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Original Research Article

Major and trace elements levels in multifloral and unifloral honeys in Croatia

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ABSTRACT

Different honey types were collected in Croatia during 2010 and 2011: 7 multifloral orchard honeys, 7 multifloral meadow honeys, 19 black locust, 9 chestnut, 11 lime and 6 sage honeys. Elements were measured using graphite (As, Cu, Cd, Pb, Se) and flame atomic absorption spectrometer (Ca, Fe, K, Mg, Na, Zn) and by mercury analyser (Hg). Significant differences in Ca, Fe, K, Mg, Zn, As and Hg levels were observed between honey types. In chestnut honey were determined (K, Ca, Mg: mg kg^{-1}; Hg, Ad, Cd: μg kg^{-1}): the highest concentrations of K 2824.4, Ca 486.7, Mg 59.1 and Hg 2.52; the lowest of As 24.1 and Cd 2.52. Lime honey has been shown the highest content of Cu (20.6 mg kg^{-1}), Zn (6.78 mg kg^{-1}), Cd (2.14 μg kg^{-1}) and Pb (810.3 μg kg^{-1}). The lowest levels of following elements were determined in black locust honey (Fe, K, Mg: mg kg^{-1}; Hg: μg kg^{-1}): Fe 2.77, K 304.7, Mg 8.02 and Hg 0.82. Sage honey had the lowest Ca and Na content (173.9 and 31.8 mg kg^{-1}). Among the multifloral honeys, the following was determined (Fe, Na, Cu: mg kg^{-1}; As, Pb: μg kg^{-1}): orchard honey – highest of Fe 5.17 and As 276.1, lowest Pb 301; meadow honey – highest Na 36.1, lowest Cu 4.38. The average Ca, Cu and Pb levels found in multifloral honey types were much higher than those reported in other European countries. Calcium levels in lime, chestnut and black locust were higher than those from other countries.

1. Introduction

Honey is a natural, sweet product used as an ingredient for sweetness, colour, flavour, caramelisation and viscosity in many types of food products (Rashed and Soltan, 2004). Honey is composed mainly of fructose and glucose (65%), and water (18%), with low protein levels (Silva et al., 2009). The mineral content in honey is low and ranges from 0.04% in pale honeys to 0.2% in dark honeys (Anklam, 1998; Fernández-Torres et al., 2005). In investigations of the environmental, geographical and botanical aspects of the mineral content in honey, botanical factors have been shown to have the greatest influence on trace element content (Bogdanov et al., 2007). Honey may contain high levels of toxic elements, such as As, Cd, Hg and Pb, as a result of the elevated metal contents in plant nectar. High concentrations of these elements have been determined in honey from industrial areas with heavy industrial activities or near busy highways (Gajek et al., 1987; Toporcák et al., 1992). Therefore, honey may be considered a bioindicator of environmental pollution (Conti and Botre, 2001; Pisani et al., 2008).

Essential metals such as copper, iron, manganese and zinc play an important role in a number of biochemical processes, but can also have toxicological implications for humans when ingested in excessive doses (Arredondo and Núñez, 2005; Fraga, 2005). In the past decade, trace metal contents have been determined in different honey types in European countries: France (Devillers et al., 2002), Italy (Conti, 2000; Buldini et al., 2001; Pisani et al., 2008), Poland (Przybyłowski and Wilczyńska, 2001), Slovenia (Golob et al., 2005; Kropf et al., 2010), Czech Republic (Lachman et al., 2007), Romania (Bratu and Beorgescu, 2005), Spain (Terrab et al., 2004; Hernández et al., 2005; Garcia et al., 2006) and Turkey (Tuzen, 2002; Tuzen and Soylak, 2005; Tuzen et al., 2007; Silici et al., 2008). Most studies have only tested the differences in element composition among regions related to the geographical origin of honey, and therefore neglected the botanical influence on honey composition. Among the many studies, most of which pertain to multifloral honeys, few studies have also been conducted on unifloral honey types. Studies conducted on different unifloral honeys demonstrated great differences in elemental composition (Fernández-Torres et al., 2005; Golob et al., 2005;

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Lachman et al., 2007; Pisani et al., 2008). In Croatia, in addition to the common production of multifloral honeys, the most common unifloral honey types are black locust (*Robinia pseudoacacia* L.), chestnut (*Castanea sativa* Mill.) and lime (*Tilia* spp.). On the other hand, unifloral honeys originating from *Lavandula stoechas* and *Salvia officinalis* are rare and are indicative of the southern geographical origin in the country.

In the present study, the element composition of honey samples of different botanical and geographical origins in Croatia were studied. Differences in trace element contents between multifloral and unifloral honey types were examined.

2. Materials and methods

2.1. Honey samples

Fifty-nine honey types of different botanical origin were collected during 2010 and 2011: multifloral orchard honeys, multifloral meadow honeys, black locust (*R. pseudoacacia* L.), chestnut (*C. sativa* Mill.), lime (*Tilia* spp.) and sage (*S. officinalis*) honeys. Honey samples were collected in five continental regions of Croatia: Centre (Zagreb and Karlovac Counties), North (Varaždin County), Northeast (Bjelovar-Bilogora and Križevci-Koprivnica Counties), Northwest (Krapina-Zagorje County) and South (Zadar and Split-Dalmatia Counties) (Table 1). The majority of samples were collected from the primarily agricultural Bjelovar-Bilogora and Križevci-Koprivnica Counties, with cultivated fields, vineyards and orchards. The region has no considerable industrial activities and vehicular traffic is rather low in comparison with European standards and the more heavily populated, urbanised and industrialised Centre region around the capital city of Zagreb. Only sage honey was collected on island locations far from urbanisation and traffic, in southern Zadar County and southwest Primorje-Gorski Kotar County.

All the honey samples were taken directly from containers used by beekeepers. Upon collection, all honey samples (500 g) were placed into clean glass bottles, labelled and transferred to the laboratory and kept at 4–8 °C until analysis.

2.2. Standard preparation

Analytical grade reagents (HNO_3 (65%, v/v), H_2O_2 (30%, v/v) $K_2Cr_2O_7$ and HCl) were purchased from Kemika (Zagreb, Croatia). All solutions were prepared and diluted with ultra-pure water (18.2 MΩ cm resistivity at 25 °C) obtained by the purification system NIRO VV UV UF 20 (Nirosta d.o.o., Water Technologies, Osijek, Croatia). Plastic and glassware were cleaned by soaking in diluted HNO_3 (1/9, v/v) and by subsequent rinsing with ultra-pure water and drying prior to use.

Standard stock solutions containing 1000 mg L^{-1} of As (1001 ± 3 mg L^{-1}), Cd (999 ± 3 mg L^{-1}), Cu (1001 ± 3 mg L^{-1}), Hg (1000 ± 3 mg L^{-1}), Se (1004 ± 3 mg L^{-1}), Pb (1001 ± 3 mg L^{-1}) were purchased from Perkin Elmer (Waltham, Massachusetts, USA). Also, standards containing 1000 mg L^{-1} of Zn (1001 ± 2 mg L^{-1}), Fe (1000 ± 2 mg L^{-1}), Ca (999 ± 2 mg L^{-1}), Mg (1000 ± 2 mg L^{-1}), Na (1001 ± 5 mg L^{-1}) and K (1002 ± 5 mg L^{-1}) were purchased from Merck (Darmstadt, Germany). The stock solution and working standards were diluted in 0.2% (v/v) HNO_3. Four calibration points were prepared for calibration curve for each element.

In the preparation of Hg working standards, 1 mL of HNO_3 conc., 0.1 mL 10% $K_2Cr_2O_7$, and 0.1 mL HCl conc. were added to all working standards and prepared in brown glass volumetric flasks. The following were used as matrix modifiers (all, Perkin Elmer, USA) in each atomisation: for Cd, Cu and Se 0.005 mg $Pd(NO_3)_2$ and 0.003 mg $Mg(NO_3)_2$, for Pb 0.050 mg $NH_4H_2PO_4$ and 0.003 mg Mg $(NO_3)_2$ and for As 0.01 mg $Pd(NO_3)_2$ and 0.005 mg $Mg(NO_3)_2$.

Ultra pure grade carrier argon (Ar, 99.9995% pure) and acetylene (99.9995% pure) were supplied by UTP d.o.o. (Pula, Croatia).

2.3. Sample preparation

Samples (0.5 g) were digested with 4 mL HNO_3 (65%, v/v) and 2 mL H_2O_2 (30%, v/v) with a Multiwave 3000 microwave closed system (Anton Paar, Germany). A blank digest was carried out in the same way. The digestion programme began at a power of 500 W, then ramped for 1 min and hold for 4 min. The second step began at a power of 1000 W ramped for 5 min and hold for 5 min. The third step began at a power of 1400 W, ramped for 5 min and hold for 10 min. Digested samples were diluted to a final volume of 50 mL with double deionised water.

2.4. Apparatus

The analyses of As, Cd, Cu and Pb were conducted by graphite furnace-atomic absorption spectroscopy (GFAAS) using an AAnalyst 800 atomic absorption spectrometer (Perkin Elmer, USA) equipped with an AS 800 autosampler (Perkin Elmer, USA). For graphite furnace measurements, argon was used as the inert gas. Pyrolytic-coated graphite tubes with a platform were used. The analyses of Zn, Fe, Ca, Mg, Na and K were conducted by flame atomic absorption spectroscopy (FAAS) using a SpectrAA 220 atomic absorption spectrometer (Varian, Australia). Gases used for flame atomic absorption spectroscopy were acetylene, air and nitrous oxide. The atomic absorption signal for GFAAS and FAAS were measured in peak area and integration measurement mode against a calibration curve.

Mercury levels in honey samples were quantified without acid digestion using the AMA-254 (Advanced Mercury Analyzer, Leco, Poland), which employs direct combustion of the sample in an oxygen-rich atmosphere. The instrumental settings and optimising temperature programs of the graphite and flame spectrometer and mercury analyser are summarised in Table 2.

2.5. Quality parameters

The limits of detection (LODs) were determined as the concentration corresponding to three times the standard deviation of twenty blanks. All specimens were run in batches that included blanks, a standard calibration curve, two spiked specimens, and one duplicate. To calculate the recovery percentage, ten honey samples spiked with known amounts of element analytical standards were processed. The limits of detection, recovery and linear range of the calibration curve for elements are presented in Table 3. The quality of data showed good accuracy, with recovery rates from 93.0% to 99.2%.

For Zn determined at levels lower than the LOD value (0.01 mg kg^{-1}), values were expressed as 0.01. Also, for Cd level lower than LOD (1 μg kg^{-1}), values were expressed as 1.

2.6. Data analysis

All calculations and statistical analysis were performed using the Statistica 6.1 software package (StatSoft® Inc., Tulsa, USA). One-way analysis of variance was used to test for differences in honey metal concentrations. Data were log-transformed to improve normality prior to analysis to meet the underlying assumptions of the analysis of variance. The differences in metal concentrations between different honey types were analysed using the *t*-test. A probability level of $p \leq 0.05$ was considered statistically significant.