meat technology
Founder and publisher: Institute of Meat Hygiene and Technology, Belgrade

UDK: 638.162:615.099
ID: 16251145
https://doi.org/10.18485/meattech.2020.61.1.6

Original scientific paper

Risk assessment of toxic elements in acacia honey

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A b s t r a c t: The element concentrations (As, Cu, Zn, Fe, Cd and Pb) of 25 acacia honeys from Serbia were analysed using inductively coupled plasma mass spectrometry (ICP-MS). Concerning the toxic element concentrations, all tested honeys met Serbian legislation. Zinc was the major element, ranging between 0.37 mg kg^{-1} and 3.95 mg kg^{-1}. Positive and significant correlations were found between Fe and Cu (r=0.567). This study showed the Serbian honey examined was good quality and met safety criteria concerning concentrations of As, Cu, Zn, Fe, Cd and Pb.

Keywords: *acacia honey, Serbia, consumption, food safety.*

Introduction

Honey bees and bee products are significant bioindicators of environmental pollution and heavy metals, since bees visit areas of up to 7 km^2 (*Spiric et al*., 2019; *Pisani et al*., 2008; *Rashed et al*., 2009; *García-Valcárcel et al*., 2016; *Matin et al*., 2016; *Ciric et al*., 2018). During harvest time, bees are exposed to different sources of contaminants through their pollen and nectar that contains heavy metals of natural and/or anthropogenic origin (*Chauzat et al*., 2009). Toxic elements are bioaccumulative, and they can be measured in honey bee tissues and bee products (*Bogdanov*, 2006; *Oroian et al*., 2016; *Gomes et al*., 2010). On the other hand, many elements have a number of important functions in chemical processes and their fingerprints can be used to determine the botanical and geographic origin of honeys (*Erbilir* and *Erdoĝrul*, 2005). Elements such as Cu, Fe, Mn, Co, Ni and Zn are essential elements that have roles in cell metabolism, but in excess amounts, they are harmful. The toxic elements that have negative impact are Pb, Cd, Cr and Hg. Pb and Cd are toxic heavy metals and are, thus, the most frequently studied (*Bogdanov et al*., 2006). Pb originating mainly from motor traffic can contaminate air and then directly nectar and honeydew. Generally, Pb is not transported by plants (*Bogdanov et al*., 2006). Pb contamination is expected to diminish, due to the increased world-wide use of car-engine catalysts. Cd originating from the metal industry and incinerators is transported from the soil to plants and can then contaminate nectar and honeydew (*Bogdanov et al*., 2006). Only a small portion of Cd might reach honey by air, mainly in the vicinity of incinerators. Also, Cd and Pb are environmental pollutants and are used as honey quality indicators.

Serbian honey production, according to *Ivanovic* et al. (2015), amounts to approximately 4,200 tons in recent years and could potentially be exported to the EU. The most common honeys produced in Serbia are the monoflorals, acacia (*Robinia pseudoacacia*), linden (*Tiliaeuropea*) and sunflower (*Helianthus annuus*), and multifloral honey (*Lazarevic et al*., 2012; *Matovic et al*., 2018). So far, existing scientific data on toxic element (As, Cu, Zn, Fe, Cd and Pb) concentrations in honeys from Serbia is very limited. In the present study, the composition of six elements was determined in acacia honey from Serbia. The objectives of this study were to: I) analyse toxic element concentrations and; II) determine potential correlations between some toxic element concentrations in acacia honey.

Materials and methods

Honey samples

A total of 25 acacia honeys (collected from different parts of Serbia during the 2018 harvest season) were examined. The botanical origin of the

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Table 1. Quality control of analysis

Elements	Limit of detection	Limit of quantification	Method repeatability/ precision as RSD (%)	Certified value[a]	Analysed value[b]
As	(µg kg^{-1}) 1.2	(µg kg^{-1}) 4	3.57	(µg kg^{-1}) 19.3±1.4	(µg kg^{-1}) 20.5±1.1
Cu	(mg kg^{-1}) 0.022	(mg kg^{-1}) 0.066	6.26	(mg kg^{-1}) 275.2±4.6	(mg kg^{-1}) 271.9±5.7
Zn	(mg kg^{-1}) 0.124	(mg kg^{-1}) 0.372	10.52	(mg kg^{-1}) 181.1±1.0	(mg kg^{-1}) 180.9±1.8
Fe	(mg kg^{-1}) 0.08	(mg kg^{-1}) 0.23	4.71	(mg kg^{-1}) 197.94±0.65	(mg kg^{-1}) 197.43±5.21
Cd	(µg kg^{-1}) 0.4	(µg kg^{-1}) 1	8.99	(µg kg^{-1}) 97±1.4	(µg kg^{-1}) 97.9±2.6
Pb	(µg kg^{-1}) 2	(µg kg^{-1}) 3.8	3.65	(µg kg^{-1}) 62.8±1.0	(µg kg^{-1}) 63.3±2.6

Legend: [a]Certified value as given by the manufacturer; [b]Data are mean±standard deviation.

honeys was established by information provided by beekeepers. Honeys (500 g) were stored in glass containers at 4–8°C until analysis.

Element concentration analyses

Approximately 0.5 g of homogenized honey was transferred into a Teflon vessel with 5 ml nitric acid (67% Trace Metal Grade, Fisher Scientific, Loughborough, UK) and 1.5 ml hydrogen peroxide (30% analytical grade, Sigma-Aldrich, St. Louis, MO, USA) for microwave digestion. The microwave (Start D, Milestone, Sorisole, Italy) programme consisted of three steps: 5 min from RT to 180°C, 10 min hold at 180°C, and 20 min cooling. After cooling, the digested honeys were quantitatively transferred into disposable flasks and diluted up to 100 ml with deionized water produced by a water purification system (Purelab DV35, ELGA, High Wycombe, Buckinghamshire, UK). Analysis of the following six elements, As, Cu, Zn, Fe, Cd and Pb, was performed by inductively coupled plasma mass spectrometry (ICP-MS) (iCap Q mass spectrometer, Thermo Scientific, Bremen, Germany).

Adjustment of physical and electronic parameters was performed before determining the elements, using calibration solution (Thermo Scientific Tune B). The calibration curve consisted of five points in two ranges (including zero). Cadmium and As were measured in the range 0.2–2.0 µg kg^{-1} and Pb, Fe, Cu and Zn in the range 2.0–20.0 µg kg^{-1}. Multielemental internal standard was introduced into the ICP-MS during the measurements. Data analysis software automatically made corrections comparing internal standards. Quality control was performed using certified reference material (CRM) NIST SRM 1577c (Table 1).

Statistical analysis

Statistical analysis was performed using the GraphPad Prism version 7.00 software. The concentrations of elements in different honey types were expressed as the minimum and maximum, mean±standard error (SE) and were subjected to analysis of variance (one-way ANOVA). The parameters were analysed using the Student's t-test at the probability of 0.05.

Results and discussion

Toxic element (As, Cu, Zn, Fe, Cd and Pb) concentrations in acacia honey from Serbia are presented in Table 2. The value presented for each element is the average concentration observed. The values of the toxic metals concentrations were compared with those established by the European Union (*European Union*, 2006) and Serbian legislation (*Official Gazette*, 2018). The As concentration was 0.004 mg kg^{-1} in the acacia honey from Serbia. The mean value of As content in honey samples was different in previous studies in Serbia (*Spiric et al*., 2018), Italy (*Pisani et al*., 2008), Slovenia (*Golob et al*., 2005) and New Zealand (*Vanhanen et al*., 2011).

Table 2. Mean±standard error of the mean, minimum and maximum levels of toxic elements in 25 acacia honeys from Serbia (mg kg^{-1})

Parameter	Mean	SEM	Min	Max
As	0.004	–	0.004	0.004
Cu	0.147	0.0175	0.070	0.300
Zn	1.57	0.2398	0.370	3.950
Fe	1.30	0.0989	0.600	2.040
Cd	0.003	0.00001	0.001	0.004
Pb	0.004	0.00001	0.004	0.004

As (arsenic) is used industrially as an alloying agent, as well as in the processing of glass, pigments, textiles, paper, metal adhesives, wood preservatives, and ammunition. As is also used in the hide tanning process and, to a limited extent, in pesticides, feed additives, and pharmaceuticals. According to Serbian legislation, the maximum residue limit for As is 0.500 mg kg^{-1}, and all tested samples of acacia honey met this legislation.

The Cu content ranged between 0.070 mg kg^{-1} and 0.300 mg kg^{-1} with an average concentration of 0.15 mg kg^{-1}. In another study of Serbian honey, the Cu concentration ranged between 94.14±41.29 ppb (µg kg^{-1}) (acacia) and 737.1±470.1 ppb (honeydew) in the 84 tested honeys (*Spirić et al*., 2018). The Cu content among honey from different countries ranged widely: 0.25 mg kg^{-1} in the case of New Zealand honeys (*Vanhanen et al*., 2011), around 900 µg kg^{-1} in the case of Italian honeys (*Pisani et al*., 2008) 0.25–1.10 mg kg^{-1} in the case of honey from Turkey (*Tuzen et al*., 2005) and 3.22 mg kg^{-1} in the case of Slovenian honeys (*Golob et al.*, 2005). Cu can be present in honey due to the copper fungicides used in agriculture. According to Serbian legislation (*Official Gazette*, 2018), honey should not contain more than 1 mg kg^{-1} of Cu. The Cu content ranged between 0.070 mg kg^{-1} and 0.300 mg kg^{-1}.

The Zn content in the acacia honeys ranged between 0.37 mg kg^{-1} and 3.95 mg kg^{-1}. Other literature data showed significant differences in element concentrations between regions in countries or between different honeys in the same regions (*Silva et al*., 2009). Any increase of Zn indicates that honey is kept in inadequate packaging or was centrifuged in a galvanized centrifuge (*Jevtic et al.*, 2012). All the investigated acacia honeys met the requirements set by Serbian regulation (*Official Gazette*, 2018), which require that the Zn content not exceed 10 mg kg^{-1}.

Fe was the most abundant analysed metal in the examined honeys. The Fe content ranged between 0.600 mg kg^{-1} and 2.040 mg kg^{-1}, with an average concentration of 1.30 mg kg^{-1}. The Fe content was in the same range as was reported in honeys from Turkey (1.8–10.2 mg kg^{-1}) (*Tuzen et al*., 2005) and another study of acacia honeys from Serbia (1.19 mg kg^{-1}) (*Spiric et al*., 2018).

The Cd content ranged between 0.001 mg kg^{-1} and 0.004 mg kg^{-1} in acacia honeys from Serbia, with a mean value of 0.003 mg kg^{-1}. The Cd content in honey differs from one country to another; in New

Table 3. Correlation between toxic elements in acacia honey

	As	Cu	Zn	Fe	Cd	Pb
As	ns	ns	ns	ns	ns	ns
Cu	ns	ns	ns	0.567*	ns	ns
Zn	ns	ns	ns	ns	ns	ns
Fe	ns	0.567*	ns	ns	ns	ns
Cd	ns	ns	ns	ns	ns	ns
Pb	ns	ns	ns	ns	ns	ns

Legend: *p<0.05; ns — not significant, p >0.05

Zealand it is 149.0 µg kg^{-1} (*Vanhanen et al*., 2011), in Italy, 305 µg kg^{-1} (*Pisani et al*., 2008), in Turkey it ranges between 0.9–17.9 µg kg^{-1} (*Tuzen et al.*, 2005) and in Poland it is 15.0 µg kg^{-1} (*Przybyłowski et al*., 2006).

The mean Pb content of our acacia honey was 0.004 mg kg^{-1}. Similar results were presented in the study by *Spiric et al*. (2019). The contamination levels of the toxic elements Pb and Cd measured in our study were low and the honeys were safe.

Many previously studies found correlations between toxic element concentrations in honey. Table 3 shows the correlations between toxic element concentrations in the Serbian acacia honeys. There were significant correlations (individual significance level is 0.05) only between the levels of Fe and Cu (r=567) in the honeys. Other toxic elements did not correlate significantly ($p>0.05$).

Conclusion

The toxic element concentrations (As, Cu, Zn, Fe, Cd and Pb) in acacia honey from Serbia were examined, and the levels of Fe and Cu correlated. The Serbian honey was of good quality according to national regulation (*Official Gazette*, 2018). Also, Zn was the major element in the tested honeys, which is in agreement with other honeys around the world.

Procena rizika toksičnih elemenata u bagremovom medu

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A p s t r a k t: Koncentracije elemenata (As, Cu, Zn, Fe, Cd i Pb) u 25 uzoraka bagremovog meda iz Srbije analizirane su primenom ICP-MS. Koncentracija toksičnih elemenata u svim ispitivanih uzorcima meda bila je u skladu sa nacionalnim propisima. Cink je bio glavni element koji se kretao između 0,37 mg/kg i 3,95 mg/kg. Utvrđene su pozitivne i značajne korelacije između sadržaja Fe i Cu (r = 0,567). Ova studija je pokazala da je bagremov med iz Srbije bio dobrog kvaliteta i ispunio bezbednosne kriterijume za koncentracije As, Cu, Zn, Fe, Cd i Pb.

Ključne reči: *bagremov med, Srbija, konzumiranje, bezbednost hrane.*

Disclosure statement: No potential conflict of interest was reported by authors.

Acknowledgments: This paper was supported by the Ministry of Education, Science and Technological Development, Republic of Serbia, through the funding of the Projects: Selected biological hazards to the safety/quality of food of animal origin and the control measures from farm to consumer (TR 31034), and; Improvement and development of hygienic and technological procedures in the production of foodstuffs of animal origin in order to obtain quality and safe products that are competitive on the world market (III 46009).

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Paper received: 27.11.2019.

Paper corrected:.3.12.2019.

Paper accepted: 3.12.2019.