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A variety of elements are present in honey at different concentrations. Although some of these heavy metals and trace elements are useful nutrition, at higher levels they could cause health problems. In order to achieve confidence about food safety, regular monitoring of food quality is necessary. In this study, heavy metals and trace element contents were determined by inductively coupled plasma atomic emission spectrometer in 10 different honey brands from Iranian markets. All heavy metal contents in these samples were within ranges as reported in literature, except for Hg, Al and As. Compared with recommended daily intakes, it is concluded that heavy metals or trace elements intoxication following honey consumption in Iran is unlikely.

Keywords: honey; heavy metal; trace element; ICP-AES; Iran

Introduction

As a natural product, honey is famous for its richness in nutrients and being a valuable remedy as it was used to treat diseases (e.g. for gastrointestinal disorders or wound healing) in Egypt and Greece (Inoue et al. 2005). The moisturising action of honey around a wound facilitates the healing process and the high viscosity of honey inhibits infections to penetrate into the body. Its anti-bacterial properties are due to its low acidity and low-level hydrogen peroxide release (Dunford et al. 2000; Lusby et al. 2002). Nowadays, honey has attracted attention for its potency in prevention and treatment of illnesses and also its ability to cause well-being (Inoue et al. 2005). Also honey, because of its flavour, colour and sweetness, could be used as an ingredient or preservative in foodstuffs; for example, it has been shown that honey is able to prevent lipid peroxidation in meat (Nagai et al. 2006).

The source of honey, made by bees, is the nectar of plants and honeydew. Honey is mainly composed of carbohydrates, water, traces of organic acids, enzymes, amino acids, pigments, pollen and wax resulting from honey maturation and bees' activity or derived from the plants. The concentration of honey minerals ranges between 0.04% and 0.2% in pale and dark honeys, respectively. This concentration could be greatly affected by the soil type of the source plants (Anklam 1998). Most abundant elements found in honey are potassium, calcium, magnesium and sodium (Lachman et al. 2007). Food safety is of great importance in health policy. Providing the best possible quality of food will protect public health and preserve consumer confidence. For this purpose, recently results for antibiotic residues in honey were published (Barganska et al. 2011). In this study, 10 of the most famous honey brands in Iran market were collected, and their heavy metal/trace element contents, specifically cadmium (Cd), lead (Pb), mercury (Hg), arsenic (As), aluminium (Al), iron (Fe), manganese (Mn), zinc (Zn), selenium (Se) and copper (Cu), were determined and compared with results of honey sample analyses published in the literature from different countries.

Materials and methods

Sample collection

Ten different honey brands (coded A–J), the most commonly consumed ones in Iran, were collected from marketplaces in March 2010. For each brand, four samples with close production dates (ranging between December 2009 and March 2010) were obtained. Limited information was available regarding the origin of each brand.

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Table 1. Wavelengths of the measured elements.

Element	Wavelength (nm)
Pb	220.3
Hg	184.9
As	189
Cu	327.4
Al	396.1
Cd	214.4
Fe	238.2
Zn	213.8
Mn	257.6
Se	203.9

Table 2. Instrumental operation conditions used for sample digestion.

	Step				
	1	2	3	4	5
Temperature (°C)	120	135	160	50	50
Pressure (bar)	40	40	40	0	0
Power (%)	30	40	50	0	0
Ramp (min)	2	2	2	1	1
Time (min)	5	5	12	1	1

Apparatus

A Varian 720-ES inductively coupled plasma atomic emission spectrometer (ICP-AES) (Agilent Technologies, Inc., Santa Clara, CA, USA) was used for elemental determination. A Speed Wave 4 microwave digestion system (Berghof Products + Instruments GmbH, Eningen, Germany), maximum pressure 40 bar, maximum temperature 230°C, with Teflon reaction vessels was applied for all digestion procedures. The reaction vessels were cleaned using 5 mL of concentrated nitric acid before each digestion. Wavelengths related to each element are summarised in Table 1.

Reagents

Standard stock solutions of different metal ions at the concentration of 1000 µg/mL were used to prepare working solutions after appropriate dilution. Standard solutions were of analytical grade (Merck, Darmstadt, Germany). Double-deionised water was used in all dilution procedures.

Sample preparation

Digestion

Microwave digestion was applied on the basis of the method described by the Speed Wave 4 microwave digestion system manufacturer. An amount of 250–300 mg of each sample was digested with 4 mL of HNO_3 (65%) and 2 mL of H_2O_2 (35%). Conditions were set as recommended by the manufacturer (Table 2). All sample solutions were clear before determination of the elements of interest using ICP-AES.

Results

Evaluation and method validation

The microwave digestion procedure for heavy metals extraction followed by ICP-AES detection was run both on consecutive days and on the same day to determine the inter-day and intra-day variances, respectively, in order to show method and instrument accuracy. To achieve this, four spiked mixtures of each heavy metal with concentrations of 50, 100, 200 and 500 ng/mL were prepared and analysed. The results of inter-day and intra-day variation are shown as CV values in Table 3, wherein limits of detection (LOD) and quantification (LOQ) are reported for each element as well. LOD was calculated as 3× standard deviation of 10 measurements in blank matrix and LOQ = 3 × LOD. After determination of elemental concentration in four standard samples, a known amount of the specific element was added to the sample, and the whole procedure was repeated to calculate recoveries.

Determination of different heavy metals in honey samples

Heavy metal content in each brand ($n = 4$) is shown in Table 4 as mean ± SD. As can be seen, the range of variation of each element could be very wide in samples from one brand, as well between different brands. Al, Fe, Hg and Zn were the most abundant metals, with an average concentration exceeding 2.5 mg/kg. Aluminium concentration was the highest (mean 9.62 mg/kg), followed by iron (5.31 mg/kg), mercury (3.03 mg/kg) and zinc (2.53 mg/kg). Average concentrations of Se, Cu, Cd, Pb, As and Mn were less than 0.5 mg/kg. The lowest level was found for lead, at a concentration of 0.11 mg/kg.

Discussion

Over the past years, there has been an increase in studies focussing on evaluation of mineral concentrations in honey. The aim of these reports is not only monitoring trace element levels in honey from the point of view of human health but also characterisation of honey adulteration (Alissandrakis et al. 2007). In addition to this, since travelling bees can cover areas as large as 50 km^2 (Ponikvar et al. 2005), honey could

Table 3. Recovery, CV, inter-day/intra-day assessment and limit of detection (LOD) of measured elements in honey samples.

Heavy metal/trace element	Recovery of metal from honey sample (% [mean ± SD])	Inter-day CV (%)	Intra-day CV (%)	LOD mg/kg	LOQ mg/kg
Cd	102.7 ± 2.1	1.69	2.41	0.008	0.020
Pb	100.7 ± 1.5	0.68	1.65	0.012	0.030
Hg	103 ± 1.4	1.48	2.46	0.010	0.025
As	101.9 ± 1.4	0.74	2.16	0.009	0.023
Al	96.2 ± 1.5	1.16	2.82	0.014	0.035
Fe	95.5 ± 2.1	1.75	3.51	0.060	0.150
Mn	98.1 ± 1.3	1.13	2.21	0.010	0.025
Zn	99.7 ± 2.2	0.97	2.83	0.007	0.018
Se	94.3 ± 3.2	1.57	2.57	0.013	0.032
Cu	96.8 ± 5.3	1.91	3.11	0.016	0.040

be an indicator of environmental pollution (Podgorski and Kanoniuk 2004). Heavy metal contamination and trace element composition could also be caused by inappropriate actions during processing and conservation steps. As a matter of fact, the acidic property of honey could lead to release of heavy metals from metallic tools and containers (Pisani et al. 2008).

Because of matrix interferences, elemental evaluation of foodstuff which is rich in sugar causes analytical difficulties. Sample pre-treatment processes usually include organic matrix removal and extracting metal ions bound in organic complexes. To achieve better extraction and increase analytical quality, lots of methods have been proposed, of which sample digestion is the one most frequently reported (Allen et al. 1997; Caroli et al. 1999; Buldini et al. 2001). Although among studies regarding heavy metal/trace element content, dry and wet ashing are the most frequently employed digesting methods, but there are several problems in these procedures, for example, time-consuming sample treatment, price and noticeable hazards. Microwave ovens, as substitutes for classical procedures, perform acid digestion of different kinds of foodstuffs efficiently. During microwave oven procedures, removal of less volatile analytes takes less digestion time in comparison to traditional methods, and the apparatus is less likely to become contaminated. It is usually preferred to use atomic spectrometric techniques in food metal content determinations. Recently, ICP-based techniques (ICP-AES and ICP–mass spectrometry) have been introduced and employed in determination of several heavy metals in honey and other sweeteners at the same time (Caroli et al. 1999). The main advantages of ICP-AES are higher sensitivity for trace analysis and its capability to simultaneously determine different metals at several spectral lines (Ioannidou et al. 2005). Based on our results, all heavy metal concentrations in honey samples were within the ranges reported in the literature from other countries except for Hg, Al and As (Table 5). Apart from possible causes as mentioned before, this could also be due to the fact that for these three elements only one other data point was found in literature.

Among these elements, heavy metals Pb and Cd are of great concern and their contamination usually has an anthropogenic source. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) recommended provisional tolerable weekly intakes (PTWIs) of Cd of 60 µg/day for a 60-kg adult (WHO 1993). The JECFA PTWI for Pb is 1.5 mg/week (=210 µg/day) for a 60-kg adult (WHO 1993). Because annual per capita honey consumption in Iran is 365 g/person, it could be calculated that Cd and Pb intake of an Iranian of 60 kg body weight is well below the recommended dose. Overall intake of copper from honey is below the provisional maximum tolerable daily intake (PMTDI) of 30 mg/day for a 60-kg person (WHO 1982a). The average daily intake of Zn from honey is estimated to be 2.53 µg/day, which is less than the recommended PMTDI of 60 mg/day (WHO 1982b). The JECFA recommended a PTWI for As of 15 µg/kg body weight (equivalent to 130 µg/day for a 60-kg adult) for inorganic As (FAO/WHO 1989), and although the average As level in our samples (0.179 mg/kg) was higher than that in other reports, it is still less than the PTWI. Accumulation of these heavy metals in plant tissues is hazardous for the food chain and may cause damages to humans or animals (Mantovi et al. 2003). Atmospheric fallout, contaminated water and chemicals from pesticides and fertilizers are some major causes of heavy metal contamination in the source plants and lands (Marcovecchio et al. 2007). Lead, cadmium, arsenic and mercury are listed as the most toxic heavy metals, and their presence in food is strongly banned in some countries. Although trace elements like copper, iron, tin, zinc, antimony, aluminium, magnesium,

Table 4. Elemental contamination in 10 honey brands.

	Fe	Mn	Zn	Se	Cu	Cd	Pb	Hg	As	Al
A	5.07 ± 0.21	0.62 ± 0.08	2.03 ± 0.72	0.17 ± 0.03	0.17 ± 0.12	0.53 ± 0.06	0.12 ± 0.06	3.47 ± 0.76	0.40 ± 0.29	15.04 ± 3.11
B	5.89 ± 0.57	0.49 ± 0.11	0.89 ± 0.44	0.06 ± 0.05	0.12 ± 0.09	0.43 ± 0.03	0.34 ± 0.04	3.51 ± 0.25	0.09 ± 0.05	17.4 ± 5.44
C	3.95 ± 0.89	0.32 ± 0.11	6.84 ± 0.74	0.08 ± 0.02	0.05 ± 0.02	0.35 ± 0.12	0.06 ± 0.03	2.79 ± 0.99	0.11 ± 0.04	13.71 ± 6.8
D	2.13 ± 0.3	0.55 ± 0.06	0.61 ± 0.23	0.1 ± 0.09	0.27 ± 0.04	0.36 ± 0.04	0.07 ± 0.03	3.08 ± 0.3	0.15 ± 0.08	1.85 ± 0.5
E	3.2 ± 0.52	0.44 ± 0.09	1.14 ± 0.39	0.07 ± 0.04	0.13 ± 0.09	0.38 ± 0.08	0.11 ± 0.05	3.28 ± 0.75	0.16 ± 0.02	3.76 ± 2.03
F	7.62 ± 3.04	0.29 ± 0.07	6.03 ± 2.61	0.18 ± 0.09	0.1 ± 0.07	0.33 ± 00.09	0.11 ± 0.08	2.37 ± 0.88	0.23 ± 0.11	8.78 ± 3.25
G	4.61 ± 0.71	0.32 ± 0.13	0.31 ± 0.37	0.31 ± 0.14	0.08 ± 0.04	0.39 ± 0.04	0.09 ± 0.07	2.79 ± 0.31	0.18 ± 0.02	2.79 ± 0.59
H	6.76 ± 1.41	0.5 ± 0.07	7.75 ± 4.28	0.26 ± 0.13	0.09 ± 0.07	0.42 ± 0.07	0.04 ± 0.01	3.04 ± 0.55	0.14 ± 0.07	11.66 ± 4.65
I	9.24 ± 0.73	0.48 ± 0.06	4.37 ± 2.97	0.19 ± 0.14	0.14 ± 0.06	0.41 ± 0.04	0.05 ± 0.05	3.09 ± 0.3	0.09 ± 0.02	4.29 ± 1.09
J	5.01 ± 0.83	0.15 ± 0.06	1.62 ± 0.56	0.18 ± 0.12	0.12 ± 0.04	0.37 ± 0.04	0.08 ± 0.07	2.84 ± 0.26	0.08 ± 0.02	16.12 ± 4.1
Overall	5.31 ± 2.29	0.42 ± 0.16	2.53 ± 2.93	0.16 ± 0.11	0.13 ± 0.08	0.39 ± 0.08	0.11 ± 0.05	3.03 ± 0.35	0.16 ± 0.13	9.62 ± 6.7

Note: Values are expressed in milligrams per kilogram as mean ± SD ($n = 4$).

Table 5. A comparison between reports regarding heavy metals/trace elements content in honey samples.

Turkey[a]	Polish standard[b]	Turkey[c] $N=34$	Turkey[d]	Czech law[e]	Ireland[f] $N=50$	Turkey[g] $N=30$	Spain[h] $N=22$	Italy[i] $N=24$	Pakistan[j]	Reference values[j]	Turkey[k] $N=15$	Present study	Results\ studies
–	–	0.08–0.3	0.00–0.33	< 0.5	–	–	–	< 15–97.1 μg/kg	–	–	10.0–23 μg/kg	0.39 ± 0.08	Cd
–	0.4	0.01–1.6	0.000–1.38	< 8.0	–	–	–	38.9–335.1 μg/kg	–	–	16.7–32.6 μg/kg	0.11 ± 0.05	Pb
–	–	–	–	< 0.5	–	–	–	–	–	–	–	3.03 ± 0.35	Hg
0.0086–0.33	–	–	–	–	–	–	–	–	–	–	–	0.16 ± 0.13	As
–	–	–	0.00–7.74	–	–	–	–	–	–	–	–	9.62 ± 6.7	Al
1.85–72	–	0.56–8.83	0.95–48.65	–	4.7–31.3	6.6 ± 3.12	3.7 ± 1.7	–	5.74 ± 0.42	3.7–9.2	1.0–5.7	5.31 ± 2.29	Fe
–	–	0.01–1.65	0.0291.48	–	0.9–10.2	1.0 ± 0.7	5.2 ± 3.1	–	–	–	0.16–1.31	0.42 ± 0.16	Mn
0.008–6.7	15	0.13–5.42	0.025–3.72	< 80.0	1.6–22.5	2.7 ± 2.5	2.0 ± 1.3	–	2.44 ± 0.17	1.3–7.8	1.0–26.1	2.53 ± 2.93	Zn
–	–	0.006–0.10	–	–	–	–	–	–	–	–	–	0.16 ± 0.11	Se
–	–	0.01–0.80	0.022–2.19	< 80.0	1–2.3	1.8 ± 1.7	0.89 ± 0.73	–	1.75 ± 0.10	0.5–2.1	0.23– 1.00	0.13 ± 0.08	Cu

Notes: From each report the maximum concentration is mentioned. All values are in milligrams per kilogram, unless stated otherwise.
[a]Sevilmi et al. (1992).
[b]As cited by Przybylowski and Wilczynska (2001).
[c]Leblebici and Aksoy (2008).
[d]Sahinler et al. (2009).
[e]As cited by Celechovska and Vorlova (2001).
[f]Downey et al. (2005).
[g]Yilmaz and Yavuz (1999).
[h]Latorre et al. (1999).
[i]Conti and Botrè (2001).
[j]Rehman et al. (2008).
[k]Tuzen and Soylak (2005).

manganese, molybdenum and chromium could be found in human diet, under certain conditions they may also cause harm (Ahmed 1999). The present study concludes that Iranian honey quality with respect to heavy metal content is satisfactory, and there is no concern about heavy metal toxicity following honey consumption in Iran.

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