Short Communication

DETERMINATION OF C VITAMIN AND SOME ESSENTIAL TRACE ELEMENTS (Ni, Mn, Fe, Cr) IN BEE PRODUCTS

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Abstract

During the last years, following the general trend of using what nature is directly offering, bee products got an increasing importance as essential natural resources in promoting healthy food. The objective of this study was to determine the ascorbic acid and the contents of trace essential elements (Cr, Fe, Mn, Ni). Ascorbic acid was first known to prevent scurvy. This disease is a rare clinical finding today. Yet, interest in ascorbic acid persists. Ascorbic acid is largely used in therapy as an anti-infections factor. It is essential to the normal functioning of cells. Ascorbic acid, $C_6H_8O_6$, is cleanly oxidized to dehidroascorbic acid by bromine. An unmeasured excess of potassium bromide is added to an acidified solution of the sample. The solution is titrated with standard potassium bromate to the first permanent appearance of excess bromine; this excess is then determined iodometrically with standard sodium tiosulfate. Instrumental method for the analysis of Cr, Fe, Mn and Ni in bee products is inductively coupled plasma atomic emission spectrometry (ICP-AES). The levels of Ni varied from 1.54 to 2.55 mg/Kg, Fe from 2.55 to 3.50 mg/Kg, Mn from 1.75 to 6.51 mg/Kg, Cr from 0.03 to 0.80 mg/Kg.

Key words: bee products, ascorbic acid, trace elements, ICP-AES

Introduction

Honey is a sweet, viscous fluid, elaborated by bees from the nectar of plants and stored in their combs as food. It is used worldwide as a basic foodstuff, either by direct ingestion or as a sweetener in a variety foodstuff. The mean content of mineral substances in honey has been calculated to be 0.17%, although this can vary within a wide range.[1] Honey contains many trace minerals that are essential to health: phosphorus, iron, aluminium, magnesium, copper, manganese, silica, chlorine, calcium, potassium and sodium. All the above components are elements of the Earth in which plants grow. Plants absorb elements and deliver them to the nectar, which is a major resource used by bees to make honey. Therefore, honey will vary in mineral content not only according to the resources in the soil (where its evolution starts), but also according

to the kind of plants from which the bees took nectar.

Honey contains low crude protein levels and also fat content is generally low. In terms of starch the honeys have only a trace and sugars are between 11.2-96.0 g/100 g dry wt.[2] Honeybees gather propolis, a resin obtained by bees from buds of plants. The liquid, which can be produced out of it, is said to be an excellent medicine for all sorts of colds.

Honey also contains Vitamin C because most flowers contain this vitamin. Vitamin C (l-keto-l-threo-hexono-g-lactone-2,3-enediol) is commonly known as ascorbic acid. It is important to the human diet because it helps the body from connective tissue, bone, teeth, blood vessel walls and assits the body in assimilating iron and amino acids. Also Vitamin C has been used for the treatment of the common cold, mental illness, infertility and cancer.[3] For the determination of ascorbic acid several analytical methods have been used, such as flame atomic absorption spectrometry [a indirect determination of ascorbic acid based on its reducing properties: Cr(VI) in acid medium was injected into ascorbic acid stream, Cr(VI) was reduced to Cr(III) and this Cr(III) formed was proportional with the ascorbic acid concentration in the sample],[4] flow injection spectrometry (UV-visible detection),[5] chromatography,[6] voltammetry[7] and titrimetry.[8]

Since 1970 honey has been proposed as an environmental indicator for the assessment of pollution in the area where beehives are placed.[9] In fact, bees in their research of food can carry, together with nectar, any other contaminant deposited on flowers.[10]

In this paper inductively coupled plasma atomic emission spectrometry (ICP-AES) has been used for the determination of Cr, Fe, Mn, Ni in honey and propolis, because of the high sensitivity, wide dynamic range and relative freedom from interferences. For the determination of ascorbic acid was used a titrimetric method with potassium bromate-bromide solution in the acid medium.

Experimental

Chemical and reagents

All reagents used were of analytical reagent grade (Merck). Deionised water was used for the preparation of all solutions. 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 deionised water. The working solutions were prepared by diluting the stock solutions to appropriate volumes.

Sample preparation

Honey and propolis samples used in the research represent several representative Romanian honey sorts and these samples were taken from local market or made by private peasants. Aliquots of ca. 1 g of studied samples were made up to 100 mL with deionized water (the dry extract of propolis was soluble in water) and then where transferred into Teflon vessels until analysis, which was done within 2-3 h.

Sample analysis

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 summarized in Table 1.[11,12] For each sample three determinations were performed and average results were reported.

Table 1. Instrument systems and operating conditions of the ICP-AES.

Plasma conditions	
Rf. frequency	27.12 Hz
Rf. power	2.5 Kw
Outer gas	Ar 17 L/min
Intermediate gas	Ar 1 L/min
Carrier gas	Ar 1 L/min
Observation height	18 mm above work coil
Plasma's temperature	8000-9000 K

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 products at background concentrations.

For the determination of ascorbic acid was used a titrimetric method with potassium bromate-bromide solution in the acid medium.[13] Ascorbic acid, $C_6H_8O_6$ is cleany oxidixed to dehidroascorbic acid by bromine:

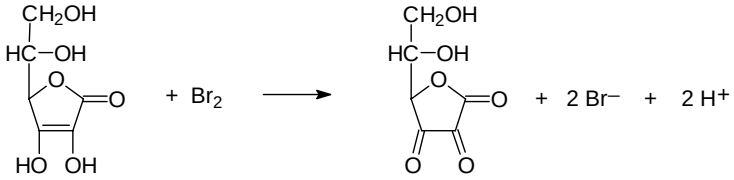

Table 2. Quality parameters of the method.

Metal	λ	LD (ng/g)
Cr	267.71	1.0
Fe	259.94	0.8
Mn	293.30	0.5
Ni	341.47	1.1

An unmeasured excess of potassium bromide is added to an acidified solution (with an amount of H_2SO_4 solution 1:1) of the sample. The solution is titrated with standard potassium bromate to the first permanent appearance of excess bromine; this excess is then determined iodometrically with standard sodium tiosulfate. The entire titration must be performed without delay to prevent air-oxidation of the ascorbic acid.[14,15]

Results and discussion

Metals are notable for their tendency to accumulate in select tissues of the human body and their overall potential to be toxic at high levels of exposure. This exposure can occur through a variety of routes and one of them is ingestion involuntarily through the food. In this purpose several types of honey samples were investigated: linden flower honey (made by private peasants coded A, honey from S.C. Honeyland coded B and honey from Apimond coded C), locust flower honey (made by private peasants coded D) and dry extract of propolis from Favisan coded E.

Chromium, iron, manganese and nickel contents of honey and propolis samples were determined with ICP-AES technique. The results obtained are given in Table 3. These show that the highest concentration of manganese (6.51 mg/Kg) exists in propolis and low concentrations are in honey samples. Also, it can be observed that concentrations of chromium, iron and nickel are higher in propolis than honey samples. So propolis is rich in essential elements and is an excellent medicine for all sorts of colds. Even the bees used the propolis at the entrance to the hive to sterilize themselves

as they come and go. Its basic ingredients (rich in amino acids and trace elements, it also has a high vitamin content) give propolis its dynamic bacteria-destroying power.

Table 3. Results obtained for the determination of Cr, Fe, Mn and Ni in honey and propolis samples.

Samples	Concentration (mg/Kg ± SD)			
	Cr	Fe	Mn	Ni
A	0.04 ± 0.07	2.04 ± 0.20	2.14 ± 0.23	1.03 ± 0.08
B	0.71 ± 0.22	2.91 ± 0,19	1.91 ± 0.24	0.90 ± 0.05
C	0.03 ± 0.11	2.33 ± 0.21	2.23 ± 0.14	2.03 ± 0.10
D	0.55 ± 0.05	2.55 ± 0.21	1.75 ± 0.08	1.54 ± 0.06
E	0.80 ± 0.17	3.50 ± 0.26	6.51 ± 0.40	2.50 ± 0.13

Iron is essential to life and plays irreplaceable roles in, for example, the functioning of critical enzyme systems. Higher concentration of this metal was found in linden flower honey from Apimond than in the locust flower honey made by private peasants.

Concentrations of studied heavy metals were a little higher in comparison to levels reported from honey samples collected in other area: near Siena (Italy).[10]

Analyses of honey samples from linden and locust flower indicate that levels of iron are comparable to those encountered in other groups of honey (blossom-multifloral and blossom-rape) and lower than concentrations measured in honeydew (forest-honey). Also the levels of manganese are comparable with those measured in these groups of honey. Data were obtained from the literature.[16]

The obtained concentrations of iron are comparable with those from the same areas (Romania) according to analyses of the other researchers in honey samples.[17]

The above-obtained concentrations of these elements are situated within the limits imposed by the last regulations of the specialized international commisions such as EC's Scientific Committee for Food. The results of heavy metals concentration in bee products were reported and published in Food Surveillance Information Sheet No. 53.[18] By Subsidiary Legislation 231.34 (1 October 1998) the levels of chromium, nickel and manganese are in the range: Cr: 0.001-0.80 mg/Kg, Ni: 0.02-2.5 mg/Kg and Mn: 0.1-12 mg/Kg. Heavy metals exposures were similar to those from the Total Diet Study.[19,20,21]

The content of ascorbic acid in bee products (honey and propolis) were determined by titrimetric method and the results are given in Table 4. It can be observed that the

ascorbic acid concentration is higher in propolis than honey samples (3.64 mg AA/g propolis). For honey samples was observed that the higher concentration is in linden flower honey made by private peasants (2.96 mg AA/g honey).

After ascorbic acid determination in these products, we can see that concentration of Vitamin C is lower in boiled solution of honey and propolis samples, because the Vitamin C is losing at the high temperature.

Table 4. Results obtained for the determination of ascorbic acid in bee products.

Samples	Ascorbic acid contents (mg AA/g bee products)	
	Solution of bee product	Boiled solution of bee product
A	2.96	1.18
B	2.77	1.10
C	2.94	1.17
D	2.26	0.90
E	3.64	1.45

Conclusions

The ICP-AES technique performs the simultaneous-sequential determination in a large number of elements from environmental and biological samples, being advantageous from the viewpoint of the short time and the low limit of detection.

The analyzed samples had low content of Cr, Fe, Mn and Ni. Although more extended investigation are necessary, it can however be stated that in the light of these data the concentrations of these elements in honey and propolis samples investigated do not pose any serious concern to human health.

It can be observed that concentrations of chromium, iron, manganese and nickel are higher in propolis than honey samples. Also the concentration of vitamin C is higher in propolis than in honey samples.

Following the results obtained was observed that linden flower honey, locust flower honey and propolis contain an important amount of Vitamin C, which is necessary for human body.

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Povzetek

Zadnja leta, sledeč trendu uporabe tistega, kar nam neposredno nudi narava, pridobivajo čebelji izdelki kot vir zdrave hrane vedno večjo veljavo. V tem prispevku smo želeli opredeliti vsebnost askorbinske kisline in sledov nekaterih esencialnih elementov (Cr, Fe, Mn, Ni). Kot vemo, askorbinska kislina preprečuje skorbut, ki je danes sicer redko bolezensko stanje, a interes za askorbinsko kislino ($C_6H_8O_6$) ostaja. V terapijah se uporablja kot protivnetni dejavnik in je ključna za normalno delovanje celice. Z bromom jo oksidiramo v dehidroaskorbinsko kislino, pri čemer v nakisano raztopino vzorca dodamo presežek kalijevega bromida, ter raztopino nato titriramo s standardno raztopino kalijevega bromata do nastanka presežnega broma, ki ga določimo jodometrično s standardno raztopino natrijevega tiosulfata. Za določitve Cr, Fe, Mn in Ni smo uporabili atomsko emisijsko spektrometrijo v povezavi z induktivno sklopljeno plazmo (ICP-AES). Vsebnost Ni je segala od 1,54 do 2,55 mg/kg, Fe od 2,55 do 3,50 mg/kg, Mn od 1,75 do 6,51 mg/kg, Cr od 0,03 do 0,80 mg/kg.