Direct Determination of Heavy Metals in Honey by Potentiometric Stripping Analysis

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Abstract: Honey is a valuable nutritious food rich in simple sugars, aminoacids, proteins and minerals. In addition, this food product contains natural phenols and flavonoids, responsible for some bioactive properties. Due to known health benefits of honey and its exquisite flavour this foodstuff is readily consumed by children and wide population imposing necessity of strict quality control. One of quality criteria is the content of heavy metals. Elevated content of heavy metals in honey may result from environmental or processing contamination, or may originate from soil or plant species that honey is derived from.

In this work twenty five samples of honey from Serbia, Montenegro and Bosnia and Herzegovina were analysed in respect to zinc, cadmium, lead and copper content. Honey samples were analysed directly, without sample preparation, by applying potentiometric stripping analysis. Zinc, cadmium and lead were quantitated by using indium as an internal standard, whereas copper was determined by standard addition method. Method accuracy was confirmed by blind analysis of standard samples and with good recovery results which, for analysed samples, were in the range 91-101%. In all analysed sampled determined metals contents were in the range permitted by Serbian regulations. Cadmium was not detected in any of the analysed samples, whereas the content of other metals were in the range of 0.01-3.6 μg/g for zinc, 0.02-0.8 μg/g for lead and 0.1-2.4 μg/g for copper, respectively.

Keywords: Cadmium, Copper, Lead, Zinc.

INTRODUCTION

Honey is highly nutritious food produced by honey bees from flower nectar or plant secretions like honeydew [1, 2]. In addition to providing instantly energy to consumers due to high content of simple sugars like fructose (27.3-44.3%), glucose (22.0-40.8%), maltose (2.7-16.0%) and sucrose (1.5-3%), honey contains other valuable nutrients, such as amino acids, proteins, vitamins and minerals [3]. The content of natural antioxidants phenols and flavonoids in honey is also high [4]. Many other beneficial health effects have been confirmed for honey, such as immunity boosting, anti-inflammatory, antimicrobial, antiviral, antifungal and others [5-10].

Due to numerous health benefits of honey this nutritious food is recommended by nutritionists as a functional food. Its safety, therefore, must be carefully monitored due to possible unwanted compounds that can be found in honey, such as hydroxymethylfurfural [11], grayanotoxin [12], plant alkaloids [13] or heavy metals [14].

Heavy metals are introduced into honey either as a result of contamination during honey production, from soil or from contaminated plants from which the honey is produced [15]. Natural acidity of honey (pH ~ 3.7) contributes to metal release from handling equipment and containers. Honey produced from nectar of aromaticplants tends to contain elevated metal contents due to ability of these plants to bioconcentrate these pollutants. The content of heavy metals, thus, can be used as an indicator of environmental pollution.

Determination of heavy metals in honey is mostly performed by spectrometric techniques, such as atomic absorption spectrometry [16] or spectroscopic techniques coupled to plasma source [17, 18]. Among electroanalytical techniques mostly anodic stripping voltammetry has been reported in the literature [19], whereas potentiometric stripping analysis was less frequently applied [20]. Electrochemical stripping techniques, on oppose to above mentioned spectroscopic techniques, allow unique possibility of metal speciation. Prior application of spectroscopic techniques complex matrix of honey must be digested to liberate free metals and to avoid interferences and errors related to atomization and dispersion. Honey matrix is normally destructed by ashing [15, 16, 18], or wet digestion [15, 18]. The process itself can cause contamination or analyte loss.

In this work honey samples were analysed directly by applying potentiometric stripping analysis to determine zinc, cadmium, lead and copper contents. Direct analysis minimises the risk of contamination, analyte loss and significantly saves the time and labour. Potentiometric stripping analysis is a sensitive

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E-ISSN: 2408-9826/14

electrochemical technique, based on analyte preconcentration in the electrode medium. In this case mercury film deposited at glassy carbon served as a working electrode. Concentrated analytes are further oxidised by dissolved oxygen producing analytical signal. During this analytical step the change in electrode potential due to oxidation process is monitored and registered. Oxidation time indicates analyte quantity and is used to quantitate the metal. Each metal dissolves at different potential and this measure is used to identify metal species. In this work zinc, cadmium and lead were determined simultaneously in one analysis, by applying internal standard method, whereas copper was quantified separately by standard addition method. Both used relative methods contributed to accuracy of measurement, avoiding the influence of sample matrix on electrode processes. In addition, both relative methods significantly reduced analysis time, avoiding tedious and, for this type of matrix erroneous, calibration curve method.

MATERIALS AND METHODS

Instrumentation

Potentiometric stripping analysis was performed using a computerised system for electrochemical stripping analysis of our own construction. Mercury film electrode was used as a working electrode. Mercury was deposited at glassy carbon by a constant current (50 μA) electrolysis from separate solution containing 100 mg/dm^3 of mercury(II) and 0.02 mol/dm^3 of hydrochloric acid. Prior deposition glassy carbon was polished with the suspension of Al_2O_3 of decreasing granulation. After polishing the electrode was rinsed with triply distilled water and sonicated in ethanol (1:1) to remove any remaining suspension particles from the pores. Electrochemical cleaning of glassy carbon implied 90 cycles of potential sweep from -0.7 V to +0.7 V with the current of 30 μA. To check electrode purity electrolysis of blank was performed from -0.7 V to +0.7 V with the current of 7 μA. The absence of potentiometric waves indicated good electrode purity.

Deposition time for electrode formation was 240 s. Working electrode prepared in this manner had mercury film thickness of 130 nm and could be used for 10-15 analyses, lasting 300 s, after what it was necessary to renew the mercury film due to its damage. Before each deposition of mercury film, glassy carbon, which served as an inert support, was cleaned mechanically with filter paper wetted first with acetone and then with triply distilled water. As a counter electrode served platinum wire (φ = 0.7 mm, l = 7 mm) and the reference was Ag/AgCl, KCl (3.5 mol/dm^3) electrode.

Chemicals

All chemicals used in this work were of ultra pure grade (″Suprapur″ - Merck, Germany). For all dilutions triply distilled water was used. All glassware, vessels and cells were washed with nitric acid (1:1), distilled and triply distilled water.

Mercury stock solution (1 g/dm^3) for electrode formation was prepared by dissolving elemental mercury in nitric acid by heating and diluting with triply distilled water. Stock solutions of zinc, cadmium, lead and copper were prepared by dissolving 1 g of the element of extra purity in nitric or hydrochloric acid with heating. Dissolved metals were subsequently diluted with triply distilled water to 1 dm^3. Stock solutions of gallium and indium were prepared in same fashion by dissolving corresponding weights of element nitrates. Prepared stock solutions were kept in polyethylene bottles in dark. Working solutions (70 mg/dm^3) of elements were prepared by diluting stock solutions with triply distilled water.

Samples

The contents of zinc, cadmium, lead and copper were determined in twenty five samples of honey collected by non-probabilistic haphazard sampling strategy. Two samples originated from Montenegro, two were from Bosnia and Herzegovina (BiH) and the rest was collected in Serbia. Of all analyzed samples twenty three were nectar type, whereas three samples were honeydew type. Among nectar type honey fourteen samples were monofloral, whereas nine samples were polyfloral honey. Analyzed monofloral honey encompassed samples of black locust (*Robinia pseudoacacia*), linden (*Tilia argentea*), false indigo-bush (*Amorpha fruticosa*), woundwort (*Stachys annua*), sunflower (*Helianthus annus*), rapeseed (*Brassica napus oleifera*), sage (*Salvia officinalis*) and sweet chestnut (*Castanea sativa*). Polyfloral honey was produced combining the honey of above mentioned plant sources, as well as meadow and forest honey. Geographical origin of analyzed honey samples is given in Table **1**.

Sample Preparation

Prior potentiometric stripping analysis honey samples were prepared by dissolving 5 g of the sample in 100 cm^3 of triply distilled water.

Table 1: Geographical Origin of Analyzed Honey Samples

Sample	Type	Geographical origin
1	Black locust	Bečej, Serbia
2	Linden	Fruška Gora, Serbia
3	False indigo-bush	Bečej, Serbia
4	Black locust	Aleksinac, Serbia
5	Black locust	Bratunac, BiH
6	Meadow	Aleksinac, Serbia
7	Blended (woundwort, sunflower)	Radičević, Serbia
8	Blended (black locust, linden)	Novi Sad, Serbia
9	Sunflower	Radičević, Serbia
10	Black locust	Petkovica, Serbia
11	Linden	Novi Sad, Serbia
12	Black locust	Tavankut, Serbia
13	Forest	Smederevo, Serbia
14	Black locust	Bečej, Serbia
15	Rapeseed	Bečej, Serbia
16	Meadow	Radičević, Serbia
17	Black locust	Petkovica, Serbia
18	Blended (black locust, linden, meadow flowers)	Tavankut, Serbia
19	Forrest	Tavankut, Serbia
20	Sage	Trebinje, BiH
21	Sweet chestnut	Loznica, Serbia
22	Pine	Subotica, Serbia
23	Mountain flowers	Cetinje, Montenegro
24	Mixture of mountain honey and dry continental fruit	Cetinje, Montenegro
25	Mixture of mountain honey and walnuts	Kopaonik, Serbia

RESULTS AND DISCUSSIONS

Potentiometric stripping analyses were performed in 0.006 mol/dm^3 hydrochloric acid as a supporting electrolyte. Preconcentration step was performed in condition of convective mass transfer, applying stirring rate of 4000 rpm with Teflon mechanical stirrer connected to the analyser. For Zn, Cd and Pb determination electrolysis potential of -1.25 V was applied, whereas to quantitate copper electrolysis was done at the potential of -0.76 V to avoid formation of the Zn-Cu intermetallic compound at the electrode. This potential was not sufficiently cathodic to reduce zinc at the electrode, thus its accumulation at the electrode was avoided. Electrolysis lasted for 600 s, after what the solution was left quiescent for 15 s to allow diffusive mass transfer in the analytical step. Zinc, cadmium and lead were quantitated by using indium (10 μg/dm^3) as an internal standard. All analyzed solutions were spiked with 150 μg/dm^3 of Ga(III) to prevent the formation of Zn-Cu intermetallic compound. For copper determination standard addition method was used.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

Limit of detection represents the lowest content of the analyte that can be determined with the accuracy in statistically-acceptable limits. For defining LOD and LOQ numerous statistical approaches can be used. One of the most frequently applied is x±3SD or 3SD, depending on whether the analyte is detected in the blank or not. If the analyte is detected in the blank its average content (x) is corrected for 3SD limit. Since Pb and Cu were detected in blanks their LODs were calculated relying on x±3SD criterion, whereas for Zn and Cd 3SD criterion was used. Correspondingly LOQs were calculated as x±10SD (Pb, Cu) and 10 SD (Zn, Cd). Calculated values for four metals are given in Table **2**.

Table 2: Calculated LODs and LOQs

Element	LOD (μg/dm^3)	LOQ (μg/dm^3)
Zn	4.3	7.6
Cd	0.77	2.1
Pb	1.62	3.4
Cu	0.38	1.2

Optimization of the Internal Standard Method

To define optimal indium content that allows accurate concentration calculation of all four elements in broad concentration range, factors (*f*) were calculated for different indium contents (10-30 μg/dm^3) in model systems containing 1-30 μg/dm^3 of the elements. The factors were calculated as following:

$$f = \frac{S_a \cdot C_s}{C_a \cdot S_s}$$

where:

S_a = is the analytical signal of the analyte.
C_s = is the concentration of the internal standard.
C_a = is the concentration of the analyte in standard solution.
S_s = is the analytical signal of the internal standard.

When applying internal standard method the factors should not change their values significantly with analyte concentrations. This ″roughness″ of factors is important when analyzing real samples where analyte concentrations are unknown. Conducted study demonstrated that in the case of cadmium and lead factors were stable in examined concentration range of indium, whereas for zinc the factors changed drastically for indium contents of 20 μg/dm^3 and 30 μg/dm^3. Indium content of 10 μg/dm^3 allowed accurate quantitation of all three elements and was chosen in all subsequent analyses.

Copper could not be quantified via indium signal even when using indium concentrations up to 120 μg/dm^3. Factors for copper were consequently calculated by using thallium as an internal standard. Unfortunately, tested thallium concentrations (200-400 μg/dm^3) neither provided stable factors. Therefore copper in honey was quantitated by standard addition method. Both relative methods used in this work allowed compensation of the matrix influence. Honey represents a matrix of a very complex composition, containing organic substances and elements that interfere electrode processes. Calibration curve method, therefore, is not recommended to be used for this matrix especially taking into consideration direct analysis and the absence of adequate blank honey matrix. Direct analysis saves time, chemicals and labour, decreasing measurement uncertainty by avoiding sample manipulation steps, like digestion, that can cause analyte loss or sample contamination.

ACCURACY

Accuracy of element determination and chosen indium concentration were tested by blind analysis of standard solutions. Solutions were analyzed under adopted experimental conditions using previously calculated factors. Used factors represented mean values of five factors calculated for different metal contents, and 10 μg/dm^3 of indium. In calculation of metal contents following factors were used: 1.037 for zinc, 0.957 for cadmium and 0.852 for lead.

Metal contents were calculated on the basis of five analyses (Table **3**).

Analysis of model solutions and content calculation by applying defined factors provided relatively good agreement of calculated contents.

Determination of Zn, Cd, Pb and Cu in Honey by Potentiometric Stripping Analysis

Unknown metal concentrations (C_{Me}) for zinc, cadmium and lead in honey were calculated according to:

$$C_{Me} = \frac{S_{Me} \cdot C_{In}}{f \cdot S_{In}}$$

where:

S_{Me} = is a measured oxidation time of particular metal.

C_{In} = is a concentration of added indium (10 μg/dm^3).

f = is a factor for a particular metal previously calculated in model solutions.

S_{In} = is an analytical signal of indium.

The content of copper was calculated by standard addition method. All samples were analysed applying electrolysis time of 600 s in triplicates. Calculated contents are presented in Table **4**.

Table 3: Metal Contents Determined in Standard Solutions

No.	Zn		Cd		Pb	
	Content (μg/dm^3)	Determined Content (μg/dm^3)	Content (μg/dm^3)	Determined Content (μg/dm^3)	Content (μg/dm^3)	Determined Content (μg/dm^3)
1	11[a]	10.1±2.2[b]	15	15.7±0.6	14	13.6±0.4
2	9	8.6±0.9	9	8.6±0.6	12	12.2±0.3
3	3	nd[c]	6	5.7±0.4	3	2.9±0.2
4	4	nd	5	4.5±0.3	2	2.2±0.1

[a] unknown measurement uncertainly of standard solution preparation
[b] mean±2SD
[c] not detected

Table 4: Calculated Metal Contents in Honey

Sample	Type	Content (μg/g)			
		Zn	Cd	Pb	Cu
1	Black locust	0.04±0.005[a]	nd	nd	0.5±0.03
2	Linden	0.6±0.03	nd	0.1±0.03	0.2±0.04
3	False indigo-bush	0.1±0.02	nd	nd	0.6±0.03
4	Black locust	0.04±0.003	nd	0.08±0.009	0.5±0.02
5	Black locust	0.2±0.001	nd	0.02±0.007	0.3±0.03
6	Meadow	3.6±0.1	nd	0.4±0.03	0.3±0.03
7	Blended (woundwort, sunflower)	0.5±0.02	nd	0.02±0.009	0.4±0.02
8	Blended (black locust, linden)	0.6±0.02	nd	0.06±0.005	0.3±0.02
9	Sunflower	0.6±0.03	nd	nd	0.3±0.03
10	Black locust	0.3±0.003	nd	0.1±0.03	0.1±0.02
11	Linden	0.2±0.02	nd	0.09±0.008	0.4±0.05
12	Black locust	0.3±0.02	nd	0.03±0.009	0.1±0.07
13	Forrest	0.6±0.04	nd	0.09±0.007	0.2±0.05
14	Black locust	0.01±0.004	nd	nd	0.3±0.04
15	Rapeseed	1.1±0.2	nd	0.03±0.006	0.4±0.01
16	Meadow	0.7±0.09	nd	0.06±0.005	0.4±0.02
17	Black locust	0.2±0.04	nd	nd	0.4±0.06
18	Blended (black locust, linden, meadow flowers)	0.09±0.01	nd	0.1±0.04	0.4±0.03
19	Forrest	0.01±0.004	nd	0.07±0.003	0.3±0.07
20	Sage	0.4±0.08	nd	0.09±0.003	0.4±0.06
21	Sweet chestnut	nd	nd	0.04±0.001	0.6±0.04
22	Pine	0.04±0.07	nd	0.1±0.07	0.2±0.01
23	Mountain flowers	nd	nd	0.1±0.06	2.0±0.2
24	Mixture of mountain honey and dry continental fruit	0.01±0.007	nd	0.1±0.07	2.4±0.2
25	Mixture of mountain honey and walnuts	1.2±0.1	nd	0.8±0.04	0.3±0.03

Defined experimental conditions for direct analysis allowed good selectivity. The signals of all elements were well defined and separated (Figure **1**) with very reproducible oxidation potentials. Relative standard deviation of oxidation potential for zinc did not exceed 0.2%, for indium 0.3%, for lead 0.3% and for copper 0.4%.

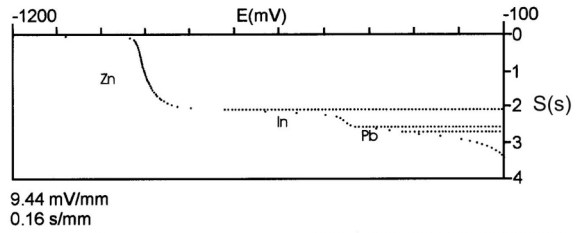

Figure 1: Original chronopotentiogram of honey sample 7.

In all analysed samples good recovery (91-101%) indicated correct sample preparation and good accuracy of measurements.

Determined metal contents were in agreement with electrochemical stripping determinations in honey reported by other authors. Analysed samples differed the most in zinc content, which was in the range 0.01-3.6 μg/g. The highest zinc content was detected in sample 6. In two analysed samples (samples 21 and 23) zinc was below the detection limit. Cadmium was below LOD in all analysed samples, indicating that honey samples were not contaminated with this environmental pollutant. The highest lead content (0.8 μg/g) was detected in sample 25 which represented a mixture with walnuts, indicating possible migration of

metals from solid parts of the mixture. For most of other samples lead content was ≤0.1 μg/g. Samples 23 and 24 exhibited similar metal contents because sample 24 was derived from sample 23 by adding dry continental fruits. These samples showed the highest copper contents (~2 μg/g) of all analysed samples.

ACKNOWLEDGEMENTS

This work was financially supported by the Provincial Secretariat for Science and Technological development of Vojvodina (144-451-3336/2013-02) and by the Ministry of Education, Science and Technological Development of the Republic of Serbia (TR 31014).

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Received on 03-08-2014 Accepted on 29-08-2014 Published on 20-10-2014

http://dx.doi.org/10.15379/2408-9826.2014.01.02.1