Ukrainian Journal of Ecology

Ukrainian Journal of Ecology, 2020, 10(2), 405-409, doi: 10.15421/2020_117

ORIGINAL ARTICLE

Contmination of heavy metals and radionuclides in the honey with different production origin

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Received: 05.04.2020. Accepted 10.06.2020

We reported the results of monitoring of heavy metals and radionuclides in the honey with different production origin from three southern regions of Odessa region (Ukraine). We founded Lead, Cadmium and 137Cs content in the samples (n=60) according to applicable domestic and international standards. Our data allow us to conclude the safety and quality of the product. Using the atomic absorption spectrometer, we founded that the sunflower honey samples were characterized by the highest levels of Lead and Cadmium – 0.24±0.01 and 0.03±0.001 mg/kg respectively. At the same time, the Lead content in the samples was within 0.13±0.01–0.24±0.01 mg/kg and Cadmium was 0.02±0.001–0.03±0.001 mg/kg. However, even minimal amounts of heavy metals indicated their active circulation in the environment, because there are no large industrial productions in the areas which were studied. Our research confirmed the importance of continuously monitoring of the radionuclides content, in particular 137Cs, in bee honey. The content of 137Cs in the bee honey samples was significantly lower than the maximum permissible values, however, within the limits, while the highest values were obtained in the polyfloral and acacia honey – respectively 6.9±0.37 and 6.3±0.36 Bq/kg, We also registered the smallest content of the radionuclides 137Cs in the sampkes of the sunflower bee honey. Thus, the 137Cs content was 3.9±0.35 Bq/kg in this group of samples. We associated this with the absence of agrotechnical measures in honey crops areas. In addition, the lower 137Cs content may be related to the radionuclide ingress and distribution whithn the honey plant. In general, the radionuclide content of the honey samples was within 3.9±0.35–6.9±0.37 Bq/kg.
Key words: heavy metals, honey, maximum permissible levels, pollution indicator, radionuclides.

Introduction

Honey is a valuable food product that is in high demand among the population worldwide because it has beneficial and healing properties. For many centuries, honey has been used by humans as a food that can be used for medicinal purposes. Only recently, the antiseptic and antimicrobial properties of honey have discovered and scientifically proven. The use of honey improves the healing of the skin wounds. It was laboratory proved its antibacterial, antifungal, antiviral and antimycobacterial properties (Israili, 2014). Also, the antibacterial effect of honey against *Staphylococcus aureus* and *Pseudomonas aeruginosa* has been proved. As we know, they are characterized by their durability (Vallianou et al., 2014). At the same time, the antioxidant activity of honey, which determines the content of vitamin C, monophenols, flavonoids and polyphenols, has been proven. It is known that antioxidants are used for the prevention of cancer, cardiovascular disease, inflammatory disorders, neurological degeneration, infectious diseases. Also, they are able to slow down the aging process of the body (Schramm et al., 2003; Khalil et al., 2010). Honey is a rich source of the phenolic bioactive molecules with the potential health benefits (Uthurry et al., 2011). It should be noted that the antioxidant activity of honey depends on its botanical and geographical origin (Kishore et al., 2011). Honey, as it is beeng produced in the environmentally friendly conditions, can be used as a "functional food" in the children, the elderly and the sick diets (Uthurry et al., 2011; Naccari et al., 2014).

The quality and safety of honey can also be affected by the microbial content of honey, although honey is a product with minimal bacterial contamination. This is due to its antibacterial properties. The presence of colibacteria, spore bacteria and yeast in honey indicate the low sanitary product quality (Snowdon & Cliver, 1996).

Honey is a complex mixture of carbohydrates and other substances, including macro- and trace elements. Excessive amounts of them can be detrimental to human health. So, monitoring of the metals content in honey helps to control its safety and quality (Aghamirlou et al., 2015). The botanical and biogeographical honey origin is an important question of the food products quality and safety (Wang et al., 2011). Usually, honey contains such minerals as Calcium, Copper, Iron, Magnesium, Manganese, Phosphorus, Potassium, Zinc (Vanhanen et al., 2011; Vallianou et al., 2014). However, some studies show that even when the heavy metals content in honey is within acceptable limits, there can be a carcinogenic rask increase (Rie et al., 2013). In addition, the determination of the trace element composition can be used to determine the geographical origin of the product, location of production. It is proved that the most accurate data is provided by the analysis of 42 indicators, but the use of 5 is also effective (Pb, Tl, Pt, Ho, Er) (Batista et al., 2012). The trace element content also can be used with the purpose to determine the product botanical origin (Czipa et al., 2015), because the honey samples origin correlates with its chemical composition (Rashen & Soltan, 2004). Romanian scientists show that the efficiency of determining the botanical origin of honey by the content of heavy metals is 80.8%, but the efficiency of determining the geographical origin is only 21.2% (Oroian et al., 2016). Croatian scientists have come to the conclusion that 40K is the main component of pollen. Therefore, its presence in the product indicates no falsification (Franić & Branica, 2019).

Despite its many useful properties and benefits, honey has been contaminated with toxic substances contained in the nectar that is collected by bees. Also, it has been contaminated by chemical substances which are used to treat bees and hives, pesticides (Naccari et al., 2014; Herrero-Latorre et al., 2017; D'Ascenzi et al., 2019). As a completely natural product, honey can play the role of an environmental pollution indicator (Pohl, 2009; Erbilir & Erdogrul, 2015). Separate studies indicate uneven accumulation of heavy metals in the bees body (Vishchur et al., 2016; 2019). This can't affect the finished product safety. Also radionuclides can accumulate in honey, in addition to heavy metals, pesticides, other pollutants. Croatian scientists point to the possibility of using honey from honey dew as an indicator of pollution by 137Cs, Cu, Rb, Cr, Ni (Barišić et al., 1999). However, other studies show that the use of honey as a bioindicator of the environment pollution isn't appropriate on the territories with the low pollution level (Bilandzic et al., 2014). The pollution of different types of honey by radionuclides has been increased significantly after the Chernobyl accident (Djuric et al., 1991). So, the maximum concentration of 134Cs and 137Cs in honey was in 1986. Their concentration has been decreasing over the next 10 years. It was founded the increase of Cs radionuclides in honey after the Fukusima-Daichi accident (Franić & Branica, 2019). At the same time the Italian scientists studies indicate that the radionuclides content in the honey samples from the supermarket chain doesn't exceed the maximum levels (Meli et al., 2016; Meli et al., 2020).

As honey are characterized by a high price, it is often falsifiable (by diluting less expensive syrups, by unauthorized use of antibiotics, by masking origin) (Strayer et al., 2014). The quality and safety of honey as an animal product is governed by the Codex Alimentarius and EU Directives 101/2001 in EU countries (Grigoryan, 2016).

The aim of our study was to investigate the heavy metals and radionuclides content in honey with different production origin from the open market in the South districts of Odessa region (Ukraine).

Materials and methods

We selected three types of honey production - acacia, sunflower, buckwheat and polyfloral. The samples selection was carried out randomly in the agro-food markets of the cities of Bolgrad, Tatarbunar and Sarata (south part of Odessa region, Ukraine). All honey samples belonged to different apiaries. A total of 36 samples (12 samples from each city) were investigated (Table 1).

Table 1. Inventory of honey samples

Area	Plants				Total
	acacia	buckwheat	sunflower	polyfloral	
Bolgrad	5	5	5	5	20
Sarata	5	5	5	5	20
Tatarbunar	5	5	5	5	20
Total	15	15	15	15	60

The studies were carried out at the Multidisciplinary Laboratory of Veterinary Medicine of Odessa State Agrarian University. The preparing of samples was performed before the study. For this purpose we put the 5g of honey in a crucible and ashed at 600 °C for 12 hours. The ash we were sorting was white. We processed it with 2 ml of 65% HNO_3 solution and 2 ml of 30% hydrogen peroxide solution. The obtained sample was placed in a 25 ml flask and brought to the mark with distilled water. The heavy metals content was determined by atomic absorption spectrometer agilent-240FS + GTA-120, and radionuclides content was registered by scintillation spectrometer by betta and gamma radiation SE-BG-01-AKP-150-63 NPP 'AKP', using the Akwin software. The samples were ashed before determining the radionuclides,. Statistical analysis of the datd was performed by Statistica v. 10 (StatSoft, Inc., USA). The data in the tables presented like mean and standard deviation.

Results

Our results indicated that in the samples from the Bolgrad district, the lead content in any of the samples didn't exceed the maximum levels. However, the highest values (within limits) were determined in the samples of acacia and sunflower honey – 0.23±0.01 and 0.18±0.01 mg/kg respectively. At the same time the lead content in buckwheat and polyfloral honey was found to be 0.13±0.01 mg/kg respectively.

In the samples, which were selected in the Tatarbunar district, the highest lead content was found in the samples of sunflower and buckwheat honey – 0.25±0.01 and 0.20±0.01 mg/kg respectively. The lowest value was found in the polyfloral honey samples – 0.12±0.01 mg/kg. In the samples from the Sarata district, the highest content of lead among all test samples was found in the sunflower honey sample – 0.29±0.02 mg/kg. The high content of this heavy metal was determined in the buckwheat honey sample – 0.24±0.01 mg/kg. As for cadmium content it was within acceptable limits in all samples. However, some samples were characterized by a value close to this indicators.

Table 2. Monitoring of radionuclides and heavy metals in honey samples

Region	Botanical origin	Number of samples	Safety performance heavy metals content, mg/kg Lead	Cadmium	^{137}Cs content, Bk/kg
Bolgrad	Acacia	5	0.23±0.01	0.01±0.001	5.7±0.34
	Sunflower	5	0.18±0.01	0.03±0.001	3.6±0.28
	Buckwheat	5	0.13±0.01	0.01±0.001	4.2±0.37
	Polyfloral	5	0.13±0.01	0.04±0.001	5.8±0.25
	average for the area		0.17±0.01	0.02±0.001	4.8±0.31
Tatarbunar	Acacia	5	0.16±0.01	0.02±0.001	6.7±0.31
	Sunflower	5	0.25±0.01	0.03±0.001	3.5±0.52
	Buckwheat	5	0.20±0.01	0.01±0.001	4.4±0.37
	Polyfloral	5	0.12±0.01	0.01±0.001	8.5±0.46
	average for the area		0.18±0.01	0.02±0.001	5.8±0.42
Sarata	Acacia	5	0.14±0.01	0.02±0.001	6.5±0.44
	Sunflower	5	0.29±0.02	0.03±0.001	4.7±0.25
	Buckwheat	5	0.24±0.01	0.04±0,002	3.8±0.54
	Polyfloral	5	0.13±0.01	0.01±0.001	6.3±0.41
	average for the area		0.2±0.01	0.03±0.001	5.3±0.41

Thus, in samples from the Bolgrad district the cadmium content in acacia honey was 0.04±0.001 mg/kg and in the samples of sunflower honey – 0.03±0.001 mg/kg. In the acacia and buckwheat honey samples an indicator of 0.01±0.001 mg/kg was established. The cadmium content in the sunflower honey samples from the Tatarbunar district was 0.03±0.001 mg/kg, while in acacia honey the content was 0.02±0.001 mg/kg, and in buckwheat and polyfloral honey – by 0.01±0.001 mg/kg. As for the Sarata district, the buckwheat honey samples were characterized by the highest cadmium content – 0.04±0.002 mg/kg. In the sunflower and acacia honey samples the cadmium content was respectively 0.03±0.001 and 0.02±0.001 mg/kg. The lowest indicator was found in the polyfloral honey samples – 0.01±0.001 mg/kg.
Regarding the 137Cs content, it was well below the limit values in the honey samples from all three districts. Thus, the polyfloral honey samples were characterized by the highest content of this radionuclide in the Bolgrad district – 5.8±0.25 Bq/kg. The high content of cesium was also in acacia honey – 5.7±0.34 Bq/kg. In the polyfloral honey samples from Tatarbunar was found 137Cs content 8.5±0.46 Bq/kg, also the high content was found in the acacia honey samples – 6.7±0.31 Bq/kg. In the honey samples which were selected in the Sarata dostrict the highest content of radioactive cesium was registered in the acacia honey samples – 6.5±0.44 Bq/kg.

Table 3. Lead, cadmium and 137Cs content in the honey samples

Botanical origin of the honey samples	Number of samples	The heavy metals content, mg/kg Lead	Cadmium	^{137}Cs content, Bk/kg
Acacia	20	0.18±0.01	0.02±0.001	6.3±0.36
Sunflower	20	0.24±0.01	0.03±0.001	3.9±0.35
Buckwheat	20	0.19±0,01	0.02±0.001	4.1±0.43
Polyfloral	20	0.13±0.01	0.02±0.001	6.9±0.37

The data from the table testify that the sunflower honey was characterized by the highest lead content – 0.24±0.01 mg/kg, at the same time the polyfloral honey was characterized by the lowest content of this heavy metal (0.13±0.01 mg/kg) in the South districts of Odessa region. Also, the sunflower honey was characterized by the highest cadmium content – 0.03±0.001 mg/kg. The content of this heavy metal in the acacia, buckwheat and polyfloral honey samples was 0.02±0.001 mg/kg.
As for radionuclides, the polyfloral and acacia honey samples were characterized by the highest 137Cs content – 6.9±0.37 and 6.3±0.36 Bk/kg respectively.

Discussion

The value of the bee honey as a food product with the healing properties has been known for humanity for a long time (Khalil et al., 2010; Israili, 2014). The scientists have also paid attention to antioxidant (Schram et al., 2003), antibacterial and antiinflammatory properties of this product (Showdon & Cliver, 1996; Vallianou et al., 2014). Considering the fact that honey is a natural product and is obtained from the flowers' nectar, it may contain a number of toxic components from the environment (D'Ascenzi et al., 2019). On this basis a number of scientists consider appropriate to use this product as an indicator of the environment pollution (Naccari et al., 2014; Erlibir & Erdogrul, 2015; Herrero-Latorre et al., 2017). We were studied 60 different botanical origin bee honey samples (the sunflower, buckwheat, acacia and polyfloral) from the South districts of Odessa region by the heavy metals content (lead and cadmium), which are regulated by the regulatory documents regarding the quality and safety of this type of product in our country, and radionuclides content. The research of foreign scientists points to a more accurate probability to establish geographical and botanical origin when the more metals are analyzed (Barišić et al., 1999; Batista et al., 2012; Czipa et al., 2015). Heavy metals and radionuclides are one of the most important environmental pollutants. Thus, Ukrainian scientists have proved that the amount of heavy metals in honey is directly depends on the botanical and geographical origin (Kovalchuk & Fedoruk, 2013; Kovalchuk et al., 2019).

Our studies indicate that the highest content of lead in honey was in the Sarata district – 0.2±0.01 mg/kg, and the lowest – in the Bolgrad district (0.17±0.01 mg/kg) We explain this by the close location of apiaries to motorways, as there is no industrial production that would pollute the environment in the study areas. It should be noted that fluctuations in the content indices were within the acceptable limits. However, the fact that a large part of heavy metals settles on the chitinous trunk of bees should be taken into account, so the content of heavy metals in the bee honey will be lower than in the environment (Kovalchuk & Fedoruk, 2013; Kovalskyi et al., 2018). The another common environmental pollutant is cadmium, which is also emitted mainly by industrial productions (Kovalchuk & Fedoruk, 2013; Aghamirlou et al., 2015). It was interesting that this heavy metal was found in the different botanical origin honey samples in all the study areas, because, as mentioned above, there are no large industrial enterprises nearby. Thus, the highest cadmium content was found in the Sarata district (within acceptable limits) – 0.03±0.001 mg/kg. This means that cadmium is a stable part of the natural chains, that allows it to spread over the long distances.

We registered the highest lead and cadmium content (within the acceptable limits) in the sunflower honey samples – 0.24±0.01 mg/kg and 0.03±0.001 mg/kg respectively. This can be attributed to the features of the heavy metals accumulation by this type of plant, because scientists have proved this fact regarding the content of radionuclides (Kryvyj et al., 2016). It should be noted that this type of honey is most often used for export. Instead, the polyfloral honey samples were characterized by the lowest content of heavy metals (lead and cadmium).

Long-term attention has been paid to the content of radionuclides in bee honey, especially after the the Chornobyl accident (Djuric et al., 1991; Meli et al., 2016; Franic´ & Branica, 2019; Meli et al., 2020). 137Cs accumulation in the different parts of the plant is not the same. In addition, the radioactive contamination of honey is characterized by the high variability. Thus, during the research of rapeseed honey, the highest 137Cs content was found in flowers (Kryvyj et al., 2016). The researches of domestic scientists show that the 137Cs content in the different botanical origin honey, obtained in different regions of Ukraine, meets the current requirements, requirements of EU standards and Codex Alimentarius.

The samples from the areas near the exclusion zone are characterized by the higher radionuclide content (Lazaryeva & Postoyenko, 2016). The results of our studies are the same as above because really the honey samples in all three districts meet the current requirements by the radionuclides content. The 137Cs content in the study areas was approximately the same and was within 4.8±0.31–l 5.8±0.42 Bq/kg. This is not surprising, as the radioactive cloud did not reach the study areas after the Chernobyl disaster. However, if we talk about the content of radionuclides in the different botanical origin honey, the highest content of 137Cs was in the polyfloral and acacia samples – respectively 6.9±0.37 and 6.3±0.36 Bq/kg (indicators correspond to the acceptable limits). We associate this fact with the polyfloral honey origin, which is produced from the plants that grows on the soils which aren'n amenable to agricultural practices, including fertilizers. They significantly reduce the radionuclide content in the plants.

Conclusion

Bee honey in the southern districts of Odessa region can be used as an indicator of the environmental pollution by such major pollutants as lead, cadmium and 137Cs. The all honey samples which were studied comply with the applicable regulations that regulate the safety and quality of this product by the lead, cadmium and 137Cs content. The highest lead and cadmium content levels was found in the samples from the Sarata district – 0.2±0.01 and 0.003±0.001 mg/kg respectively. The highest content of radionuclides (137Cs) was found in the polyfloral honey samples – 6,9±0,37 Bq/kg. However, the indicator was within acceptable limits.

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Citation:

Piven, O.T., Khimych, M.S., Salata, V.Z., Gutyj, B.V., Naidich, O.V., Skrypka, H.A., Koreneva, Z.B., Dvylyuk, I.V., Gorobey, O.M., Rud, V.O. (2020). Contamination of heavy metals and radionuclides in the honey with different production origin. *Ukrainian Journal of Ecology, 10*(2), 405-409.