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Elemental composition of acacia honey samples from Serbia

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ABSTRACT

This study examines the concentration of essential minerals and toxic heavy metals in acacia honey samples collected in Serbia, highlighting their health implications and environmental influences. Among the 23 elements analyzed, calcium levels ranged from 38.35 to 1148.5 mg/kg, averaging 411.5 mg/kg, higher than previous findings for acacia honey, underscoring its nutritional value. Potassium averaged 208 mg/kg, consistent with the literature, whereas Na content was notably higher than the values reported in the literature. Zinc levels were higher than typical (average 11.57 mg/kg), likely due to pollution of acacia plant. Lead was the only detected toxic metal, with concentrations below the European safety threshold of 1 mg/kg. The findings highlight honey's nutritional benefits and emphasize the need for monitoring environmental contaminants to ensure honey's safety and quality.

Keywords: acacia honey, elemental composition, ICP OES

Introduction

Honey is a natural sweetener produced by *Apis mellifera* (honey bees) from the nectar of flowers. It has been valued not only for its flavor but also for its nutritional and medicinal properties. Honey is a source of simple carbohydrates. Its composition on average is 17.1% water, 82.4% total carbohydrate and 0.5% proteins, amino acids, vitamins and minerals. (Khan et al., 2007) Researches indicates that honey have several health-beneficial effects including antioxidant, anti-

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inflammatory, antibacterial, antidiabetic, respiratory, gastrointestinal, cardiovascular, and nervous system protective effects. (Samarghandian et al., 2017) Understanding the elemental composition of honey is essential for assessing its quality, geographical origin, and potential health benefits.

Inductively Coupled Plasma (ICP) techniques, such as ICP-OES (Optical Emission Spectroscopy) and ICP-MS (Mass Spectrometry), have become indispensable techniques in the precise determination of trace elements in honey samples (Solayman et al., 2015). Given the growing concern over food safety and authenticity, applying ICP techniques to investigate the elemental profile of honey can provide critical insights into its traceability and quality.

The elemental composition of honey can be influenced by various factors, including the botanical and geographical origin of the nectar, environmental conditions, and anthropogenic activities. Relatively small mineral content (0.1–1.0% w/w) in honey can be a reliable indicator for tracing its geographical and botanical origins. Trace elements such as Na, K, Ca, Mg, Fe, Zn and Cu are naturally present in honey and contribute to its nutritional value. However, the presence of toxic elements like lead, cadmium, and arsenic can indicate environmental contamination and pose health risks. Various studies have been conducted to evaluate the elemental composition of honey samples worldwide (Pisani et al., 2008; Chudzinska & Baralkiewicz, 2010, 2011; Marghitas et al., 2010; Golob et al., 2005; Fernandez-Torres et al., 2005; Bilandzic et al., 2011; Spiric et al., 2019).

This study aims to analyze the elemental composition of five acacia honey samples using the ICP-OES technique to identify their mineral content and detect potential contaminants. By comparing the elemental profiles of honey from different regions, we seek to establish patterns that may aid in the authentication and quality control of honey products. Additionally, this research contributes to a broader understanding of how environmental and geographical factors impact the elemental composition of honey.

Experimental

Honey samples

Honey samples were purchased from local productors in vicinity of Niš, Serbia. The areas where samples were collected are relatively unpolluted, because of the distance between large industrial plants and major railways and highways. Samples were collected in a steel container and kept a few days until maturation is achieved. After that, samples were bottled in decontaminated glass vessels and stored at room temperature until analysis.

Chemicals and instruments

Hydrochloric acid, nitric acid (65%), and hydrogen peroxide (30%), were purchased from Merck (Darmstadt, Germany). Multi - element standard solutions (20.00 ± 0.10 mg L-1) used for ICP

analysis was purchased from Ultra Scientific (North Kingstown, RI, U.S.A.). Samples were prepared using Milestone ETHOS EASY advanced microwave digestion system.
The measurements were carried out with an ICP-OES iCAP 6000, Thermo Scientific.

Samples preparation

Digestion was done using a Milestone ETHOS EASY advanced microwave digestion system. Samples (0.3 g) were mixed with 9 mL of HNO_3 and 1 mL of H_2O_2 and subjected to digestion. The instrumental parameters used are shown in Table 1.

Table 1. Instrumental parameters for the microwave oven digestion.

Step	Power (W)	Temperature (°C)	Time (min)
1	1800	210	20
2	1800	210	15

After digestion, the PTFE vessels were left to cool to room temperature. The samples were quantitatively transferred into 25 mL volumetric flasks and filled up to the mark with ultrapure water. The honey samples were digested in triplicate. Blank samples were prepared with the same procedure.

Parameters of ICP-OES instrument and characteristics of the calibration curve

The contents of the tested elements in soil and plant material samples were determined by the ICP-OES technique (iCAP 6000 series, ThermoScientific, Cambridge, United Kingdom) at the following optimal instrument parameters: flush pump rate-100 rpm, analysis pump rate-50 rpm, RF power-1150 W, nebulizer gas flow-0.7 L/min, coolant gas flow-12 L/min, auxiliary gas flow 0.5 L/min, plasma view-axial, washing time-30 s. Multielement certified standard solution IV (Al, As, Ba, Be, B, Cd, Cr, Co, Cu, Fe, Pb, Mn, Ni, Se, Tl, V, and Zn; TraceCERT, Fluka Analytical, Switzerland) was used. The correlation coefficient (r), the limit of detection (LOD), and the limit of quantification (LOQ) of the working calibration curve for each tested element are shown in Table 2.

Table 2. ICP-OES validation parameters

Element	λ (nm)	r	LOD (ppm)	LOQ (ppm)
Al	308.215	0.991447	0.001955	0.006516
As	189.042	0.999202	0.002142	0.007139
B	249.678	0.998918	0.000871	0.002902
Ba	455.403	0.999804	0.000027	0.000090
Be	234.861	0.999777	0.000060	0.000199
Ca	393.366	0.997494	0.000147	0.000490
Cd	226.502	0.999744	0.000085	0.000282
Co	228.616	0.999677	0.000216	0.000721

Cr	283.563	0.99971	0.000429	0.00149
Cu	324.754	0.997565	0.000399	0.001329
Fe	259.940	0.999494	0.000370	0.001234
Hg	184.950	0.995259	0.000341	0.001136
K	766.490	0.997001	0.050090	0.166965
Mg	279.553	0.998979	0.000160	0.000532
Mn	257.610	0.998056	0.000068	0.000228
Na	589.592	0.998974	0.016701	0.055670
Ni	221.647	0.996075	0.000216	0.000720
P	177.495	0.999885	0.003288	0.010959
Pb	220.353	0.999355	0.001051	0.003504
Se	196.090	0.999167	0.003321	0.011068
Si	251.611	0.996822	0.001034	0.003448
Tl	190.856	0.999842	0.001340	0.004468
V	309.311	0.99982	0.000285	0.000950
Zn	213.856	0.998308	0.000054	0.000181

Results and Discussion

The analysis of the elemental composition of acacia honey samples revealed the presence of various essential and trace elements. Table 1 shows the mean content of detected elements of three consecutive measurements ± standard deviation SD (mg/kg of honey) for each of the tested samples.

Out of 23 elements analyzed, four of them (Cd, Co, Hg and Tl) were below the limit of detection.

The calcium content ranged from 1148 – 38.3 mg/kg, with an average concentration of 411.5 mg/kg. Calcium is essential for bone health and metabolic functions. Alvarez-Suarez et al. (2014) showed calcium levels between 10 to 45 mg/kg for Cuban honey samples, indicating variability based on geographical and botanical origin. Concentrations of Ca in acacia honey samples found by Alvarez-Suarez et al. (2014) were lower, compared to our results. Two samples from our study showed extremely high content of Ca (773 and 1148 mg/kg), whereas the other three were in the range found in the literature (Elbagerma et al., 2019). Various factors affect Ca content in honey samples. Different plants have varying capacities for absorbing and accumulating calcium, which is reflected in the honey produced from their nectar. Soil composition and environmental factors in different regions affect the mineral content of plants, and consequently, the honey (Schmidlová et al., 2024). The calcium levels found in honey reinforce its role as a nutritious food product with additional health benefits. The recommended daily intake (RDI) of calcium for adults ranges from 1000 to 1300 mg, depending on age and gender (National Institutes for Health). While acacia honey is not a primary source of calcium, its regular consumption can contribute to the overall dietary intake, particularly when included as part of a balanced diet.

Table 3. The mean content of detected elements ± standard deviation SD (mg/kg) in acacia honey samples

Sample (mg/kg)	Acacia honey 1	Acacia honey 2	Acacia honey 3	Acacia honey 4	Acacia honey 5
Al	17.8±0.9	4.59±0.06	3.07±0.04	9.4±0.1	0.801±0.005
As	0.013±0.001	0.020±0.002	0.132±0.005	0.187±0.004	0.127±0.005
B	5.23±0.06	11.9±0.6	2.73±0.09	31.8±0.8	2.18±0.06
Ba	1.25±0.05	1.34±0.03	0.191±0.005	0.227±0.002	5.02±0.06
Ca	773±4	1148±23	46±1	51±2	38.3±0.9
Cd	n.d.	n.d.	n.d.	n.d.	n.d.
Co	n.d.	n.d.	n.d.	n.d.	n.d.
Cr	0.099±0.001	0.19±0.08	0.081±0.003	0.033±0.002	0.028±0.003
Cu	3.99±0.04	5.13±0.05	0.213±0.004	0.374±0.002	0.309±0.003
Fe	49±1	46.1±0.8	13.5±0.6	8.01±0.06	11.20±0.06
Hg	n.d.	n.d.	n.d.	n.d.	n.d.
K	197±6	231±7	183±6	217±8	209±8
Mg	27±0.9	44.2±0.8	12.00±0.09	9.89±0.06	6.36±0.04
Mn	0.877±0.009	0.998±0.007	0.279±0.003	0.294±0.002	0.246±0.001
Na	222±4	301 ±6	218±6	235±5	235±3
Ni	1.68±0.05	0.654±0.005	0.242±0.002	0.661±0.006	0.450±0.005
P	25.5±0.4	32.3±0.3	32.7±0.6	57.7±0.7	28.1±0.8
Pb	0.178±0.003	0.398±0.005	0.110±0.001	0.140±0.002	0.021±0.004
Se	0.237±0.005	0.472±0.004	0.191±0.002	0.153±0.004	0.211±0.003
Si	29±1	353±7	29±2	253±8	68±4
Tl	n.d.	n.d.	n.d.	n.d.	n.d.
V	0.158±0.004	0.155±0.002	0.037±0.001	0.027±0.002	0.028±0.001
Zn	3.52±0.06	9.057±0.07	5.45±0.06	37.95±0.03	1.877±0.01

n.d. – below LOD

Potassium is the most abundant element in the honey samples, found in literature, with an average concentration of 350 mg/kg. Our study reported an average value of 208 mg/kg, which is in agreement with the values reported in the literature. Alqarni et al. (2014) found 450 mg/kg of K in Saudi Arabia acacia honey samples, which is almost twice as higher than values reported in our study, indicating that K level can reflect geographic origin of honey samples. A high level of potassium is typical in honey and is beneficial for human health, playing a crucial role in maintaining fluid balance and normal cell function. Studies have shown that acacia honey generally contains lower levels of potassium compared to darker honey varieties such as buckwheat or heather honey (Bogdanov et al., 2004). This is attributed to the floral source, as darker honey often come from plants that grow in mineral-rich soils.

Interestingly, sodium level was higher in honey samples analyzed in our study, compared to literature data. Pseudo Acacia honey exhibited the lowest Na content (15.69 mg/kg), whereas Somrah honey had the highest (26.93 mg/kg). (Alqarni et al., 2014). Those values were ten times lower compared to values found in our study (average value 243 mg/kg). Na content in a study by Pavlin et al. (2023) was in broad concentration range from 2.4 ± 10% mg/kg to 142 ± 3% mg/kg.

Sodium level might be more subjected to geographical origin than botanical, so it could not be used to distinguish honey samples based on Na content data.

Magnesium is one of the essential minerals found in honey, although its concentration can vary depending on the floral source, geographical origin, and environmental factors. (Schmidlová et al., 2024) Magnesium content in honey is an indicator of its nutritional quality and can provide health benefits when consumed as part of a balanced diet. Magnesium plays several critical roles in maintaining health, including muscle and nerve function, bone health, cardiovascular health, energy production and blood sugar control. (Volpe, 2013) Mg content in honey samples analyzed in our study varied between 6.36-44.22 mg/kg, with an average value of 20.11 mg/kg. Content of Mg varied between199.30 mg/kg and 80.70 mg/kg in a study by Alqarni et al. (2014), whereas Pavlin et al. (2023) reported Mg values 6.8-110 mg/kg. Such differences in Mg concentrations might be a consequence of Mg concentrations in soil, as one of main soil element, where plants for honey production are grown.

Trace elements in honey, though present in small amounts, are crucial for human health. They contribute to various physiological functions, offer antioxidant protection, and support the immune system. (Barreiros et al., 2024) Understanding the factors influencing the concentration of these trace elements can help in selecting high-quality honey and maximizing its health benefits. Trace elements in honey include minerals such as iron, zinc, copper, manganese, selenium, and chromium.

Iron content in analyzed acacia honey samples varied between 8.01 to 49 mg/kg, with an average value of 20.11. mg/kg. Atanassova et al. (2012) reported Fe values in Bulgarian honey acacia (0.8 mg/kg), linden (1.6 mg/kg), and coriandrum (1.3 mg/kg), which is lower than our findings. Iron helps prevent anemia and boosts energy levels and immune function. The recommended daily intake (RDI) of iron varies by age, sex, and physiological status (e.g., pregnancy). According to the National Institutes of Health (NIH) RDI for iron varies between 7 and 27 mg per day. While honey does contain iron, its contribution to the daily iron intake is relatively modest due to its low concentration. Consuming honey alone would not be sufficient to meet the RDI for iron, especially for groups with higher iron requirements such as pregnant women and adolescent girls. Therefore, it is essential to include a variety of iron-rich foods in the diet, to meet the recommended daily intake of iron.

On average, the zinc content in honey typically ranges from 0.1 to 3.5 mg/kg (Terab et al., 2004; Tuzen and Soylak, 2005), which is lower than average value for Zn content in our study (11.57 mg/kg). Industrial pollution, agricultural practices, urbanization, and waste disposal can lead to increased levels of zinc in honey. Understanding these influences is essential for maintaining the quality and safety of honey as a dietary source of zinc. Tuzen et al. (2007) concluded that trace element concentrations in honey are generally correlated with the degree of trace element contamination of the environment. Cu content in analyzed honey samples varied between 0.213 to 5.13 mg/kg, which is in accordance for Cu values found in literature. (Sitarz-Palczak et al., 2015)

The presence of toxic heavy metals in honey is influenced by environmental pollution, from anthropogenic activities. Regular monitoring and assessment of honey for heavy metals are essential to ensure its safety and quality. While honey can serve as an environmental bioindicator, the potential health risks from heavy metal contamination highlight the need for stricter regulations and pollution control measures. Heavy metals that have no proven biological function and are considered to be toxic are Hg, Cd and Pb. Out of the mentioned elements, only Pb was found in analyzed acacia honey samples, in concentrations between 0.021 and 0.398 mg/kg. The European Commission suggests an acceptable maximum level of 1 mg/kg for Pb (Bogdanov et al., 2007), which is higher than values for Pb concentration in our study, confirming acacia honey analyzed in this study is safe for usage regarding toxic heavy metals.

Conclusion

This study provides a comprehensive analysis of the elemental composition of acacia honey samples, emphasizing both essential minerals and toxic heavy metals. The findings highlight the nutritional benefits of honey, particularly its contributions of calcium, potassium, magnesium, and zinc, which play vital roles in various physiological functions and overall health. The significant variability in mineral content among samples underscores the influence of geographical origin, botanical source, and environmental factors. Notably, the calcium content in our samples was considerably higher than previously reported values, suggesting a robust potential for honey to contribute to dietary calcium intake. Magnesium and zinc concentrations further demonstrated the impact of environmental conditions and pollution on mineral content. The detection of lead as the only toxic heavy metal, with concentrations below the European Commission's safety threshold, indicates that the analyzed acacia honey samples are safe for consumption regarding heavy metal contamination. This reinforces the importance of regular monitoring and stringent quality control measures to ensure honey safety. Overall, this study confirms honey's role as a nutritious food product, offering essential minerals while highlighting the need for continuous assessment of environmental pollutants to safeguard its quality.

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Conflict-of-Interest Statement

The authors declare no conflict of interest.

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