ORIGINAL PAPER

INVESTIGATION OF PESTICIDES AND HEAVY METALS FROM DIFFERENT VARIETIES OF HONEY

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Abstract. *Bee products are essential natural resources in promoting a healthy diet with very important therapeutic properties, therefore they are used in medicine and cosmetics. That is why we set out to determine the degree of contamination with organic and inorganic pollutants in various bee products. For this purpose, different samples of honey were investigated, as follows: linden honey from Romanian accredited beekeepers (1); linden honey from H Company (2); linden honey from A Company (3) and acacia honey from accredited beekeepers (4). The concentrations of eight pesticides and five heavy metals were analyzed in the four honey samples taken. According to experimental data, there have been identified honey varieties in which the maximum permissible limits for pesticides (A Company) and heavy metals (H Company) were exceeded. As a result, a more rigorous control is required regarding the honey assortments sold on the market for consumer safety.*

Keywords: *honey; pesticides; pollutants; heavy metals.*

1. INTRODUCTION

Honey is the product made by bees exclusively from the nectar of flowers or from the sweet juices collected by bees from certain plant species, substances that bees collect, process, enrich with their own substances and store them in honeycomb cells. Depending on the raw materials used by bees to produce honey, there are only two types of honey: floral and manna. A family of bees can raise up to 150 kg of honey in a season [1, 2].

Floral honey can be monofloral, when it comes from a single species of nectariferous plants (acacia honey, linden, sunflower, etc.), or polyfloral, when the nectar comes from several species of plants. Due to the diversity of honey varieties depending on the botanical

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origin, they differ by: appearance, sensory (organoleptic) perceived qualities and composition [3,4].

For a very long time in human history, honey has been an important source of carbohydrates and also the only sweetener available until the emergence of the sugar industry after the year 1800. Honey contains mainly carbohydrates. The glycemic index of honey is between 32 and 87, depending on its botanical origin and fructose content. Numerous recent studies have shown that changing sugar with honey as a sweetener in the daily diet can significantly reduce the risk of diabetes, obviously in the case of a rational consumption [5,6].

Honey also contains various biologically active elements: small amounts of proteins, enzymes, amino acids, aromatic compounds and polyphenols. In addition to its main energy components (fructose and glucose), honey also contains a large number of constituents in small quantities or in the form of traces that have many nutritional and biological actions such us: antimicrobial, antioxidant, antiviral, antiparasitic, anti-inflammatory, immunostimulator, and antitumor [7-11].

Chemical contamination of food is usually due to pollution and improper practices in agriculture. Heavy metals like lead, cadmium, arsenic or mercury are present in soil and water and can accumulate in various food. Food contamination can occur naturally or as a result of cultivation practices or production processes [12,13]. To protect public health, maximum levels of contaminants in food, such as nitrates, heavy metals and dioxins, have been set and are regularly reviewed.

Pesticides, veterinary medicines and chemicals used in agriculture have been considered hazards if they aren't used properly. Food residues may also come from animals involved in the production of food that had been administered veterinary medicinal products or had been exposed to pesticides or biocidal products. Maximum residue limits shall be established and regularly updated. Food containing unacceptable amounts of contaminants are not allowed to be marketed in the EU. In addition, there are rules regrding materials that come into contact with food, used for transport or to process food, but also materials used as packaging or crockery [14].

Contamination of bee products with various pollutants is mainly due to environmental pollution with pesticides from agricultural activity or heavy metals from intense industrial activity or pollutants released by various machines or equipment [15-18].

There are numerous studies in the literature that highlight the presence of contaminants with high toxic potential in bee products harvested from areas with high levels of pollution. These studies draw attention to the danger of consuming such contaminated products for the health of the population [19-24].

The aim of the paper is to analyze some pollutants with toxic potential on consumers (pesticides and heavy metals) from different varieties of honey sold on the Romanian market.

2. MATERIALS AND METHODS

In order to analyse the content in pesticides and heavy metals, different samples of honey were investigated as follows: linden honey from Romanian accredited beekeepers from the Prahova region (1); linden honey from H Company (2); linden honey from the A Company (3) and acacia honey from accredited beekeepers (4). The two mentioned companies sell assortments of honey on the Romanian market.

GC - ECD - gas chromatography with electron capture detector was used to determine the concentration of pesticides in bee products.

In order to establish the degree of pollution of bee products with pesticides, we aimed to determine the following organochlorine pesticides: HCB (hexachlorobenzene), p,p'-DDT (dichlorodiphenyltrichloroethane), p,p'-DDD (dichlorodiphenyldichloroethane), p,p'-DDE (dichlorodiphenyldichloroethylene), dieldrin, eldrin, linden and aldrin.

Thus, the Hewlett Packard HP 5890 Gas Chromatograph (Agilent Technologies, Santa Clara, CA, USA), equipped with an electron capture detector (ECD), a Split / splitless injector operating in splitless mode and an HP 5 capillary column (Hewlett Packard) with the following dimensions was used: 30 m × 0.32 mm × 0.25 μm. The compounds were separated on column passage and detected in turn by ECD. The identification of the analyte was made by comparing the retention time. The chromatographic peak was characterized with the data corresponding to a series of standard substances.

The chromatographic measurement conditions for GC-ECD were: one μL of the obtained extracts was injected, the column temperature was programmed as follows: one minute at 60°C, followed by an increase with 20°C / minute up to 300°C where it was maintained for 10 minutes.

The honey was solubilized in deionized ultrapure water and brought to a 100 mL volumetric flask. Then, the sample was passed into a separatory funnel, 10 mL of hexane were added and the mixture was stirred for 2 minutes.

The organic layer was allowed to separate and the aqueous phase was passed into a beaker, then the extract was collected. The operation was repeated 4 times. Purification was performed on a column of fluorisil and anhydrous sodium sulfate, eluted with hexane and then with a mixture of hexane: dichloromethane in a 3:1 ratio. The extract thus obtained was concentrated on a water bath using a Kuderna-Danisch concentrator. Fluorisil and anhydrous sodium sulfate were activated at 120°C for 12 hours.

A Shimadzu AA 6500 atomic absorption spectrometer (Shimadzu, Kyoto, Japan) was used to determine five heavy metals: cadmium (Cd), copper (Cu), zinc (Zn), manganese (Mn), ion (Fe) from the honey samples. An air / acetylene flame was used as the atomizing source, for all elements. Copper was measured with a multi-element cavity cathode lamp. Single-element cavity cathode lamps were used for Cd, Fe, Mn, Zn. The used fuel was acetylene with a purity of 99.999%, and the oxidizer was air with a flow rate of 1.8 - 2.0 L/min.

Before being analyzed, the honey samples without visible granules, were shaken for homogenization purposes, and those containing crystallized sugar (four samples) were heated to 65° C in a water bath for 30 minutes to homogenize and solubilize the crystals. From the homogenized samples, 1 g of honey was digested with 4 mL of 65% (v/v) HNO_3 and 0.5 mL of 35% (v/v) H_2O_2 in PTFE vessels. The vessels were placed into CEM MARS 5 microwave accelerated reaction system (CEM, Matthews, NC, USA). A blank digest was carried out in the same way. Digestion conditions for the microwave system applied were as follows: up to 120°C for 15 min and then constant for 10 min; up to 160°C in 20 min and constant for 15 min; finally, a cooling stage (30 min) was carried out to 22°C and diluted to 50 mL with deionized ultrapure water. The sample was then analysed with a flame atomic absorption spectrometer [13].

Three determinations were made for each investigation. Data was statistical evaluated.

All used reagents were of analytical reagent grade (Merck, Germany).

Statistical analysis of the collected data was performed using the open-source software (R Core Team 2019). Statistical significance was accepted for alpha-level 0.05. Because some samples from our study were too small for a classical approach, we applied a robust ANOVA version for comparing our datasets [25]. Eleven samples from each type of honey were analyzed. Results were expressed as mean ± S.D (standard deviation).

The linear correlation coefficients (R^2), limits of detection (LODs) and limits of quantification (LOQs) for each polutants analysed are presented in Table 1.

Table 1. Linear correlation coefficients (R^2), Limits of Detection (LOD) and Limits of Quantification (LOQ) for polutants

Element	R^2	LOD [μg/g]	LOQ [μg/g]
Cadmium	0.992	1.8 x 10^{-3}	19 x 10^{-3}
Copper	0.998	44 x 10^{-3}	134 x 10^{-3}
Zinc	0.988	62 x 10^{-3}	176 x 10^{-3}
Manganese	0.995	52 x 10^{-3}	164 x 10^{-3}
Iron	1.000	49 x 10^{-3}	146 x 10^{-3}
HCB	0.999	54 x 10^{-3}	184 x 10^{-3}
p,p'-DDT	0.994	1.35 x 10^{-3}	15.38 x 10^{-3}
p,p'-DDD	1.000	1.41 x 10^{-3}	16.35 x 10^{-3}
p,p'-DDE	0.996	1.48 x 10^{-3}	17.44 x 10^{-3}
Dieldrin	0.984	1.21 x 10^{-3}	14.22 x 10^{-3}
Eldrin	0.998	1.12 x 10^{-3}	12.18 x 10^{-3}
Aldrin	0.994	1.18 x 10^{-3}	12.46 x 10^{-3}

3. RESULTS AND DISCUSSION

Table 2 shows the concentrations of pesticides determined by the GC - ECD technique in the analyzed honey samples. Based on the obtained results it can be observed that the values of the concentrations of organochlorine pesticides were small, in samples of honey harvested from accredited beekeepers.

The highest concentrations of pesticides have been registered in the linden honey samples from the A Company, and they were above the maximum allowed limit (MAL) by International Standard (Codex Alimentarius Standard of F.A.O. / O.M.S Commission) [23] for p,p'- DDT and p,p'-DDD. These high values may be due to the fact that bees collect from plants treated with these pesticides. The lowest concentrations of pesticides were observed in linden honey samples from accredited beekeepers where only small amounts of Aldrin were founded (0.001 μg /g), the other pesticides analyzed not being detected. Pesticide contamination may indicate that the hives are located in areas with intensive agricultural activity, areas that are usually polluted with such substances.

Table 2. Concentrations [μg/g] of pesticide in the analyzed honey samples

Elements	Sample	Mean ± SD	Lower CI 95%	Upper CI 95%	MAL - International Standard [26]
Lindan	Linden honey H Company	0.002± 0.001	1.4 x 10^{-3}	3.1 x 10^{-3}	0.01
	Linden honey A Company	-	-	-	
	Linden honey from accredited beekeepers	-	-	-	
	Acacia honey from accredited beekeepers	-	-	-	

Elements	Sample	Mean ± SD	Lower CI 95%	Upper CI 95%	MAL - International Standard [26]
HCB	Linden honey H Company	-	-	-	0.2
	Linden honey A Company	0.044± 0.021	2.3 x 10^{-3}	4.2 x 10^{-3}	
	Linden honey from accredited beekeepers	-	-	-	
	Acacia honey from accredited beekeepers	0.032± 0.011	1.8 x 10^{-3}	3.9 x 10^{-3}	
p,p’-DDT	Linden honey H Company	-	-	-	0.05
	Linden honey A Company	**0.083± 0.018**	4.8 x 10^{-3}	9.2 x 10^{-3}	
	Linden honey from accredited beekeepers	-	-	-	
	Acacia honey from accredited beekeepers	0.035± 0.054	1.9 x 10^{-3}	4.1 x 10^{-3}	
p,p’-DDD	Linden honey H Company	-	-	-	0.05
	Linden honey A Company	**0.074± 0.015**	4.3 x 10^{-3}	8.2 x 10^{-3}	
	Linden honey from accredited beekeepers	-	-	-	
	Acacia honey from accredited beekeepers	0.016± 0.022	1.8 x 10^{-3}	3.1 x 10^{-3}	
p,p’ DDE	Linden honey H Company	-	-	-	0.05
	Linden honey A Company	0.043± 0.028	2.2 x 10^{-3}	4.2 x 10^{-3}	
	Linden honey from accredited beekeepers	-	-	-	
	Acacia honey from accredited beekeepers	0.009± 0.038	2.3 x 10^{-3}	4.5 x 10^{-3}	

Elements	Sample	Mean ± SD	Lower CI 95%	Upper CI 95%	MAL - International Standard [26]
Dieldrin	Linden honey H Company	-	-	-	0.2
	Linden honey A Company	0.031± 0.012	1.7 x 10^{-3}	3.5 x 10^{-3}	
	Linden honey from accredited beekeepers	-	-	-	
	Acacia honey from accredited beekeepers	0.002± 0.015	2.3 x 10^{-3}	4.2 x 10^{-3}	
Endrin	Linden honey H Company	-	-	-	0.2
	Linden honey A Company	-	-	-	
	Linden honey from accredited beekeepers	-	-	-	
	Acacia honey from accredited beekeepers	-	-	-	
Aldrin	Linden honey H Company	0.002± 0.014	1.3 x 10^{-3}	2.4 x 10^{-3}	0.2
	Linden honey A Company	0.041± 0.011	2.2 x 10^{-3}	4.5 x 10^{-3}	
	Linden honey from accredited beekeepers	0.001± 0.036	1.1 x 10^{-3}	2.1 x 10^{-3}	
	Acacia honey from accredited beekeepers	0.037± 0.018	1.9 x 10^{-3}	3.8 x 10^{-3}	

Note: SD –standard deviation; CI – confidence interval

Table 3 shows the values of metal concentrations determined in different honey samples. The highest concentrations were recorded in linden honey samples from H Company for Cu and Fe above the maximum allowed limit (MAL) by International Standard [26], and the lowest concentrations in heavy metals in acacia honey samples collected from accredited beekeepers. Contamination of honey samples with copper and iron can also be due to containers and utensils used in the process of collecting and storing honey.

Table 3. Concentrations [μg/g] of heavy metal in the analyzed honey samples

Elements	Sample	Mean ± SD	Lower CI 95%	Upper CI 95%	MAL - International Standard [26]
Cu	Linden honey H Company	**0.58± 0.18**	0.4775459	0.5424541	0.5
	Linden honey A Company	0.49± 0.28	0.5642253	0.6341080	
	Linden honey from accredited beekeepers	0.32± 0.11	0.2751124	0.3702144	
	Acacia honey from accredited beekeepers	0.26± 0.25	0.1952262	0.2464404	
Cd	Linden honey H Company	0.015± 0.11	0.0883468	0.1389581	0.02 – 0.2
	Linden honey A Company	0.018± 0.12	0.0945128	0.2431852	
	Linden honey from accredited beekeepers	0.005± 0.19	0.0027645	0.0084512	
	Acacia honey from accredited beekeepers	0.002± 0.48	0.0018246	0.0032175	
Zn	Linden honey H Company	1.38± 0.18	1.7451202	2.9158142	1.00-3.00
	Linden honey A Company	0.92± 0.14	0.5114818	1.0118152	
	Linden honey from accredited beekeepers	0.83± 0.44	0.4621482	0.9821516	
	Acacia honey from accredited beekeepers	0.72± 0.13	0.3814414	0.8114064	

Elements	**Sample**	**Mean ± SD**	**Lower CI 95%**	**Upper CI 95%**	**MAL - International Standard [26]**
Mn	Linden honey H Company	0.84± 0.23	0.4811211	1.0114082	-
	Linden honey A Company	0.56± 0.33	0.3631841	0.8145144	
	Linden honey from accredited beekeepers	0.48± 0.12	0.3112337	0.8772246	
	Acacia honey from accredited beekeepers	0.42± 0.16	0.2441168	0.8518661	
Fe	Linden honey H Company	1.33±0.13	0.7208103	2.8433010	1.00
	Linden honey A Company	0.91± 0.26	0.4663001	1.4056022	
	Linden honey from accredited beekeepers	0.78± 0.12	0.5022603	1.4881253	
	Acacia honey from accredited beekeepers	0.67± 0.22	0.3880117	1.3550371	

Note: SD –standard deviation; CI – confidence interval

According to the obtained results, it was noticed the presence of potentially toxic contaminants in high concentrations in some types of honey sold for consumption. The presence of contaminants indicates in particular that the locations of the hives are in polluted areas. The small accredited producers stated that they took into account the degree of pollution of the areas where they placed the hives.

Bee honey is one of the healthiest and most complex food, having an impressive number of benefits on health, and the contained substances are extremely easy to assimilate. Bee products are essential natural resources of a healthy diet and have important therapeutic properties, therefore they are used in medicine and cosmetics [27]. The determination of pesticides in honey is particularly important because these compounds are highly toxic to animals and humans.

4. CONCLUSIONS

Honey and bee products are natural products with therapeutic potential highly valued by people since antiquity. At the same time, honey is one of the most counterfeit products. In addition, in the current context of increasing global pollution, there is a risk of contamination with highly toxic pollutants. The present study aimes to evaluate the safety in consumption of some varieties of honey sold on the Romanian market.

The highest values of the concentration of the analyzed organochlorine pesticides were observed in linden honey from the A Company above the maximum allowed limit for total DDT, and the lowest in linden honey from authorized beekeepers, most of the pesticides not being detected in the latter.

Heavy metals are notable for their tendency to accumulate in human body tissues and for their potential to be toxic in small amounts.

The highest concentrations in heavy metals were detected in linden honey samples from H Company for Cu and Fe above the maximum allowed limit for Cu and Fe, and the lowest concentrations in heavy metals in acacia honey from authorized producers.

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