COMMUNICATIONS

DETERMINATION OF SOME TRACE ELEMENTS IN PROPOLIS BY ATOMIC ABSORPTION SPECTROMETRY

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Abstract

The results from the determination of trace elements Cu, Mn, Pb, Cd, Co, Ni, Ag and Cr in propolis by atomic absorption spectrometry are presented. Two procedures for mineralization of propolis are suggested. The procedures are checked by a standard addition method. Recovery values for all examined elements ranged from 87.8 to 105.2% for the wet procedure and from 90.4 to 101.9% for the dry one. The trace elements presented in Macedonian propolis are as follows: Mn>Cu>Pb>Cr>Ni>Co>Cd>Ag. Great variations in the amounts of some elements are probably due to differences in propolis origin, plant sources, ecology, climate and other factors.

Keywords: *Propolis, Trace elements determination, Atomic absorption spectrometry*

Introduction

People receive trace elements from food and water. Most of the trace elements in hive products are also discovered in blood and some organs of the human body. It has been established that there are 24 trace elements in human blood; 22 of them represented in hive products were also discovered in the human body. If some trace elements as Fe, Co, Cu, Mn, Ni and Zn are insufficient, the process of hematopoiesis will be disturbed.

The biological activity of many trace elements is linked with their synergistic action together with enzymes and vitamins. There is a strict dependence between the amount of vitamin B_{12} and Co. The presence of Cu and Co is necessary during the formation of the bone tissue. It was confirmed by experimental investigation that addition of honey, iodine and Co intensifies the phagocytosis of white blood corpuscles, increases the resistance of the body to infection diseases. That is why the regular use of hive products increases the resistance of the body, not only due to the vitamin content but also due to the trace element content [1]. Hive products such as honey, bee venom, royal jelly and propolis play an essential role in the prophylaxis and treatment of some diseases of men because they contain aminoacids [2], phenols [3], flavonoids [4-6], carbohydrates [9], terpenoids [10] and trace elements [11-14].

Recently, we reported some data concerning the composition of some macro- and microelements in Macedonian propolis [15] ethanol and water extracts [16].

The aim of this work is to determine trace elements as Cu, Mn, Pb, Co, Cr, Ni, Cd and Ag in Macedonian propolis by use of atomic absorption spectrometry (AAS).

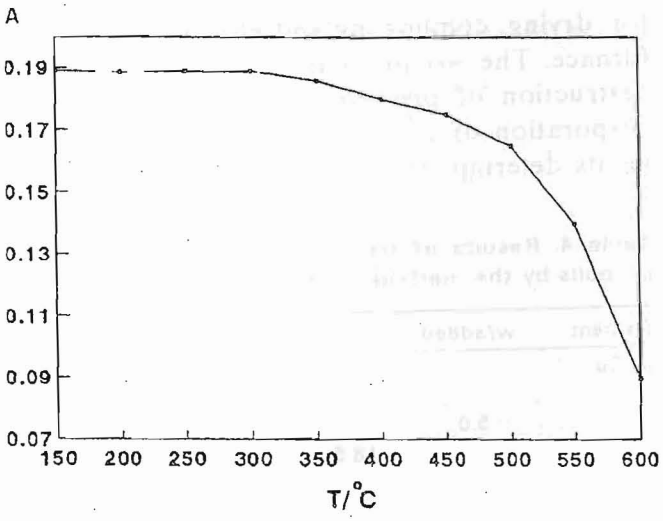

Fig.1. Determination of optimal char temperature for cadmium

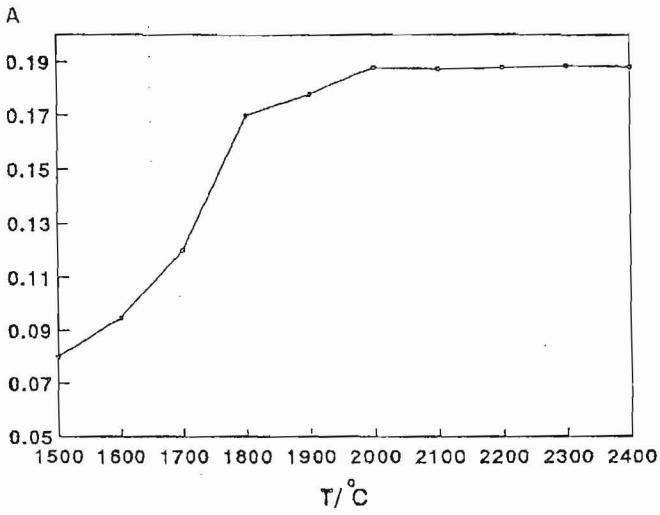

Fig.2. Determination of optimal atomize temperature for cadmium

Experimental

Apparatus

A Perkin-Elmer (USA) Model 370 flame atomic absorption spectrophotometer and a Model 303 atomic absorption spectrophotometer, equipped with a deuterium background corrector and HGA-400 graphite furnace were used. A mixture of acetylene-air was used. Optimal conditions for Cu, Mn, Pb, Co, Cd and Cr determination by flame atomic absorption spectrometry are given in Table 1. Optimal conditions for Co, Cd, Ag and Ni determination by electrothermal atomic absorption spectrometry (ETAAS) are given in Table 2. These conditions were determined by absorbance measurement for samples with equal concentrations of trace elements at different char and atomize temperatures. The highest temperature at which the maximum absorbance was registered was taken as an optimal char temperature and the lowest temperature provided maximum absorbance value was taken as an optimal atomize temperature. The procedure for determination of optimal conditions for ETAAS for Cd is illustrated in Figs. 1 and 2, which represent char and atomize curves for Cd, respectively.

Table 1. Optimal conditions for Cu, Mn, Pb, Co, Cd, Ni and Cr determination by AAS

Element	Wavelength (nm)	Slit (nm)	Lamp Current (mA)
Cu	324.7	0.7	15
Mn	279.5	0.2	10
Pb	283.3	0.7	10
Co	240.7	0.2	30
Cd	228.8	0.7	4
Ni	232.0	0.7	25
Cr	357.9	0.7	10

Samples

Samples of propolis were collected from different areas in Macedonia. They were cooled,

Table 2. Optimal conditions for Co, Cd, Ag and Ni determination by ETAAS

Parameters	Co	Cd	Ag	Ni
Wavelength (nm)	240.7	228.8	328.1	232.0
Slit (nm)	0.2	0.7	0.7	0.7
Lamp Current (mA)	30	4	10	25
DRY				
Temperature, °C	110	110	80	110
Time, s	20	20	20	20
Ramp time, s	2	2	2	2
CHAR				
Temperature, °C	900	300	400	900
Time, s	20	20	20	20
Ramp time, s	1	1	1	1
ATOMIZE				
Temperature, °C	2400	2000	1600	2400
Time, s	5	5	5	5
Ramp time, s	0	0	0	0
CLEANING				
Temperature, °C	2700	2700	2700	2700
Time, s	3	3	3	3
Gas	argon	argon	argon	argon

milled, transferred into plastic bags and stored at low temperature (in fridge) until they were used for determination.

Reagents

All reagents were of analytical grade. Stock standard solutions with concentration of 1 g/dm^3 were prepared using commercial solutions of all investigated elements, Merck (Darmstad, Germany). Standard solutions were prepared by dilution with redistilled water.

Procedures

Dry procedure - it was carried out in a porcelain crucible where 5 g of propolis were heated on a hot plate for 2 h, then glowed in muffle furnace at 150°C for 30 min and at 500°C for 6 h. The obtained mineral residue was dissolved in 10 ml of 4% solution of HNO_3.

Wet procedure - it was carried out in a beaker where 5 g of propolis were wetted with concentrated HNO_3 and heated in boiling water-bath for 5 h, covered with watch glass. After that, heating was prolonged on the hot plate until it was almost dry. A little amount of concentrated H_2SO_4 was added and heated until white vapors stopped. Complete mineralization was carried out by adding a few ml of concentrated H_2O_2. The obtained mineral residue was dissolved in 10 ml of 4% solution of HNO_3.

Results and Discussion

Because of the complex composition of propolis, the process of mineralization is longer than normally. The dry procedure takes about 20 h for: drying, combusting and glowing in a muffle furnace. The wet procedure takes two days, for destruction of propolis matrix as well as the evaporation of acids. The results of trace elements determination in one sample of propolis

Table 3. Trace elements content in propolis determined by AAS including dry and wet procedures for mineralization of samples

Element	Dry procedure		Wet procedure	
	w (µg/g)	RSD (%)	w (µg/g)	RSD (%)
Cu	9.55	4.76	14.9	9.83
Mn	24.1	1.66	25.8	2.71
Pb	8.24	8.49	10.5	3.81
Co	0.44	4.54	0.56	8.93
Cd	0.71	9.13	0.76	6.58
Ni	2.40	4.65	2.64	7.84
Cr	2.93	6.42	2.45	8.87

Table 4. Results of trace element determination in propolis by the method of standard additions, in µg/g

Element	w/added	w/calc.	w/determ.	R(%)
Cu				
1′	-	-	8.47	-
2′	5.0	13.5	13.6	100.0
3′	10.0	18.5	17.0	91.8
1″	-	-	8.47	-
2″	5.0	13.5	13.6	100.7
3″	10.0	18.5	16.5	89.3
Mn				
1′	-	-	20.70	-
2′	10.0	30.7	29.9	97.4
3′	20.0	40.7	36.8	90.4
1″	-	-	19.50	-
2″	10.0	29.5	26.4	89.5
3″	20.0	39.5	34.5	87.3
Pb				
1′	-	-	15.80	-
2′	2.0	17.8	17.8	100.0
3′	4.0	19.8	19.3	97.5
Cd				
1′	-	-	0.43	-
2′	0.4	0.83	0.78	94.1
1″	-	-	0.56	-
2″	0.2	0.76	0.73	95.5
Co				
1′	-	-	0.76	-
2′	0.4	1.06	1.07	101.0
3′	1.0	1.76	1.79	101.1
1″	-	-	0.72	-
2″	1.0	1.72	1.61	93.4
Ni				
1′	-	-	2.77	-
2′	4.0	6.77	6.90	101.9
3′	10.0	12.8	12.5	97.7
1″	-	-	3.40	-
2″	4.0	7.40	7.29	98.4
3″	10.0	13.4	11.9	88.8
Cr				
1′	-	-	2.56	-
2′	2.0	4.56	4.62	101.3
3′	10.0	12.6	12.4	98.4
1″	-	-	2.06	-
2″	2.0	4.06	4.27	105.2

1′, 2′ and 3′ - determinations related to the dry procedure
1″, 2″ and 3″ - determinations related to the wet procedure

milled, transferred into plastic bags and stored at low temperature (in fridge) until they were used for determination.

Reagents

All reagents were of analytical grade. Stock standard solutions with concentration of 1 g/dm^3 were prepared using commercial solutions of all investigated elements, Merck (Darmstad, Germany). Standard solutions were prepared by dilution with redistilled water.

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Element	w/added	w/calc.	w/determ.	R(%)
Cu				
1'	-	-	8.47	-
2'	5.0	13.5	13.6	100.0
3'	10.0	18.5	17.0	91.8
1''	-	-	8.47	-
2''	5.0	13.5	13.6	100.7
3''	10.0	18.5	16.5	89.3
Mn				
1'	-	-	20.70	-
2'	10.0	30.7	29.9	97.4
3'	20.0	40.7	36.8	90.4
1''	-	-	19.50	-
2''	10.0	29.5	26.4	89.5
3''	20.0	39.5	34.5	87.3
Pb				
1'	-	-	15.80	-
2'	2.0	17.8	17.8	100.0
3'	4.0	19.8	19.3	97.5
Cd				
1'	-	-	0.43	-
2'	0.4	0.83	0.78	94.1
1''	-	-	0.56	-
2''	0.2	0.76	0.73	95.5
Co				
1'	-	-	0.76	-
2'	0.4	1.06	1.07	101.0
3'	1.0	1.76	1.79	101.1
1''	-	-	0.72	-
2''	1.0	1.72	1.61	93.4
Ni				
1'	-	-	2.77	-
2'	4.0	6.77	6.90	101.9
3'	10.0	12.8	12.5	97.7
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2''	4.0	7.40	7.29	98.4
3''	10.0	13.4	11.9	88.8
Cr				
1'	-	-	2.56	-
2'	2.0	4.56	4.62	101.3
3'	10.0	12.6	12.4	98.4
1''	-	-	2.06	-
2''	2.0	4.06	4.27	105.2

1', 2' and 3' - determinations related to the dry procedure
1'', 2'' and 3'' - determinations related to the wet procedure

(from Skopje region) by two suggested procedures are given in Table 3.

Satisfactory results were obtained for the relative standard deviation (RSD). The best results were achieved for Mn – RSD is 1.66% for the dry procedure and 2.71% for the wet one, respectively. Better results for Cu, Cd, Ni and Co were obtained by the dry procedure while for Pb and Co by the wet procedure.

The suggested procedures were checked by the method of standard additions. The results of this determination are presented in Table 4.

Recovery (R,%) values ranged from 87.2 to 105.2% for the wet and from 90.4 to 101.9% for the dry procedures. Better results for R were obtained by the dry procedure for all examined elements. Because of that, as well as the simplicity of the dry procedure, the latter was chosen as highly recommended in our further studies of trace elements.

Trace elements Cu, Mn, Pb, Co, Ni, Ag and Cr were determined for twelve different samples of propolis collected from different areas in Macedonia. The results of this determination are given in Table 5.

It is shown that for the majority of the samples the highest amount of Mn (7.09 - 36.8 μg/g) was found (Table 5). The content of Cu was from 2.29 to 9.08 μg/g, except for two of the samples which contained 20.7 and 67.8 μg/g. For a few samples the content of Pb ranged from 1.30 to 6.83 μg/g but for others – from 16.6 to 21.8 μg/g. Trace elements as Co, Cd, Ni, Cr and Ag were presented in a very low concentration. The content for Cr was 1.50 - 17.2 μg/g, for Ni 0.62 - 2.42 μg/g, for Co 0.21 - 0.98 μg/g, for Cd 0.04 - 1.48 μg/g and for Ag 0.09 - 0.42 μg/g, respectively.

The correlation between the obtained results is less than it was expected, mostly due to the differences in the areas where the propolis was collected. They were more or less equal for the samples obtained from the same region but in two or three following years (two samples from Veles, two from Ohrid, three from Skopje). They were also very different for the samples from Skopje, Kumanovo and Tetovo, in spite of the proximity of the collection sites. Besides, the plant sources, climate, ecological, and other factors probably

Table 5. Contents of trace elements in Macedonian propolis determined by AAS and ETAAS, in μg/g (RSD in %, n=5)

Origin	Cu	RSD	Mn	RSD	Pb	RSD	Co	RSD	Cd	RSD	Ni	RSD	Cr	RSD	Ag	RSD
Skopje 1992, a.	3.32	5.42	35.1	1.73	4.55	9.23	0.25*	9.13	0.05*	6.90	2.35	4.60	3.60	4.32	-	
Skopje 1992, b.	0.08	4.76	12.3	2.07	5.53	9.45	0.28*	9.15	0.09*	5.55	2.01*	4.56	1.90	6.73	0.018*	8.92
Veles 1992	4.34	5.17	12.3	2.06	19.5	4.16	0.61*	9.00	0.82*	2.34	1.62	4.67	2.45	6.40	0.056*	8.23
Ohrid 1992	3.52	5.54	17.5	1.96	4.55	9.23	0.49*	9.06	0.10*	5.57	1.10	5.67	2.70	6.24	0.039*	9.79
Ohrid 1993	3.67	5.56	10.5	2.34	13.7	5.73	0.21*	9.12	0.07*	6.83	0.62	6.89	5.60	1.45	0.042*	8.76
Berovo 1992	4.08	5.23	36.8	1.67	2.93	9.87	-		0.14	5.56	1.14	5.66	3.75	1.87	-	
Strumnica 1992	67.9	1.45	8.77	2.67	6.83	9.06	0.18*	9.11	0.07*	6.78	2.42	4.44	2.66	6.24	0.014*	8.76
Kicevo 1993	3.62	5.05	9.93	2.54	16.6	5.83	0.55*	9.05	0.06*	6.89	0.84*	5.82	17.3	1.03	0.008*	8.97
Kumanovo 1993	20.07*	2.45	4.37	3.95	4.49	9.27	-		-		1.48*	5.03	2.41	6.40	-	
Resen 1993	3.42	5.42	24.6	1.89	10.4	8.75	0.98*	8.80	0.08*	6.83	1.25*	5.22	5.50	1.45	0.009*	9.87
Tetovo 1993	2.29	3.24	7.09	2.86	1.30	9.95	0.32*	8.98	0.04*	6.73	0.75*	5.45	1.50	1.45	0.009*	9.23

* - Determined by ETAAS

have a significant influence on the composition of propolis as well as the composition of trace elements. Accordingly, it was determined high level for Pb and Cd in two samples of propolis collected from Veles area. These high levels of Pb and Cd are probably connected with the presence of a lead and zinc smelting plant in this area.

Remarkably good resemblance was found for some of the elements in comparison to the literature data. According to Chanisev et al. [11] 2.59 mg/g of Ni, 3.04 μg/g of Pb, 2.41 μg/g of Co and 0.65 μg/g of Cr were spectrographically determined in a Siberian propolis. In the same propolis the Mn content was much higher (45-140 μg/g) than in the Macedonian one. Similar comment could be made about other results concerning German and Polish propolis [12].

Conclusions

Atomic absorption spectrometry is a convenient analytical method for determination of trace elements in propolis. Mineralization of propolis could be provided by dry or wet procedure but for the majority of trace elements dry procedure gives better RSD values. By checking the procedures by the method of standard additions, higher recovery values were obtained for the dry procedure.

The contents of trace elements (Cu, Mn, Pb, Ni, Co, Cd, Ag and Cr) in twelve different samples of propolis collected from different areas of Macedonia varies depending on the differences in the collection areas, plant sources, climate and other factors.

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