ESTIMATION OF VITAMIN *C* AND Cd, Cu, Pb CONTENT IN HONEY AND PROPOLIS♦

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Abstract: The bee colony collect for their own developmental needs the nectar, honeydew, resinous substances (to produce propolis), pollen and water from the environment that unfortunately is often exposed to various contaminants that can be found in the human consumed foods. Vitamin C and three heavy metals (Cd, Cu and Pb) were determined in honey samples produced from different plants (sun flower, conifers, multifloral, mountain flowers, pine tree forest, acacia and linden tree) from 14 regions of Romania (Babadag, Bragadiru, Brezoi, Calafat, Câmpulung, Constanța, Dâmbovița, Drăgăşani, Deva, Pecineaga, Periş, Podişul Transilvaniei, Timiş, Râmnicu Vâlcea) and in propolis produced from Timiş region and in two commercial samples. The investigated samples were collected from beekeepers and local market during 2002 - 2004. Vitamin C content was analyzed using a titrimteric method with potassium bromate-bromide solution in acidic medium and the heavy metals were determined in bee products by flame atomic absorption spectrometry (FAAS) and inductively coupled plasma atomic emission spectrometry (ICP-AES) and the results were compared. The results of the FAAS analysis are in good agreement with those given by ICP-AES.

Keywords: *vitamin C, Cd, Pb, Cu, honey, propolis*

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INTRODUCTION

Honeybees contain proteins, amino acids, carbohydrates, lipids (fatty acids, sterols), vitamins, minerals (salts) and water, and these nutrients must be in the diet in a definite qualitative and quantitative ratio for an optimal nutrition. Honey is a remarkable viscous liquid, prepared by the bees from the nectars of various plants. It has occupied a prominent place in traditional medicines throughout world history. The ancient Egyptians, Assyrians, Chinese, Greeks and Romans employed honey for wounds and diseases of the gut. When the Children of Israel were in Egypt or journeying through the desert, their promised goal was a *'land flowing with milk and honey'*.

In recent years, scientific support is beginning to emerge confirming the beneficial effects of honey on certain medical and surgical conditions. Uncontaminated honey is a healthy, easily digestible, natural and energy rich food.

Honey is widely available in most communities but its medical potential remains grossly underutilized. Its mode of action remains incompletely understood and the healing properties of honey in other clinical and laboratory situations require further evaluation. Ali studied the miraculous beneficial properties of honey in 1989 [1]. The antioxidative effects of honey and propolis were evaluated by many scientists [2, 3].

Vitamin C (ascorbic acid) is associated with health and vital force, and therefore food products are well accepted by the consumer when a high content of vitamin C is indicated. The ascorbic acid is quite unstable, reacting very easily with oxygen, especially in the presence of heavy metal ions and light, forming dehydroascorbic acid and further degradation products [4]. Vitamin C was first isolated in 1928 by the Hungarian biochemist and Nobel Prize winner Dr. Albert Szent-Gyorgyi. Vitamin C plays an important role as a component of enzymes involved in the synthesis of collagen and carnitine. Vitamin C intake markedly reduces the severity of a cold; it also effectively prevents secondary viral and bacterial complications. Vitamin C works by stimulating the immune system and protecting against damage by the free radicals released by the body in its fight against infection. The official Recommended Daily Allowance (RDA) is 60 mg/day.

The aim of the work was to study the content of cadmium, copper, lead and vitamin C in honey and propolis from different sources and locations from Romania. The heavy metal concentrations in these samples were determined by ICP-AES and vitamin C content was analyzed using a titrimteric method with potassium bromate-bromide solution in acidic medium.

MATERIALS AND METHODS

Chemicals and reagents

The reagents used for the vitamin C determination have been: $Na_2S_2O_3$ 0.5 N, $KBrO_3$-KBr 0.05 N, $K_2Cr_2O_7$ 0.5 N, H_2SO_4 1 N, H_2SO_4 1:2, KI, starch indicator 1%. All reagents were of analytical grade (Merck) and all solutions were prepared using distilled-deionized water. All metal stock solutions (1000 mg/L) were prepared by dissolving the appropriate amounts of the metals or compounds in dilute acids (1:1) and then diluting them with deionized water. The working solutions were prepared by diluting the stock solutions to appropriate volumes.

Sampling

The samples of honey produced from different plants (sun flower, conifers, multifloral, mountain flowers, pine tree forest, acacia and linden tree) in 14 regions of Romania (Babadag, Bragadiru, Brezoi, Calafat, Câmpulung, Constanța, Dâmbovița, Drăgăşani, Deva, Pecineaga, Periş, Podişul Transilvaniei, Timiş, Râmnicu Vâlcea) and propolis produced in Timiş region and two commercial samples were collected from beekeepers and local market during 2002 - 2004.

A titrimetric method with potassium bromate-bromide solution in acidic medium was used for the determination of ascorbic acid and the optimization of the method was described in a previous paper [5].

In order to determinate the contents of Cu, Cd and Pb, aliquots of ca.1 g of honey samples were made up to 100 mL with deionized water and then where transferred into Teflon vessels until analysis, which was done within 2-3 h.

A flame atomic absorption spectrometer Shimadzu AA6200 was used for the determination of three heavy metals (Cd, Cu, Pb). An air-acetylene flame was used for all elements. Copper was measured using multi element hollow cathode lamp. For Cd and Pb monoelement hollow cathode lamps were employed. The acetylene was of 99.999 % purity at a flow rate 1.8 – 2.0 L/min. The characteristics of metal calibration are presented in Table 1. Analyses were made in triplicate and the mean values are reported.

Table 1. *Characteristics of metal calibration curves*

Metal	λ, nm	Concentration range (ppm)	Correlation coefficient
Cadmium	228.8	0.008 – 1.600	0.9999
Copper	324.7	0.010 – 1.200	0.9990
Lead	282.3	0.020 – 6.000	0.9950

Also a “Spectroflame P” apparatus provided by Spectro Company, Germany analyzed the resultant solutions. The instrumental components and operating conditions in the ICP-AES measurements are presented in a previous paper [6]. For each sample three determinations were performed and average results were reported.

The used method ICP-AES showed the detection limits in Table 2 (defined as the concentration equivalent to three times the standard deviation of the analytical blank signal). The detection limits of the method are good and permit the determination of the elements in bee product at background concentrations.

Table 2. *Quality parameters of the method*

Metal	λ	LD (ng/g)
Cadmium	226.50	0.6
Copper	324.75	0.9
Lead	220.35	0.5

RESULTS AND DISCUSSION

The results of vitamin C analyses of the studied honey and propolis are presented in Table 3, and these results showed that the investigated samples contain an important amount of vitamin C, which is necessary for human body. The highest concentration of vitamin C was obtained in propolis and the lowest concentration in multifloral honey from Transilvania (see Table 3). In commercial honey samples were determined higher concentrations of vitamin C (2.77 and 2.90 mg AA/g honey) that those determined in honey samples collected from beekeepers. This situation appears probably due to the use of some preservatives or even the use of ascorbic acid in these samples. Is possible that these substances to interfere in the determinations of vitamin C, and a study about this was presented in a previous paper [7].

Table 3. Results obtained for the determination of ascorbic acid in bee products

Bee product	Production region and year	Ascorbic acid content (mg AA/g bee product)
Multifloral honey	Peris, 2004	0.96
	Pecineaga, 2003	0.75
	Transilvania, 2004	0.61
Pine tree forest honey	Dragasani, 2003	0.89
Conifers honey	Ramnicu Valcea, 2004	1.08
Sun flower honey	Timis, 2004	0.79
Mountain flowers honey	Deva, 2004	0.87
Acacia honey	Calafat, 2004	0.77
	Brezoi, 2003	0.99
Acacia honey with propolis	Dambovita, 2003	2.26
Linden tree honey	Babadag, 2003	1.00
	Bragadiru, 2004	0.82
	Commercial, 2002	2.77
	Commercial, 2003	2.90
Propolis	Timis, 2003	3.64

On the other hand, heavy metals present in the atmosphere can deposit on the bodies of the bees and be brought back to the hive with pollen, or they may be absorbed together with the nectar of the flowers, or through the water or the honeydew. A persistent contamination induces higher absorption of pollutants, by inhalation or ingestion, into bee bodies [8].

A comparison of cadmium, copper and lead obtained from honey samples using two different techniques (FAAS and ICP-AES) is presented in table 4. It can be observed that the results obtained for copper concentrations by both techniques are in good agreement. Is not possible to make a comparison for cadmium and lead because their concentrations are under the detection limit by FAAS.

The results show that honey contain low concentrations of lead according with those reported and published in Food Surveillance Information Sheet No. 53 (0.04 – 0.20 mg/kg) [9]. Cadmium concentrations for studied samples were also low, lying between 0.15 mg/kg and 0.25 mg/kg. These concentrations are comparable with the reported surveillance data (0.04 – 0.18 mg/kg) [9] with an exception for acacia honey with propolis (0.25 mg/kg). This concentration of cadmium in honey can be attributed to the propolis content (0.07 – 3.8 mg/kg indicated in surveillance data).

***Table 4**. Results obtained for the determination of Cd, Cu and Pb in honey and propolis*

Honey samples	Concentration (mg/kg)					
	Cd		Cu		Pb	
	FAAS	ICP-AES	FAAS	ICP-AES	FAAS	ICP-AES
Linden tree honey, Constantza area	< LD	0.20	2.00	2.04	< LD	0.05
Linden tree honey, commercial, 2002	< LD	0.19	1.85	1.91	< LD	0.04
Linden tree honey, commercial, 2003	< LD	0.20	1.97	2.03	< LD	0.05
Acacia honey, Constantza area	< LD	0.15	1.25	1.55	< LD	0.03
Acacia honey with propolis, Campulung area	< LD	0.25	2.90	2.63	< LD	0.06
Propolis, Timis area	< LD	0.25	3.78	3.63	< LD	0.06

LD – limit of detection

The heavy metals concentrations in acacia honey were lower than the concentrations in linden honey. Therefore comparison with literature is difficult because the literature data report only the contents of the individual heavy metals without sorting the honey samples by their botanical origin.

The concentrations of the three studied heavy metals in Romanian honey found by us are in agreement with those found in honeydew and multifloral honey from Czech Republic [10]. Analyses of honey and propolis samples indicate that cadmium, copper and lead concentrations are lower than those measured in acacia and eucalyptus honey [11]. Data from literature [12, 13] show that lead concentrations in our samples correspond with Brazilian honey and copper concentrations are higher than those in Italian honey.

The above-obtained results make it possible to state that concentrations of heavy metals found in honey samples might be due to:

- the use of improper collecting container (made of various metallic alloys not recommended for preservation of honey);
- exhaust gas from vehicles (which use various fuels containing lead compounds);
- residues emanated or discharged by various industries.

CONCLUSIONS

The proposed spectrometric methods are rapid, accurate and convenient for determination of cadmium, copper and lead from honey samples. These heavy metals concentrations are situated within the limits imposed by the last regulations of the specialized international commissions.

Based on our experimental findings, it should be concluded that heavy metals are presented at low levels in honey and propolis samples. More than that the investigated samples contain an important amount of vitamin C, which is necessary for human body. So, the consumption of investigated Romanian honey samples is not a hazard to health.

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REFERENCES

1. Ali, A.T.M.M.: The Pharmacological Characterization and the Scientific Basis of the Hidden Miracles of Honey; *Saudi Medical Journal*, **1989**, **10**(3), 177-179.
2. Nagai, T., Sakai, M., Inoue, R., Inoue, H., Suzuki, N.: Antioxidative activities of some commercially honeys, royal jelly, and propolis, *Food Chemistry*, **2001, 75**(2), 237-240.
3. Choi, Y.M., Noh, D.O., Cho, S.Y., Suh, H.J., Kim, K.M., Kim, J.M.: Antioxidant and antimicrobial activities of propolis from several regions of Korea, *Food Science and Technology*, **2006**, **39**, 756.
4. Gensler, M., Rossmann, A., Schmidt, H.L.: Detection of Added L-Ascorbic Acid in Fruit Juice by Isotope Ratio Mass Spectrometry, *J. Agric. Food Chem*., **1995**, **43**, 2662-2666.
5. Matei, N., Birghila, S., Dobrinas, S., Capota, P.: Determination of C vitamin and some essential trace elements (Ni, Mn, Fe, Cr) in bee products, *Acta Chim. Slov.,* **2004**, **51**, 169-175.
6. Birghila, S., Popescu, V., Dobrinas, S., Capotă, P., Matei, N., Soceanu, A.: Determination of Al, Mg, P, Sb, Si, Sn and Ti in honey and propolis by ICP-AES technique", *13th Romanian International Conference on Chemistry and Chemical Engineering RICCCE*, **2003**, **1**, 113-117.
7. Matei, N., Magearu, V., Birghila, S., Dobrinas, S.: Determinarea vitaminei C din cirese si visine, *Revista de Chimie*, **2004**, **55** (5), 294-296.
8. Porrini, A.G., Sabatini, S., Girotti, S., Ghini, P., Medrzycki, F., Grillenzoni, L., Bortolotti, E., Gattavecchia, G., Celli, A.: *Apiacta*, **2003**, **38**, 67.
9. * * * Ministry of Agriculture, Fisheries and Food, **1995**, Analysis of bee products for heavy metals, *Food Surveillance Information Sheet No. 53*.
10. Vorlova, L., Celechovska, O., *Acta. Vet. Brno*, **2002**, **71**, 375-378.
11. Forte, G., D'Ilio, S., Caroli, S., *J. AOAC Inter.,* **2001**, **84,** 1972-1975.
12. Lima, E.C., Krug, F.J., Arruda, M.A.Z., *Spectrochim. Acta B*, **1998**, **53**, 610.
13. Caroli, S., Forte, G., Iamiceli, A.L, Galoppi, B., *Talanta*, **1999**, **50**, 331.