POLYCYCLIC AROMATIC HYDROCARBONS AND HEAVY METALS CONTAMINATION IN HONEY FROM CLUJ COUNTY, ROMANIA

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Abstract. Several honey samples from Cluj County and one sample from its territorial limit were investigated in order to establish their contamination degree in relation with polycyclic aromatic hydrocarbons (PAH) and heavy metals. High Performance Liquid Chromatography (HPLC) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) techniques were used in order to perform the proposed analysis. The HPLC analysis showed that the investigated honey samples have close concentrations of PAH. Benzo[a]pyrene, the representative marker of PAH in food have a concentration lower than limit of detection. Low concentration of heavy metals (As, Cd, Pb and Sr) in honey samples were obtained by ICP-MS analysis. The obtained results suggest that the investigated honey samples are appropriate for human consumption.

Keyword: honey, PAH, heavy metals, HPLC, ICP-MS

INTRODUCTION

Bee products such as honey are used in human diet for centuries. The use of honey is mentioned even in old and new testaments of Bible and Holy Quran [1]. Honey is a natural supersaturated sugar solution, which contains certain minor constituents like enzymes (glucose oxidase, catalase, phosphatases), glucose and sucrose (65–75% of total soluble solids), proteins, amino and organic acids, vitamins, lipids, volatile chemicals, flavonoids, phenolic acids, and minerals [2]. The biochemical properties of honey and its quality are related to honey maturity, climatic conditions, production methods, processing and storage conditions, as well as the nectar source of the honey [2]. Although for general population there is no doubt that honey is a natural, clean and healthy product, the number of scientific papers presenting real data on honey contaminants is increasing. Since honeybees are able to fly even 4 km per day for collecting nectar, it is possible to detect certain undesirable compounds and/ or residual molecules from different human activities. Likewise, abiotic factors such as air, water, and soils may be polluted with metals and they may play an important role in transferring residues to honey [3]. This conclusion was reported by several authors who consider honey as an environmental marker due to its ability to contain harmful pollutants such as Polycyclic aromatic hydrocarbons [4] and trace elements [5].

Polycyclic aromatic hydrocarbons (PAHs) are a large group of chemical contaminants [6] with properties negatively impacting the human organism such as carcinogenicity, mutagenicity, etc. [7]. The main exposure source of PAHs for most of the population is inhaled air and foodstuffs [8]. It is considered that the major route for non-smokers is consumption of food 9, 10], which can be contaminated from environmental

sources, from industrial food processing and from some domestic cooking practices [6]. Metals are listed among the pollutant residues that can be toxic for human beings if found at high levels, due to damages to physiological functions of living systems and their persistence through the food chains [3]. In honey, the presence of metals is associated with the presence of hives close to contamination sources, such as factories and highways and /or usage of agrochemicals [3]. Having in view the dangerous consequences on human health due to long exposure to PAH and metals, the purpose of this study is to present the results for these parameters obtained by studying the honey from several sources from Cluj County, Romania.

MATERIAL AND METHODS

Sampling. In order to achieve the proposed purpose, several honey samples were bought from local producers from Cluj County, Romania (Table 1). The position of the honey sources can be followed on Cluj County map [10] presented in Figure 1.

Table 1

Sampling location

Sample name	Location
Sample 1	Chinteni, Cluj County
Sample 2	Gherla, Cluj County
Sample 3	Beclean, Bistrita-Nasaud County (near Cluj County)
Sample 4	Dealul Iclodului, Cluj County
Sample 5	Pata, Cluj County
Sample 6	Dej, Cluj County

Fig. 1. Cluj County map [10]

Reagents and standards

PAH. PAH Calibration Mix containing 10µg/ml of each compound (Naphthalene, Acenaphthene, Fluorene, Phenanthrene, Anthracene, Fluoranthene, Pyrene, Benz[a]anthracene, Chrysene, Benzo[b]fluoranthene, Benzo[k]fluoranthene, Benzo[a]pyrene, Dibenz[a,h]anthracene, Benzo[ghi]perylene, Indeno[1,2,3-cd]pyrene) in Acetonitrile was acquired from Supelco. Hexane for HPLC (purity ≥99.9) was acquired from Sigma – Aldrich, Florisil (Merck) was used after heating overnight at 120°C. 0,45 µm filtration cartridge for syringe where acquired from Phenomenex, Ultra pure water was obtained using Ultraclear TWF UV UF T from Evoqua Water Technology - Siemens.

Metals. Analytical grade HNO_3 65%, H_2O_2 30% and ICP multi-element standard IV (1000 mg/L of Ca, Cu, Fe, Mn, Mg, Na, K and Zn in 0.2% (v/v) HNO_3) were purchased from Merck (Darmstadt, Germany Merck (Darmstadt, Germany. All dilutions were prepared using ultrapure water (18.2 MΩ/cm) obtained from a Millipore Direct-Q3 UV system (Millipore, France) was used.

Extraction method

PAH. 10 g of homogenized samples was extracted using 25 ml hexane in an ultrasonic bath for 60 min. The supernatant was purified on a Florisil clown and then evaporated to dry in a stream of nitrogen. The sample was reconstituted using 1 ml of acetonitrile. Before being injected the sample was filtered using a 0,45 µm filtration cartridge.

Metals. 0.2 g of honey samples were weighted in Teflon reaction vessel for microwave assisted digestion. A volume of 7.5 mL of HNO_3 65% and 3 mL of H_2O_2 30% were added to each sample. The samples were digested using a 5 step heating program. After mineralization, the samples were quantitatively transferred to 25 mL volumetric flasks and diluted with deionized water.

Analytical methods

PAH. Liquid chromatography conditions. The method was developed using a Perkin Elmer 200 Series High Performance Liquid Chromatograph (HPLC) with FLD detector. System Parameters: Flow Rate: 1,4 ml/min, Gradient mobile phase of H_2O and Acetonitrile, Column Temp: 24°C, Injection Volume: 50 µL, Column: INERTSIL ODS-P 5um 15cm X 0.46cm, TEKNOKROMA, different wavelengths appropriate for each compound for the FLD detector.

Metals. Inductively coupled plasma mass spectrometry (Elan DRC II instrument, Perkin-Elmer, Canada) was used for determining elemental composition of samples. A quantitative method was used for As, Cd, Cd, Cr, Co, Cu, Mn, Zn, Pb and Hg determination, while a semi-quantitative method for other elements was used. For the quantitative method, a calibration curve was developed (10 µg/L, 20 µg/L, 50 µg/L and 100 µg/L). If sample concentration exceeded the highest point on the calibration curve, the sample was properly diluted. Semi-quantitative method used response factor calibration curve for proper calibration before analysis. All the calibration procedures were performed before each sample batch. Daily parameter checks ensured the instrument run within specification (oxides level and double ionized species below 3%, background for low and high mass below

2 cps) and optimization procedures ensured high signal/noise ratio (using a solution of 1 µg/L of Indium for most of the optimization methods). Nickel skimmer and sampler cones were used and RPa was set to 0 for all determined elements and RPq parameter was 0.25. Dynamic reaction cell – (DRC) was used in rf-only mode (not pressurized).

RESULTS AND DISCUSSIONS

PAH. The results obtained for PAH analysis in honey samples are presented in table 2.

Table 2

Results obtained for PAH in honey sample

No.	Name of compound	Sample 1 (µg/kg)	Sample 2 (µg/kg)	Sample 3 (µg/kg)	Sample 4 (µg/kg)	Sample 5 (µg/kg)	Sample 6 (µg/kg)
1.	Naphthalene	<LQ	0.2054	<LQ	0.1461	0.1887	0.1461
2.	Acenaphthene	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
3.	Fluorene	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
4.	Phenanthrene	<LQ	0.0901	0.0870	<LQ	<LQ	<LQ
5.	Anthracene	<LQ	<LQ	0.0630	<LQ	<LQ	<LQ
6.	Fluoranthene	<LQ	<LQ	<LQ	0.0726	<LQ	0.0726
7.	Pyrene	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
8.	Benz[a]anthracene	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
9.	Chrysene	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
10.	Benzo[b]fluoranthene	<LQ	<LQ	0.1140	0.1193	<LQ	0.1193
11.	Benzo[k]fluoranthene	<LQ	<LQ	0.0845	<LQ	<LQ	0.0685
12.	Benzo[a]pyrene	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
13.	Dibenz[a,h]anthracene	<LQ	<LQ	0.1090	0.0837	<LQ	0.0837
14.	Benzo[ghi]perylene	<LQ	<LQ	0.2785	0.1216	<LQ	0.1216
15.	Indeno[1,2,3-cd]pyrene	<LQ	<LQ	<LQ	<LQ	<LQ	<LQ
16.	PAH TOTAL	<LQ	0.2955	0.7359	0.6119	0.1887	0.6119

*LQ = 0.05 µg/kg

The results presented in Table 2 suggest that there is no significant difference between the honey samples. Benzo[k]fluoranthene was found in concentration between 0.0685 – 0.0845 µg/kg. Benzo[b]fluoranthene was found in concentration between 0.1140 – 0.1193 µg/kg. Naphthalene was found in the highest concentration 0.2054 µg/kg. Although benzo[a]pyrene is considered to be is a representative marker of total PAHs in food [11], its presence in studied honey samples wasn't confirmed. In this moment there is no legislation regarding the permitted maximum level of PAH in honey. Considering that benzo[a]pyrene is used to evaluate the level of total PAHs contaminants for several food products, it can be concluded that the consumption of studied honey samples do not present any risk to human health. Comparable results were obtained by Dobrinas S. [4].

Metals. The results obtained for metals determination in honey samples are presented in Table 3. While Sr and Rb were found in honey samples in very low

concentration, the value obtained for As and Cd was under detection limit (LOD). These results shows that, regarding heavy metals contamination, the investigated honey is appropriate for human consumption.

Table 3

Results obtained for metals in honey sample

No.	Name of compound	Sample 1 (mg/kg)	Sample 2 (mg/kg)	Sample 3 (mg/kg)	Sample 4 (mg/kg)	Sample 5 (mg/kg)	Sample 6 (mg/kg)
1.	Li	0.06	0.05	<LOD	0.08	0.07	<LOD
2.	Na	12.47	5.17	6.95	6.15	8.83	9.91
3.	Mg	8.92	13.45	19.78	5.40	5.08	4.38
4.	Al	0.72	0.21	1.03	0.51	0.29	0.16
5.	K	176.12	358.80	223.28	156.70	149.03	142.48
6.	Cr	0.13	0.11	0.39	0.27	0.11	0.15
7.	Mn	0.32	0.55	0.20	0.33	0.05	<LOD
8.	Fe	0.24	<LOD	<LOD	<LOD	<LOD	<LOD
9.	Cu	<LOD	0.13	0.08	<LOD	<LOD	<LOD
10.	Zn	0.28	0.43	0.57	0.26	0.10	0.08
11.	Rb	0.26	0.45	0.70	0.21	0.13	0.12
12.	Sr	0.05	0.05	0.12	<LOD	<LOD	<LOD
13.	As	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
14.	Cd	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD
15.	Pb	<LOD	<LOD	0,09	0,06	0,19	<LOD

*LOD = 0.03 – 0.06 mg/kg

CONCLUSIONS

Several honey samples from Cluj County area were investigated in order to determine the level of PAH and metals. Benzo[a]pyrene, the representative marker of total PAHs in food, was not detected. The elemental analysis show levels of heavy metals under limit of detecton (LOD), much lower than the permitted maximum level.

*All authors had equal contribution to this study.

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