PSA and ZrO_2-based sorbents for determination of pesticides in bovine milk samples by HPLC-DAD. Food Chem. 2017;217:225–33.
[11] Rejczak T, Tuzimski T. Method development for sulfonylurea herbicides analysis in rapeseed oil samples by HPLC–DAD: comparison of zirconium-based sorbents and EMR-lipid for clean-up of QuEChERS extract. Food Anal Methods. 2017;10:3666–79.
[12] Hernandez OM, Fraga JMG, Jimenez AI, Jimenez F, Arias JJ. Characterization of honey from the Canary Islands: determination of the mineral content by atomic absorption spectrophotometry. Food Chem. 2005;93:449–58.
[13] Somenath M. Sample preparation techniques in analytical chemistry. Hoboken: John Wiley & Sons Inc; 2003. p. 227.
[14] De Andrade CK, dos Anjos VE, Felsner ML, Torres YR, Quinaia SP. Direct determination of Cd, Pb and Cr in honey by slurry sampling electrothermal atomic absorption spectrometry. Food Chem. 2014;146:166–73.
[15] Bertoncelj J, Polak T, Kropf U, Korosec M, Golob T. LC-DAD-ESI/MS analysis of flavonoids and abscisic acid with chemometric approach for the classification of Slovenian honey. Food Chem. 2011;127:296–302.
[16] Vial J, Jardy A. Experimental comparison of the different approaches to estimate LOD and LOQ of an HPLC method. Anal Chem. 1999;71(14):2672–7.
[17] International Honey Commission. Harmonised methods of the International Honey Commission; 2009.
[18] Tosi E, Ciappini M, Re E, Lucero H. Honey thermal treatment effects on hydroxymethylfurfural content. Food Chem. 2002;77:71–4.
[19] Zarei M, Fazlara A, Tulabifard N. Effect of thermal treatment on physicochemical and antioxidant properties of honey. Heliyon. 2019;5(6):e01894.
[20] Petrović S, Mrmošanin J, Pavlović J, Alagić S, Tošić S, Stojanović G. The influence of agricultural soil preparation methods on the pseudo-total element content determined by ICP-OES. Stud U Babes-Bol Che. 2022;LXVII(1):43–60.
[21] Petrovic SS, Mrmosanin JM, Arsic BB. Extraction of selected elements from commercial fertilizers. Chem Naissensis. 2021;4(2):77–88.
[22] Sedik P, Horska E, Adam S, Miskeje M. Mineral content as an aspect of nutrition marketing: Case study of honey market in Slovakia. J Food Nutr Res. 2020;59(3):185–92.
[23] Ciric J, Spiric D, Baltic T, Brankovic I, Trbovic D, Parunovic N, et al. Honey bees and their products as indicators of environmental element deposition. Biol Trace Elem Res. 2021;199:2312–9.
[24] Louppis AP, Karabagias IK, Papastephanou C, Badeka A. Two-way characterization of beekeepers' honey according to botanical origin on the basis of mineral content analysis using ICP-OES implemented with multiple chemometric tools. Foods. 2019;8(6):210.
[25] COMMISSION REGULATION (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006, Official Journal of the European Union.
[26] Gašić UM, Milojković-Opsenica DM, Tešić ŽLj. Polyphenols as possible markers of botanical origin of honey. J AOAC Int. 2017;100(4):852–61.
[27] Tomás-Barberán FA, Truchado P, Ferreres F. Flavonoids in stingless-bee and honey-bee honeys. In: Vit P, Pedro S, Roubik D, editors. Pot-Honey. New York: Springer; 2013.
[28] Nesovic M, Gasic U, Tosti T, Trifkovic J, Baosic R, Blagojevic S, et al. Physicochemical analysis and phenolic profile of polyfloral and honeydew honey from Montenegro. RSC Adv. 2020;10:2462–71.
[29] Ciucure CT, Geana E-I. Phenolic compounds profile and biochemical properties of honeys in relationship to the honey floral sources. Phytochem Anal. 2019;30(4):481–92.
[30] Flanjak I, Strelec I, Kenjeric D, Primorac LJ. Croatian produced unifloral honey characterized according to the protein and proline content and enzyme activities. J Apic Sci. 2016;60(1):39–48.
[31] Bentabol Manzanares A, Hernandez Garcia Z, Rodriguez Galdon B, Rodriguez Rodriguez E, Diaz Romero C. Differentiation of blossom and honeydew honeys using multivariate analysis on the physicochemical parameters and sugar composition. Food Chem. 2011;126(2):664–72.
[32] Bartakova K, Vorlova L, Titera D, Lutzova M. Physicochemical parameters and botanical origin of Czech honeys. J Food Nutr Res. 2007;46(4):167–73.