Chem. Chem. Technol., 2017, Vol. 11, No. 3, pp. 285–290

Chemical Technology

STRIPPING VOLTAMPEROMETRIC DETERMINATION OF HEAVY METALS IN HONEY SAMPLES

***Olena Kupchyk*[1, *]**

https:

Abstract. The content of zinc, cadmium, lead, and copper in honey varieties was determined by the method of stripping voltammetry. Samples preparing was made by three different methods: dry and hydro ashing, as well by "hydro" mineralization and dry ashing. It was found that all methods of samples preparing can be used for destroying honey matrix, but the combined method of samples preparing is the most efficient one for ions of heavy metals extracting and provides accuracy of the definition. Method of stripping voltammetry can be used in practice of quantitative determination of heavy metals in honey samples.

Keywords: honey, heavy metals, stripping voltammetry, nitric acid, mineralization, ashing.

1. Introduction

Honey is the most valuable food item. Owing to its complex chemical composition (enzymes, glucose and sucrose, proteins, amino and organic acids, vitamins, lipids, flavonoids, phenol acids, and mineral components) it has a therapeutic effect on human organism. Regular use of honey increases organism's resistance to various infections and cold-related diseases. Honey is widely used in food, medical, and cosmetic industries. Honey and other beekeeping products should be of good quality and safe, should correspond to the requirements of current normative documents concerning maximal allowed level of the substances which are potentially harmful for the consumers' health [1-3].

Investigations of the last years show that bees and beekeeping products can selectively accumulate some heavy metals, radioactive substances, pesticides, and other contaminants. Extent of heavy metals accumulation on the territory of Ukraine, as toxicants of techno genic origin, unfortunately grows. Contamination of the farmland by heavy metals is due to harmful emissions of industrial enterprises and auto transport into the atmosphere, waste of livestock farms and use of fertilizers and pesticides [4]. The most dangerous toxicants with prolonged influence are such heavy metals as lead, mercury, cadmium, arsenic, zinc, nickel, copper, *etc.*, which are emitted to the environment and are accumulated in grounds. Then they migrate into natural waters, absorbed by plants and enter the food chain. Toxic influence of heavy metals in the human organism is realized slowly, leading to impaired functioning of separate systems and organs, immunodeficiency state of human body and causing mutagenic, teratogenic and embryo toxic effect [5].

Modern analytical and sanitary-hygienic service of heavy metals control in food and environmental objects puts the following requirements to the instrumental methods of the analysis: high accuracy and sensitivity; possibility to conduct the analysis by minimal quantity of preparatory actions; automatization and computerization of the analysis processes [5].

Despite considerable progress in the development of physical and chemical methods of bee products analysis, for instance, spectroscopic [1, 2, 6-13] and chromatographic ones [14], such analyses require complicated and valuable equipment, and as a result are expensive.

Electrochemical analysis methods are characterized by high sensitivity and selectivity, quick response to the composition change of the analyzed object, are easy in automatization, do not require expensive analytical equipment [5, 14, 15]. Thus, method of stripping voltammetry, which allows to determine zinc, copper, cadmium, and lead in the sample on condition of their simultaneous presence, seems to be more promising for analysis of the consumer goods.

To determine tracer quantity of heavy metals it is necessary to convert them into solution, thereby destroying the organic matrix of honey sample. Unfortunately, because of the big amount of the samples types there is no universal methodology for samples preparation that would meet all the demands. For full destroying of the organic matrix of

[1] Chernihiv National Pedagogical University named after T.G.Shevchenko,
33, Hetman Polubotka St., 14013 Chernihiv, Ukraine
[*] *kupchik.olena@gmail.com*

honey samples dry and hydro ashing is usually used. It is performed mostly by acids: nitric acid [1, 8], chloric [11], mixture of nitric and chloric acids [8], mixture of nitric and sulfate acids [9], as well as by nitric acid and hydrogen peroxide [2, 7, 14] and mixture of nitric acid and magnesium acetate [10, 13]. Besides traditional samples heating at their mineralization, a more advanced method is using devices with microwave heating for hydro ashing under pressure, which is actually described in works [2, 7]. However, it remains unclear what composition of acid/acid mixture is more rational for the apparatus used and each definite analytical method of investigation.

Therefore, the purpose of this work was the determination of such heavy metals as zinc, lead, cadmium, and copper in honey types by the method of stripping voltammetry, as well as correlation of the efficiency of three methods of samples preparing – dry and hydro mineralization and their combination.

2. Experimental

The investigation was performed with honey samples which were bought in the specialized honey shop and from beekeepers in different parts of Chernihiv region. Honey samples were fresh and not pasteurized, *i.e.* they were not thermally treated. The 100 g of honey samples were gathered for the analysis in glass jars at room temperature without light admission. Researches were conducted with the sampling averaged from 3 samples of each of the following types of honey: lime (1), polyfloral (2), floral with thistle (3), acacia and raspberry (4), rape (5), clover (6), sunflower (7), meadow (8), forest (9), buckwheat (10), and field (11).

In order to dissociate the organic component of matrix and to transfer the investigated components into solution in electrochemically active forms, the programmable two-chamber furnace was used PDP–Lab (Research and Development Enterprise "Tomanalit" RF). The preparing of 1 g samples was made by three methods:

1. "Dry" ashing

Sample weight of honey was mixed with magnesium acetate (1 mg/ml), then it was dried in porcelain cup at 373 K for 2 h. 2.5 ml of nitric acid was added to the dried sample (5H). Then the cups with samples were put in cold muffle oven and the samples were gradually heated up to the temperature of 873 K, and then they were ashed at the temperature of 873 K for 4 h. If ashing of the sample failed (the sample contained dark inclusions) after cooling 2.0 ml of nitric acid (5H) were added to each sample and the process of drying and ashing (at 873 K for 15 min) was repeated until getting homogeneous ash of white, yellow or grey color.	+ $MgAc_2$; + 2.5 ml HNO_3 (5H); + 2.0 ml HNO_3 (5H) – replication 2-3 times

2. "Hydro" ashing

Sample weight of honey was mixed with 10 ml of concentrated nitric acid in 50 ml ashing dish and heated up to the temperature of 303–313 K until gassing completion. Then 3.0–3.5 ml of concentrated nitric acid and 1.5–2.0 ml of 30 % solution of hydrogen peroxide were added and the mixture was evaporated at the temperature of 393–403 K. The process of adding hydrogen peroxide, nitric acid and evaporation was repeated three-four times until getting homogeneous ash of white, yellow and grey color.	+ 10 ml HNO_3 (conc.); + 3.0 ml HNO_3 (conc.); 1.5 мл H_2O_2 (30%) – replication 3-4 times

3. "Hydro" mineralization and dry ashing

Sample weight of honey was mixed with 2.5 ml of concentrated nitric acid in quartz glass, heated up to the temperature of 423 K until gassing completion and evaporated up to 1/3 of the initial volume. Then 2.0 ml of concentrated nitric acid and 1.0 ml of 30 % solution of hydrogen peroxide were added and the mixture was evaporated until dry state for 60–70 min under the temperature of 423–623 K. The sample was ashed at 723 K for 30 min. The process of nitric acid, hydrogen peroxide adding, evaporation and ashing was repeated two-three times until getting homogeneous ash of white, yellow or grey color.	+ 2.5 ml HNO_3 (conc.); + 2.0 ml HNO_3 (conc.); 1.0 мл H_2O_2 (30%) – replication 2-3 times

Ash was dissolved in 1 ml of concentrated formic acid and solved by double distilled water up to 10 ml. In quartz electrochemical chamber 10 ml of distilled water, 0.2 ml of concentrated formic acid and 0.5 ml of sample aliquot were added.

The content of heavy metals was determined by the voltammetry analyzer TA-Lab (Research and Development Enterprise "Tomanalit" RF) in a three-electrode electrochemical cell. As an indicator electrode amalgam electrode was used. Silver chloride electrode filled with the 1M solution of potassium chloride was used as an electrode for comparison and as an auxiliary electrode.

The analysis was performed on background electrolyte, which contains 200 µl of concentrated formic acid (chemically pure), on the following conditions: electrochemical purification of the indicator electrode at the potential +0.050 for 15 s, metals accumulation on the surface of the indicator electrode at the potential of 1.500 for 30 s, solution calming at the potential of 1.300 V for 5 s, anodic oxidation at a liner potential scanning with the speed of 80 mV/s.

Probe of each honey sample was analyzed in three parallel experiments. Metals definition was performed by the method of additives using standard solutions, which contain 1 or 10 mg/l of each of the defined metals, which were prepared based on state standard samples and bidistilled water. Calculation of the metals concentration was performed with the help of method of one addition, presented by the manufacturer of device TA-Lab (specialized computer program, version 3.6.10) by the following formula:

$$X_n = \frac{I_n \cdot c_д \cdot V_д}{(I_д - I_n)} \cdot \frac{V}{m \cdot V_{al}} \qquad (1)$$

where: X_n – content of the determined element in sample under investigation, mg/kg; I_n – current (peak height) of the element on volt-ampere program of the sample, µA; $I_д$ – current (peak height) of the element on volt-ampere program of the sample, µA; $c_д$ – additive concentration of calibrated solution of the element, mg/l; $V_д$ – additive volume of calibrated solution of the element, ml; V – mineralizer volume, ml; V_{al} – aliquot volume, *i.e.* volume sampled from mineralizer volume and added to analyzer cell to conduct measurements, ml; m – weight of the sample taken for analysis, g.

Values of the detection limit and quantitative determination are given from technical documentation of the device. Method accuracy was determined by the honey sample probe, divided into two parts, one was left without changes and to the other the determined elements were added.

3. Results and Discussion

In Fig.1 typical samples of voltammetry curves of the background (1), honey samples without additives (2) and with additive (3) of the analyzed metal, obtained for the honey sample, are presented.

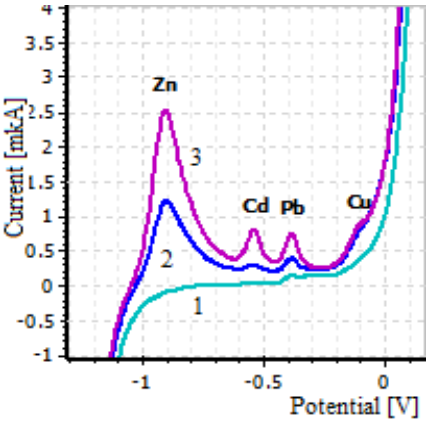

Fig. 1. Voltammetry curves of the honey sample No. 2: background (1), honey without additives (2) and with additives (3)

One can see from Fig. 1 that on the voltammetry curves of the background solutions in the intervals of the potentials from – 1200 to +100 mV there are no currents peaks of the oxidation (*curve* 1). This indicates the purity of the background electrode, in particular the absence of zinc, cadmium, lead, and copper in it, because in terms of voltammetry curve registration there is possible anodic dissolution of these metals, which were earlier concentrated on the indicator electrode. On the voltammetry curves of the sample No. 2 there are four maxima of currents – at potentials -900, -550, -320, and -50 mV, which correspond to the processes of anodic oxidation of zinc, cadmium, lead, and copper, respectively. When adding the additive probe of the standard solution, which includes all metals, current peaks of zinc, cadmium, lead, and copper oxidation on the voltammetry curves increase proportionally to the increase of these metals concentration.

The same voltammetry curves are registered for other samples of honey under investigation.

Results of the determined zinc, cadmium, lead, and copper content in honey samples prepared by different mineralization methods are given in Fig. 2.

As one can see from the data in Fig. 2, all honey samples contain heavy metals, except white honey, where such toxic metal as lead was not found. The biggest content of lead was determined in the sample of sunflower honey, which is not higher than EPC (extreme permissible convention) level ($C(Pb^{2+})$ = 1.0 mg/kg) [16, 17]. Cadmium content was below the limit of determination in buckwheat honey sample, whereas the highest content was in rape honey sample. As compared to the Ukrainian standards ($C(Cd^{2+})$ = 0.05 mg/kg) [16] the increased

cadmium content was found in two samples of honey: rape and sunflower, while the content in field and clover honey samples is on the EPC level. However, all the samples meet the requirements of honey safety in the EU countries ($C(Cd^{2+})$ = 0.1 mg/kg) [17]. Thus, the highest contamination with cadmium and lead was determined in sunflower honey, and with cadmium – in rape and sunflower honey.

The content of zinc and copper in honey are not standardized. However, it is interesting that zinc content in the majority of samples is less than 1 mg/kg, *i.e.*, is below the limit of the element determination. Maximum zinc amount is in flower honey with thistle, and the minimum one – in meadow honey. The highest content of copper was found in forest honey, and the lowest – in polyfloral honey. The obtained data on the content of heavy metals in honey are within the values known from the literature.

Relative standard deviation (S_r) for zinc, cadmium, lead, and copper determination in honey samples is not higher than 2.8, 5.3, 4.4 and 1.8 after dry ashing; 1.7, 4.1, 3.4 and 1.7 % after hydro ashing; and 1.5, 3.1, 2.9 and 1.6 % after combined method of samples preparation, respectively.

Analytical results of the degree of extracted spiked metals in honey by different methods of samples preparing are given in Table 1.

One can see from Table 1 that all methods of honey samples preparing are satisfactory. However, degree of lead ions extraction after dry ashing is higher than in other methods and higher compared with the accepted limit (75–125 %). In other words, the hydro and the combined methods of honey samples preparing are satisfactory and produce more similar indexes. However, the method of hydro ashing is more reagents consumption. That is why "hydro" mineralization and dry ashing can be recommended as relatively inexpensive, easy and fast way of honey samples preparing for further quantitative determination of heavy metals by the method of stripping voltammetry.

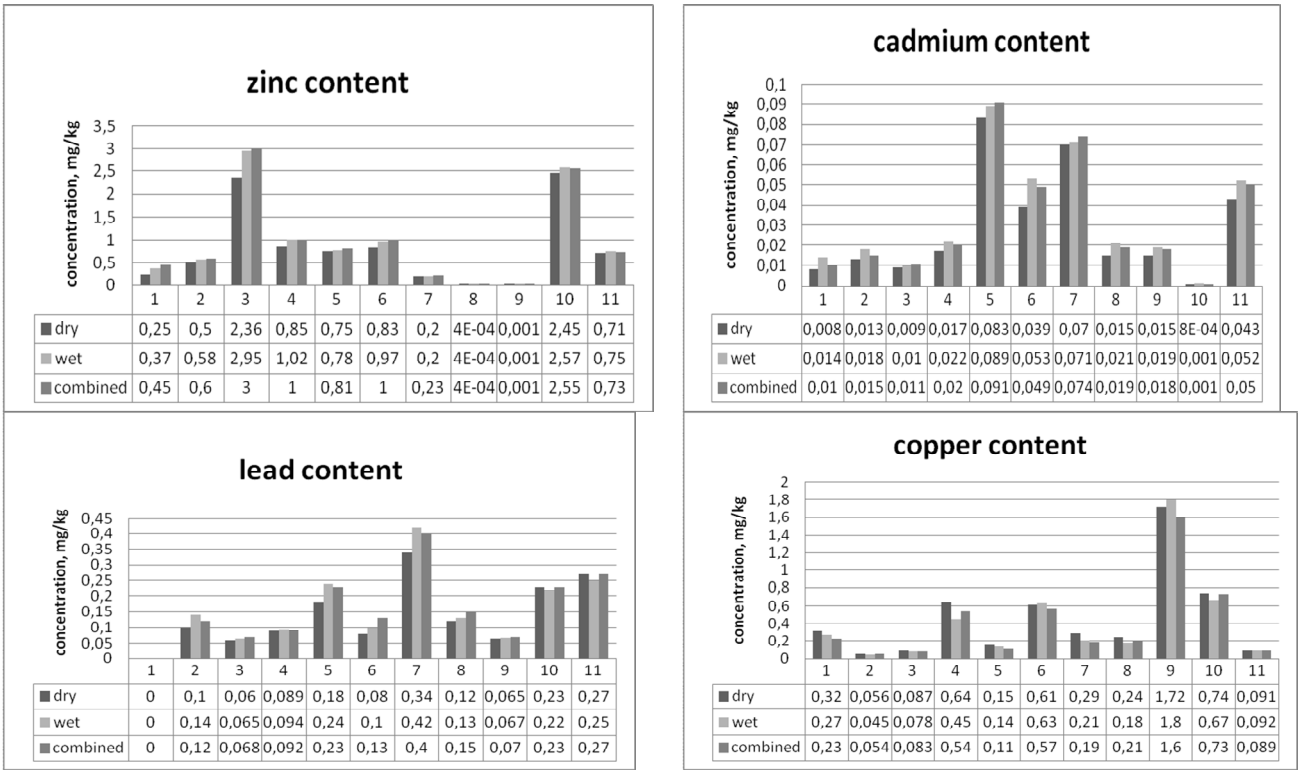

Fig. 2. Heavy metals in honey samples

Table 1

Degree of heavy metals extraction, % (n = 3)

Mineralization method	Zn	Cd	Pb	Cu
dry	86.9	85.2	125.3	112.2
hydro	103.9	93.7	94.3	93.2
combined	100.2	105.1	96.5	98.5

Table 2

Metrological characteristics of the methods of cadmium and lead content determination in honey samples

Method	Limits of detection (LOD), mg/kg	Limit of quantitation (LOQ), mg/kg	Recovery (R), %	Relative standard deviation (S_r), %
Stripping voltammetry method				
Cd	0.00010	0.002	94.1	4.2
Pb	0.00010	0.02	96.5	3.8
Atomic adsorption method [1]				
Cd	0.0014	0.01	100	2.5
Pb	0.0061	0.02	98.7	3.9
Ion chromatography [14]				
Cd	0.015	0.05	-	-
Pb	0.04	0.05	-	-
Voltammetry method [14]				
Cd	0.005	0.01	-	-
Pb	0.01	0.02	-	-

Benchmarking of metrological characteristics of analytical methods of toxic heavy metals determination, such as cadmium and lead, in honey samples is given in Table 2.

So, stripping voltammetry method gives possibility to decrease limit of detection by 1-2 times and determine cadmium concentration in honey samples next lower order comparing to other methods described in literature, however the methodology accuracy somewhat yields to atomic absorption method, but does not exceed 5 %.

4. Conclusions

Chemical composition and nutritional value of honey vary and depend on the type of honey plant, honey maturity, climate conditions, and industrial methods of treatment and storage. On the basis of the conducted investigation the following conclusions can be made:

1. To define the content of heavy metals zinc, cadmium, and lead in honey samples the method of stripping voltammetry can be used with relative deviation of no more than 4.2 % and determination regularity of about 95 %.

2. Honey samples preparation preceding stripping voltammetry determination of heavy metals content can be conducted by three methods. It was defined that the method that combines hydro mineralization and dry exposure is the most efficient in destroying organic matrix of honey samples and ions of heavy metals in solution extraction, and provides high accuracy of determination.

Acknowledgements

The author wish to thank Prof., D.Sc., V.V. Kazymyr, Vice-Rector on Scientific Work of Chernihiv National Technological University, Laureate of the State Prize of Ukraine in Science and Technology, Corresponding Member of Engineering Academy of Ukraine for providing the possibility to conduct the experiment.

References

[1] Naccari C., Macaluso A., Giangrosso G. *et al.*: J. Food Res., 2014, **3**, 107. https://doi.org/10.5539/jfr.v3n2p10712.
[2] Aghamirlou H., Khadem M., Rahmani A., *et al.*: J. Environ. Health Sci. Eng., 2015, **13**, 39. https://doi.org/10.1186/s40201-015-0189-8
[3] Skripka G.: Visnyk Sumy Nats. Agrar. Univ., 2014, **1**, 48.
[4] Kovalchuk I., Fedoruk R., Saranchuk I.: Nauk. Visnyk NUBiP Ukrainy, 2012, **172**, 113.
[5] Melnik O., Ivanov S., Mank V. *et al.*: Yakist i Bezpeka Kharchovykh Produktiv, Ukraina, Kyiv 2013.
[6] Bibi S., Husain S., Malik R.: Pak. J. Bot., 2008, 507.
[7] Tuzen M., Soylak M.: J. Food Drug Anal., 2005, **4**,343.
[8] Fredes C., Montenegro G.: Cien. Inv. Agr., 2006, **33**, 50.
[9] Shah A., Sikandar F., Ullah I., Shah A. *et al.*: J. Food Nutrit. Res., 2014, **2**, 532.
[10] Mahmoudi R., Mardani K., Rahimi B.: J. Chem. Health Risks, 2015, **5**, 251.
[11] Singh C., Shubharani R., Sivaram V.: J. Pharm. Pharm. Sci., 2014, **3**, 509.
[12] Ioannidou M., Zachariadis G., Anthemidis A., Stratis J.: Talanta, 2005, **65**, 92. https://doi.org/10.1016/j.talanta.2004.05.018
[13] Saghael S., Yarsan E., Ekici H., Tumer L.: Kafkas. Univ. Vet. Fak. Derg., 2012, **18**, 281.
[14] Buldini P., Cavalli S., Mevoli A., Sharma J.: Food Chem., 2001, **73**, 487.
[15] Osipova E.: Soros. Obrazovat. Zh., 2001, **2**, 47.
[16] www.lab.biz.ua/apps/dstu_4497-2005.pdf
[17] Council Directive 2001□110□ES of 20 December 2001 relation to honey: Off. J. Eur. Communities, 2002, **10**, 47.

Received: August 03, 2016 / Revised: November 23, 2016 / Accepted: March 12, 2017

ІНВЕРСІЙНО-ВОЛЬТАМПЕРОМЕТРИЧНЕ ВИЗНАЧЕННЯ ВАЖКИХ МЕТАЛІВ У ЗРАЗКАХ МЕДУ

Анотація. *Вміст цинку, кадмію, свинцю і міді у різновидах меду визначено методом інверсійної вольтамперометрії. Пробопідготовка зразків була виконана трьома способами: сухим та мокрим озоленням, а також «мокрою» мінералізацією і сухим озоленням. Встановлено, що всі методи підготовки проб можна використовувати для руйнування медової матриці, але комбінований метод підготовки зразків є більш ефективним для вилучення йонів важких металів та забезпечує точність визначення. Метод інверсійної вольтамперометрії може бути використаний в практиці кількісного визначення важких металів у зразках меду.*

Ключові слова: *мед, важкі метали, інверсійна вольтамперометрія, нітратна кислота, мінералізація, озолення.*