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Review paper

Comparative overview of microelements and toxic elements in honey regarding the international criteria

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ABSTRACT

Honey is source of energy for bees and natural sweet substance with many benefits in human consumption. It is also used as a part of apitherapy, because it is rich in carbohydrates enzymes, trace elements, organic acids, dextrins, phytochemicals (flavonoids, phenolic acids, essential oils), vitamins and even prebiotics oligosaccharides. The stated use of honey demands high quality and safety of honey and honey products. Criteria for quality of honey is precise and regulated in Codex Alimentarius, where are defined honey, types of honey and parameters for quality of honey. For European Union (EU) members Council Directive 2001/110/EC is in effect, for United States of America (USA) it is United States Standards for Grades of Extracted Honey Effective date May 23, 1985, for China it is National Standards of the People's Republic of China, GB 14963-2011, National Food Safety Standard, for Serbia Rulebook on quality and other requirements for honey, other bee products, preparations based on honey and other bee products (Pravilnik o kvalitetu meda i drugih proizvoda pčela („Sl. glasnik RS" 101/2015). Regarding microelements and toxic elements there are criteria for EU countries, similar to USA and China, defining criteria only for content of Pb in honey set to 1 mg/kg maximum residue level (MRL). National criteria for several microelements and toxic elements were present in our legislative, but over the time national criteria has been synchronized with EU criteria.

1. Introduction

Honey is complex natural product that is created by the collection of nectar by insects, mainly *Apis mellifera*. Honey bees are interacting with environment while collecting nectar, pollen and water, foraging up to 6 km from their hives. They are considered to be bioindicators, due their capacity to deposit microelements, contaminants and residues of pollutants in their bodies. They also bring nectar, pollen and water that contain diverse substances, including hazardous components, and store it in the hives. Honeybees are considered as cumulative bioindicators regarding deposition of minerals in their bodies. They have capacity to retain number of certain microelements above naturally present concentrations in their bodies. In the case of residue monitoring in bee bodies, these organisms are observed as effective bioindicators. Pesticide concentration in dead bees samples and mortality of honeybees are monitored during blooming season for misapplication detection of pesticides during plant production (*Calatayud-Vernich et al*., 2019). The presence and amount of microelements in honey can indicate the type of honey and its geographical origin. Microelements are stable and characteristic of the soil and melliferous flora. Honey bees have large area of habitat compared to their body size and they are reflecting nature with their mineral composition and composition of their products

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around hives up to 6 km during good weather (*Atanassova et al.*, *Bilandžić 2016; et al.*, 2019; *Bogdanov et al.*, 2007). From flower nectar as raw material to the final product, honey undergoes a complex process of sugar conversion by bee enzymes and maturation in combs. Presence and quantity of microelements in nectar depends on the mineral composition of the vegetation. There are metabolic processes of plants resulting in avoidance or tolerance of certain elements, including toxic elements. Plants have several evolution mechanisms for surviving in polluted environment, or to control the level of toxic elements intake.

2. Mineral composition of honey

From flower nectar as raw material to the final product, honey undergoes a complex process of sugar conversion by bee enzymes and maturation in combs. The mineral composition of nectar depends on the mineral composition of the vegetation. There are metabolic processes of plants resulting in avoidance or tolerance of certain microelements, including toxic elements. Plants have several evolution mechanisms for surviving in polluted environment, or to control the level of toxic elements intake. The major minerals in honey have nectar-producing plants origin. Mineral composition of honey indicates type of honey. Honeydew, dark and forest honey is richer in minerals comparing to monofloral honey. The presence of heavy metals (Pb and Cd) and toxic elements (Cr and As) in honeydew honey has been reported in some studies suggesting its origin from environmental, soil contamination, pest treatment, and unsuitable procedures in the processing and conservation of honey (*Atanassova et al.*, 2016). Range of minerals in honey can be from 0,04% in pale honey, e.g., black locust to 0,20% in darker, forest honey (*Bogdanov et al.*, 2007). Likewise, content of minerals in honey is reflection of environment, mineral content of soil, water, pollen and nectar of plants. Serbian Regulative is synchronized with (EU) 2023/915, setting **maximum residue level** (MRL) for Pb in honey, 0,1 mg/kg. Regarding data from different studies (Table 1), average values of Pb are considerably below MRL, except for higly contaminated mining areas from Kosovo* (*Kastrati et al.*, 2023). Microelements are distributed in honey in sense that Zn is the most abundant compared to other elements, as potassium is the most abundant macroelement. Average content of minerals and toxic elements in honey from some studies is presented in Table 1.

3. Mechanisms of toxic element intake control

“Avoidance” is the first line of defence to restrain uptake of toxic elements and limit their distribution into plant tissues through root system. It consists of extracellular mechanisms, such as metal ion precipitation, and metal exclusion (*Dalvi and Bhalerao*, 2013). Exposure to heavy metals, can cause excretion of organic acids and amino acids by root system that make stable complexes that stay in soil and can´t enter the plant, or change the pH of soil and precipitations of heavy metals occurs. Existence of mycorrhizas can obstruct absorption of toxic elements into the plant, leaving them in rhizosphere by absorption, adsorption, or chelation. (*Dalvi and Bhalerao*, 2013). Embedding the heavy metals in the plant cell walls is another mechanism of heavy metal avoidance (*Memon and Schröder*, 2009). Cell wall has carboxylic groups of polygalacturonic acid are negatively charged and can bind heavy metals. In this way, the uptake of toxic elements into plant tissue is prevents thanks to the cell wall. Once the toxic element ions entry the cytosol, tolerance strategy is adopted by the plants to cope with the toxicity of accumulated metal ions. It is the second line of defence at intracellular level through various mechanisms such as inactivation, chelation, and compartmentalization of toxic element ions.

4. Potential health hazards resulting from honey consumption

Besides environmental concerns regarding honey element composition, quality control of honey is also important given the increase in total honey production and demands of the largest honey market of EU (*Bilandzic et al.*, 2012). Parameters for honey quality are defined in Codex Alimentarius and transferred into national regulations. In that manner essential composition and quality factors are (moisture content, fructose and glucose content (and ratio), sucrose content, water insoluble solids content, free acidity, diastase activity, hydroxymethylfurfural, electrical conductivity), toxic elements, residues of pesticides and veterinary drugs, hygiene, declaring (CXS 12–1981). There are no specific national regulations regarding the presence of toxic elements, whose origin in honey is usually anthropogenic. A Codex Alimentarius statement is that honey shall be free from metals in amounts which may result in a hazard to human health and this statement is included in the

Table 1. Average content of microelements and toxic elements in honey from different studies, expressed as mg/kg

Honey, Contry of origin	Mark of microelements and toxic elements										Author
	Fe	Mn	Cu	Zn	Co	Ni	Pb	As	Cr	Cd	
Black locust, Italy	3.67–5.39	0.1–0.6	<0.05–0.05	<0.05	<0.05	0.51–066	<0.002–0.006	<0.01	0.66–0.74	<0.01	(Meli et al., 2018)
Wildflower, Italy	3.46–7.28	0.5–2.5	0.11–0.49	<0.5–1.6	<0.05	0.42–0.69	0.005–0.017	<0.001–0.01	0.53–0.96	<0.01	
Multiflower, Poland	<0.002–1.256		0.002–0.464	0.004–1.048	0.001–0.007	0.0022–0.266	Nt**	0.00008–0.004	0.003–0.0037	Nt	(Ligor et al., 2022)
Multiflower, Kosovo*	0.57–15	0.15–8.0	0.52–9.50	0.75–14	0.0042–0.240	0.024–1.2	0.05–2.1	0.0021–0.025	0.06–2.1	0.0098–0.27	(Kastrati et al., 2023)
Black locust, Serbia	1.57±1.58	1.55±6.01	0.22±0.12	2.8±11.3	0.11±0.31	0.067±0.056	Nt	Nt	0.068±0.089	0.003±0.007	(Jovetic et al., 2017)
Linden, Serbia	1.44±1.01	1.56±0.89	0.25±0.08	1.4±1.0	0.07±0.14	0.025±0.026	Nt	Nt	0.051±0.061	0.001±0.003	
Multiflower, Brasil	<0.72–47.0	0.29–4.62	<0.07–0.95	<1.1- 2.94	Nt	Nt	Nt	<1.6–3.25	Nt	<0.04–0.29	(Oliveira et al., 2019)
Multiflower, Croatia	1.3±1.1	2.1±4.8	0.37±0.3	1.4±1.4	0.019±0.01	0.193±0.108	0.009±0.019	<0.002	0.05±0.074	<0.001	(Bilandzic et al., 2019)
Black locust, Croatia	0.57±0.5	0.22±0.2	0.12±0.1	0.63±0.6	0.051±0.031	0.281±0.26	0.0078±0.012	<0.002	0.024±0.097	<0.001	
Linden, Croatia	1.3 ± 1.0	0.94±0.7	0.24 ± 0.2	0.63±0.2	0.099±0.006	<0.033	0.023±0.012	<0.002	<0.004	<0.001	
Linden, Serbia	2.25–5.59	0.21–1.12	Nd***–0.71	1.12–20.36	Nt	Nd	Nd	Nd	Nd	Nd-0.02	(Mracevic et al., 2020)
Multifloral, Serbia	0.79–3.54	0.31–2.05	Nd	0.77–7.68	Nt	Nd	Nd	Nd	Nd	Nd-0.15	
Black locust, Serbia	3.16–3.91	0.54–0.66	Nd	0.38–1.08	Nt	Nd-0.05	Nd	Nd	Nd	Nd-0.02	
Black locust, Serbia	1.3	Nt	0.147	1.57	Nt	Nt	0.004	0.004	Nt	0.003	(Ciric et al., 2020)
Black locust, Hungary	0.43	Nt	0.13	1.58	Nt	Nt	Nt	Nt	14.5	Nt	(Czipa et al., 2015)

Nt** – not tested; Kosovo* – This designation is without prejudice to positions on status, and is in line with UNSCR 1244/1999 and the ICJ Opinion on the Kosovo declaration of independence; Nd*** - not detected (bellow limit of detection)

Codex Alimentarius (*Codex Alimentarius*, 2001) and incorporated as well, in Regulation of countries. The Codex maximum level (ML) for a contaminant in a food or feed commodity is the maximum concentration of that substance recommended by the Codex Alimentarius Commission to be legally permitted in that commodity. For toxic elements considered by the Directive 96/23/EC, no limits have apparently been established (EC, 1996) in honey. The Directive 2001/110/EC of the EU Commission relating to honey includes some general and specific properties of honey composition but no guidelines with respect to the content of toxic elements (*European Commission*, 2001). The regulation EC 1881/2006, which set the maximum levels for certain contaminants in foodstuff, does not address honey bee products (*European Commission*, 2006), except for the ML of Pb (0.10 mg/kg). Consequently, there are currently no legal criteria to compare the results obtained in presented studies (Table 1). A recommendation from the Joint Food and Agriculture Organization of the United Nations and Worl Health Organization (FAO/WHO) Expert Committee on Food Additives (JECFA) concerning the maximum tolerable or allowable intake, based on a full evaluation of an proper toxicological database, should be the main basis for decisions by Codex member. However, to have a frame for comparison, the maximum limits of Pb and Cd permitted by EC Regulation 1881/06 in bivalve mollusks (1,5 ppm for Pb and 1 ppm for Cd) were taken as reference considering the characteristics of bioaccumulation in bees and annual per capita consumption of honey. Former legislation of Serbia had prescribed MRL values for certain elements in honey. According to the Rulebook (*Official Gazette of the FRY* 5/92, 11/92, 32/02, 25/10 and 28/11), the criteria for the MRL quantities were prescribed in honey, respectively for Pb, Cd, Zn, As, Cu and Fe are 0,5; 0,03; 10; 0,5, 1 and 20 mg/kg. These criteria are withdrawn due to alignment process of Serbian and EU regulative. Before EU regulative criteria, each country had its own MRLs for toxic elements in honey, e.g., Polish standards were 0,4–0,5 mg/kg for Pb, 0,1 mg/kg for Cd, and 10 mg/kg for Cu in pollen and honey. In Finland, criteria for toxic elements in honey as category of "other food" were 0,3 mg/kg for Pb, 0,1 mg/kg of Cd, 10 mg/kg of Cu, 0,5 mg/kg of Hg and 50 mg/kg of Zn (*Fakhimzadeh and Lodenius*, 2000).

China has established MRL value for lead in honey 1 mg/kg (CH18025), and Vietnam etsablished MRL for As to 1,0 mg/kg, for Cd to 1,0 mg/kg, for Pb to 2,0 mg/kg and for Hg to 0,05 mg/kg. Russian regulative regarding toxic elements is not revised since SSSR period, and does not have criteria for toxic elements in honey (*Dudarev et al*., 2019). USA Food and Drug Administration (FDA) has set general criteria for toxic elements in food: toxic elements as lead (as Pb), not more than 10 parts per million; arsenic (as As), not more than 3 parts per million, mercury (as Hg), not more than 1 part per million (CFR FDA, 2023). The reason for lack of MRLs in honey for toxic elements could be explained in several manners. Honey is nutritious and precious food used as sugar source, and minor component of meals. It is more important for major ingredients to have set and met MRL values. Honey as well as other foodstuff is rather studied in sense of intake per kg of body mass, that includes multielement approach and nutritive aspect. Studies and forming of different food databases enabled risk management and knowledge of what contaminants can be expected in certain food, including toxic elements. Though, it is expected to detect mercury in fishery products, so the limits are set for this group of foods, and probably honey is proven over the years that is not common grocery to accumulate mercury. Studies showed that mercury was not detected in any sample of bees or apiary products tested (*Spiric et.al*, 2019, *Ciric et al*., 2020). It is also important to consider that honey is source of energy in hive, and bees as well as other living creatures have tendency to decontaminate source of energy and life to enhance survival and wellbeing of next generations.

FDA organized Total Diet Study (TDS) to monitor toxic elements in the USA food supply from traders from all states. From testing 3,241 samples of 305 foods during 2018 through 2020, cadmium was reported in 61% of samples from the TDS survey, including 98% of vegetable samples and 28% of fruit samples. Predominant highest mean concentrations of cadmium were detected in sunflower seeds, spinach, potato chips, leaf lettuce, and French fries. Lead and arsenic were detected in 15% and 43% of the food samples. Arsenic was detected at higher levels than Cd. The highest concentrations of inorganic arsenic were reported for certain foods, including crisped rice cereal, white rice, and baby foods such as puffed snacks, dry rice cereal and multi-grain cereal (*USDA*, 2017). The Joint FAO/WHO Expert Committee on Food Additives has designed for Ni a Provisional Tolerable Weekly Intake (PTWI) of 2,45 mg (*JECFA*, 2006). For inorganic As, a PTWI of 1,05 mg. Value of 0,3 mg Hg was indicated as PTWI for a 70 kg person (*FAO/WHO*, 2010a). A Provisional Tolerable Monthly Intake (PTMI) of 1,75 mg was outlined for Cd (*FAO/WHO*, 2010b) and for Pb a PTWI of 1,75 mg was created. Therefore, although the honey samples are not completely pollution free, toxic element intake from honey is considerably bellow the recommended dose and, from this point of view, the consumption of these honey products is not considered dangerous for human health. (*Meli et al*., 2018). According to the standard values determined by Codex Alimentarius Commission, the maximum Fe value that can be found in sweet nutrients such as sugar and honey is reported as 15 µg/g. The maximum Pb value that must be found in sweet substances such as sugar and honey is determined as 0,3 µg/g by Codex Alimentarius Commission. According to the standards determined by the Codex Alimentarius Commission, the maximum Zn value that must be found in sweet nutrients such as sugar and honey is 5 µg/g.

5. Conclusion

Haevy metals and other toxic elements in honey are naturally occurring. They are found in smaller amount than in other bee products, because honey is the main source of energy for hive, so there is trend in minimizing exposure of beehive comunity to toxic elements through energy source. That sort of decontamination provides clean source of food for bees and healthy swarm of bees. Honey produced in that manner by bees is very desirable food product and ingredient, acceptable for pharmacy industry as well for nutrition of sensitive part of population, with the exception of children up to the age of one. Existing data from studies of toxic elements and haevy metals in honey show that their content is lower than ML values by Codex Alimentarius (Table 1).

Uporedna analiza međunarodnih kriterijuma u pogledu mikroelemenata i toksičnih elemenata u medu

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INFORMACIJE O RADU

Ključne reči:
Med
Mikroelementi
Toksični elementi
Zakonska regulativa

APSTRAKT

Med je osnovni izvor energije za pčele i prirodni zaslađivač za ljudsku ishranu. Koristi se i u apiterapiji, jer je bogat ugljenim hidratima, mikronutrijentima, vitaminima i prebioticima. Ovakva specifična upotreba zahteva visok kvalitet i bezbednost meda i proizvoda od meda. Kriterijumi za kvalitet meda su standardizovani i regulisani Codex Alimentariusom, gde su definisani med, vrste meda i granice kvaliteta meda. Za članice Evropske unije (EU) na snazi je Direktiva Veća 2001/110/EC, za Sjedinjene američke države (SAD) to su američki standardi za klasifikovanje meda koji su stupili na snagu 23. maja 1985., za Kinu su nacionalni standardi Narodne Republike Kine, GB 14963–2011, Nacionalni standard o bezbednosti hrane, za Srbiju Pravilnik o kvalitetu i drugim zahtevima za med, druge pčelinje proizvode, preparate na bazi meda i druge pčelinje proizvode (Pravilnik o kvalitetu meda i drugih proizvoda pčela („Sl. glasnik RS" 101/2015). Što se tiče toksičnih elemenata, postoje kriterijumi za zemlje EU, slični SAD i Kini, koji definišu kriterijume samo za sadržaj Pb u medu postavljen na 1 mg/kg maksimalni rezidualni nivo (MRL). Nacionalni kriterijumi za nekoliko toksičnih elemenata bili su prisutni u zakonodavstvu, ali su tokom vremena nacionalni kriterijumi sinhronizovani sa kriterijumima EU.

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