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Chapter 14

Analytical Procedures for Determining Heavy Metal Contents in Honey: A Bioindicator of Environmental Pollution

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Abstract

Metals are pollutant residues detectable in honey and in fact account for most of the inorganic pollutants found in this food product. Metal pollutants can be accumulated through the food chain and, at levels exceeding safe thresholds, can be toxic to humans and even damage physiological functions. During the honey-making process, bees can transport pollutants to the beehive following contact with polluted botanic species or from drinking contaminated water. Detecting very low concentrations is a persisting challenge to accurately measure these elements in honey. Additionally, since honey is a complex organic matrix, treatments are needed prior to applying any classical chemical methods for metal determination, such as inductively coupled plasma and atomic absorption spectroscopy. Therefore, optimal results are dependent on adequate sample conditioning prior to heavy metal content analyses. Chemical pretreatments include calcination processes and/or acid digestion. Regarding execution, the last steps of any metal detection methodology are the primary determinants of result quality, where any loss of mass is reflected by unreliable values.

Keywords: heavy metals, metals in honey, sample preparation, analytical methods

1. Introduction

The internationally recognized Codex Alimentarius Commission defines honey as a naturally sweet substance produced by bees through the collection of flower nectar or secretions from living plants and the subsequent transformation of these collected materials with substances inherent to bees. This mix is deposited and dehydrated for storage, a process that results in the maturation of honey [1].

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Honey is principally composed of a complex mix of carbohydrates, among which fructose and glucose account for 85–95% of the total sugars. Since glucose is less soluble than fructose, the proportions of these sugars in honey determine overall granulation, with higher fructose quantities lending to honey that remains longer in the liquid state [2, 3]. Other more complex sugars are formed through the bonding of two or more fructose/glucose molecules with trace polysaccharide residues. Honey also contains other substances to lesser degrees, including organic acids, amino acids, proteins, enzymes, minerals, fat-soluble vitamins, flavonoids with antioxidant properties, and hydroxymethylfurfural, a compound that indicates honey freshness [4–8]. Finally, honey can be further classified by melissopalynological analysis as either monofloral or polyfloral in origin. Monofloral honey is of greater commercial value due to 45% of solid residues being single-pollen in origin [9–12]. Altogether, the quality of honey depends on the presence and concentrations for each of the aforementioned compounds, as well as on classification as either mono- or polyfloral.

The close source-product association between plants-honey means that all honey inherit various characteristics of and share biological properties with their respective botanic sources [13]. Due to this, undesirable compounds or residues can be found in honey if the source plants were exposed to these substances, including those of anthrophic origin. Among the residues that alter the natural composition of honey are metals, which, depending on their concentration in food, can pose as a human health risk [14]. The most common route through which humans ingest and are exposed to metals is through the diet, although the presence of these chemical elements in the air also means intake through inhalation.

Some heavy metals are essential elements for normal growth of plants such as Co, Fe, Mn, Ni, Zn, and Cu and they have important roles in metabolism, but at higher concentrations, the same metals become toxic. Those increased levels can cause a decrease in percentage of biomass in vegetables and in many other cases, they lead to plant death. On the contrary, some heavy metals such as Pb, `Cd, Cr, and Hg have been marked with high toxicity for plants [15].

Metals have a density, (d) > 5 g/mL and atomic number > 20, with the exceptions of alkaline and alkaline earth metals. No more than 0.1% of the earth's crust contains metals. Although the term "heavy metals" primarily refers to elements with elevated cellular toxicity, this definition now extends to include micronutrients that, at high concentrations, represent a risk to human health. Heavy metals without known biological functions are the most dangerous due to high toxicities, including barium (Ba), cadmium (Cd), mercury (Hg), lead (Pb), strontium (Sr), and bismuth (Bi). Trace elements, or micronutrients, toxic at increased concentrations include boron (B), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), magnesium (Mn), molybdenum (Mo), nickel (Ni), selenium (Se), and zinc (Zn) [16]. Due to the human health risk presented by these heavy metals and micronutrients, regulations exist for the maximum residual limits permitted in various foods destined for human consumption [17, 18].

2. Metals in soils: impacts on apiculture

The origin of heavy metals in soils can be anthropic or natural, and may be associated with different fractions of soil, which determine the mobility and availability of these metals to

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the surrounding ecosystem. It could affect honeybees or its habitat by polluting plants and water. The availability and mobility of these contaminants could be modified in relation to the physicochemical properties of the soil, for example, pH and organic matter content, among others.

Undoubtedly, this will have an important effect in the metal content in honey, since honeybees are able to take water from these polluted sources. Moreover, bees may transport these pollutants to beehives by fixing them to their bodies after their contact with the polluted plant species.

Soils systems are complex and vary in traits based on mineral and organic residue compositions. In particular, heavy metals of both anthrophic and geochemical origins can affect soil characteristics. For example, Chile is the leading producer of copper worldwide, with this metal constituting a primary source of both net national income and employment. Nevertheless, copper mines generally overlap with the Transverse Valleys of the "Norte Chico" region of Chile, which is also an important region for agriculture and apiculture. Due to this spatial crossover, controversies exist between the mining and apiculture industries regarding mining-produced wastes. Specifically, these toxic residues are discharged into the air, soil, and water of valleys with human populations and with ranch, farm, and apiculture productions.

The presence of heavy metals in soils is not only due to external contamination, but can also be of geochemical in origin. Indeed, high copper contents can be found internationally in a number of soils [19]. This can occur due to mixed causes, such as abnormal native geochemical contents being complemented by mining contaminants. Generally, copper contamination is accompanied by high contents of other metals, such as arsenic, lead, cadmium, and zinc. Soils are open systems that exchange energy and organic matter with proximal environments. These exchanges are typified by a heterogeneous mix of three principal components—solid, gaseous, and liquid fractions of organic, inorganic, and microorganic components [20]. Several analytical approaches exist for determining total heavy metal contents or the fraction of total soil content represented by these elements. This fraction can be used to determine metal availability and mobility. Element availability in soils is the most representative way to estimate total element content as this fraction facilitates establishing assumptions of mobility, plant absorption, and possible contamination [21]. The availability of distinct contaminating elements depends on properties inherent to each element, including the tendency to form complexes with organic material; mineral chemisorption; precipitation as insoluble sulfides, carbonates, phosphates, and oxides; and co-precipitation in other minerals [22].

One of the most important chemical processes in soils is adsorption. This process determines the quantities of nutrients, metals, pesticides, and other organic chemical components retained on the soil surface. Due to these functions, adsorption clearly participates in regulating nutrient and contaminant transport in soil. Chemical and physical forces act during adsorption in direct relation to soil-surface functional groups and the ion or molecule of the solution. The interplay between both these relations gives rise to surface complexes that can be classified as either internal or external sphere complexes. Internal sphere complexes are established by chemical forces that are generally irreversible and slightly affected by changes in ionic strengths. In turn, external sphere complexes primarily involve Coulombic interactions that, through a reversible process, are affected by ionic strengths in the aqueous phase [23].

Most soils are heterogeneous and constituted by different minerals, solids, and organic compounds. Various interaction mechanisms of soil with heavy metals have been described, including diffusion through micropores and adsorption at sites with variable reactivity. It is not possible to discriminate between these mechanisms, being more appropriate to use the term "sorption" in order to describe the retention of heavy metals by these three pathways [24]. The type of sorption and metal-binding mechanisms depend on various factors, such as ionic radius, electronegativity, surface type, valence electrons, and ionic strength of the solution. Currently, strict regulations exist for metals due to residual accumulations and persistence in the environment, as supported by findings after specific contamination events [25, 26]. Furthermore, a number of studies have established the threat posed by the possible contamination of water and soil resources destined for agricultural ends. Any subsequently produced plants would represent healthy risk to consumers [27–29].

3. Metals in honey

The presence of metals in honey has been associated with the presence of hives close to contamination sources, such as factories, highways, volcanoes, or mines/mine tailings. Contamination sources can also include agrochemicals that contain cadmium and arsenic, among others [30–32]. Due to this association, extensive research has been conducted in honey to determine the relationship between heavy metal contents and quality indicators or biological markers [33, 34]. Frequently, heavy metal concentrations in honey are low, complicating the analysis of these elements. This complication is directly evidenced in the quality of obtained results, where any loss during the analytical processing of samples influences the concentration values determined for each metal [35].

Related to the analysis of honey, Przybyłowski and Wilczyńska [36] conducted research on polyfloral honey produced in Poland to evaluate possible relationships between parameters such as pH, the glucose:fructose ratio, moisture, electric conductivity, and hydroxymethylfurfural concentration, among others, and the presence of cadmium, lead, and zinc. These relationships were determined based on methodologies established by the Association of Analytical Communities [37] for processing organic samples and performing posterior metal assessments. While no clear relationships were found between the measured parameters and the metals studied, discrete cadmium and lead concentrations were found in all of the studied samples. This finding indicates a degree of environmental contamination. Similarly, relationships did exist between plant origin and the presence of zinc in samples. Further research was conducted by Hernández et al. [38], who analyzed the metal contents in 81 honey samples from the Canary Islands and compared results against 35 additional samples from zones in Spain and Europe in general. Analyses established that the concentrations of alkaline and alkaline earth metals were within specific ranges that discriminated between Canary Islands and European mainland honey. The authors therefore concluded that this type of analysis can be used to certify the source of a honey. Hernández et al. [38] also suggested that the presence of metals could indicate the production of honey in areas contaminated by these metals.

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Another study on avocado honey from Spain also showed a common pattern between the measured concentration of alkaline and alkaline earth metals. Just as with the Cannery Island honey, Terrab et al. [39] suggested that honey origin could be established based on the concentrations of certain metals. However, the correlation between botanic origin and the presence of metals has been difficult to establish in other parts of the world. For example, Fredes and Montenegro [40] studied the possible origin-metals correlation for honey from distinct regions of Chile, but were unable to establish an association between the presence of the measured elements and the botanic/geographic origin of the analyzed honey samples.

On the other hand, an objective established by a number of researchers has been to correlate the presence of metals with the biological properties of honey. One such investigation by Küçük et al. [41] analyzed three honey samples with different botanic sources in Turkey to evaluate a possible relationship between the concentrations of alkaline, alkaline earth, and other metals with the antibacterial properties of honey. The obtained results were able to establish that honey with higher concentrations of all the studied metals also presented greater antibacterial activities. In the three samples, the metal concentrations did not exceed permitted limits. Nevertheless, no clear link was found between the measured metal concentrations and other biological properties of honey, such as phenolic compound levels.

4. Sample treatments

Before assessing the metal contents in honey, samples need to be pretreated to eliminate the majority of organic matrix components that can interfere in obtaining results. One method used in determining metal contents is solid phase extraction. This method can remove the predominant sugars from honey, thereby allowing for the collection of concentrated metal extracts that can then be analyzed through atomic absorption spectroscopy (AAS), inductively coupled plasma optical emission spectrometry (ICP-OES), or inductively coupled plasma mass spectrometry (ICP-MS).

Solid phase extraction can be useful in fractioning extracts of an element, zinc for example, that could be present in honey as hydrophobic complexes or as cationic species. Resins, such as Amberlite XAD-16 and Dowex-x8-200, must be used in these cases to accurately separate metal species [42]. Other strong cation-exchange styrene-divinylbenzene resins, including Amberlite IRP-69, Dowex 50W x8-400, and Dowex HCR-W2, have been used to determine and fractionate manganese and zinc contents in extracts [43]. Similarly, Dowex 50W x8-400 and Dowex HCR-W2, together with the Diaion WT01S resin, have been used to satisfactorily detect copper and zinc species [44]. Solid phase extraction is advantageous because it destroys all of the organic materials present in honey samples, thereby reducing analysis time and risks of analyte loss that could affect result reliability. However, application of this method is limited when a mix of various metals is needed for subsequent analyses.

Recently, a new chelating resin of poly[2-(4-methoxyphenylamino)-2-oxoethyl methacrylate-co-divinylbenzene-co-2-acrylamido-2-methyl-1-propanesulfonic acid] was synthesized for determining Cd(II), Co(II), Cr(III), Cu(II), Fe(III), Mn(II), Pb(II), and Zn(II) ions. This resin

showed good performance in separation and preconcentration of those trace metals with acceptable recovery values (higher than 95%) in comparison with other reported methods [45].

Another methodology with a purpose similar to solid phase extraction is wet digestion, which applies strong acids to digest organic material in honey. Specifically, samples must be heated for 3–4 h at 105°C to remove as much water as possible. Following this, digestion takes place at 45°C through the addition of an aliquot composed of an acid mix (i.e., HNO_3/HCl 1:1) until the organic matter is fully destroyed. Excess acid is then evaporated through drying. Finally, the obtained ashes are suspended in 10 mL HNO_3 10% v/v. The resulting solutions can be directly measured via AAS, ICP-OES, or ICP-MS [46]. On variation of wet digestion is calcination in a muffle furnace, which produces ashes that can then be suspended in a solution of 0.1 M HNO_3 and H_2O_2 at 3–30% v/v [47, 48]. A noted advantage of this method is that it permits measurement of diverse analytes through only one approach. However, a disadvantage is the risk for cross-contamination between samples and the time of analysis, so close supervision is needed during the execution of experimental procedures. Another variation on wet digestion that has been implemented with notable success is that of using microwaves to induce wet digestion [49].

Tuzen et al. [50] evaluated the efficiency of calcination with a muffle furnace as compared to other ash-generating techniques, such as wet digestion using inorganic acids and through microwave. For this, various honey samples were assessed and submitted to three digestion procedures. The obtained results for copper, magnesium, zinc, iron, lead, cadmium, and nickel, among others, were classified according to the standard deviation obtained for each measurement. From the resulting values, the authors concluded that microwave digestion gave the best results, followed by direct wet digestion. Finally, calcination via a muffle furnace resulted in the least precise and most disperse results.

Currently, no technique has been validated for determining and measuring metals specifically in honey. The AOAC [37] lists calcination in a muffle furnace as the official method for determining metals in any organic sample. However, the application of this technique to honey is limited due to the chemical properties of distinct metals and the different ranges in which each type of metal can exist in a honey sample. The behavior of any sample during calcination is fundamentally determined by the organic composition of the sample. Preventing losses in the interior of the muffle furnace is a complicated process to control, directly affecting the distribution of the data obtained from muffle furnace measurements. Furthermore, although metals are often collectively referred to as a single group of elements, metals present important physico-chemical differences. These variations constitute another challenge during calcination via a muffle furnace. Specifically, the chances of cross-contamination within the muffle furnace are high, ultimately influencing the distribution of the obtained values.

Likewise, the toxicity to human health presented by metals varies from one element to the next. Some heavy metals, such as lead, mercury, and cadmium, are highly toxic and are found at much lower concentrations than other elements. Although there are not maximum residue levels for these elements, the World Health Organization and Food and Agriculture Organization have established acceptable levels for honey (i.e., Pb: 25 μg/kg ; Hg: 5 μg/kg; and Cd: 7 μg/kg; [51]). Therefore, sample loss during the process of obtaining ash can result in imperceptible differences between the actual and recorded values for the aforementioned

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elements. This is a relevant issue when considering the low maximum residue levels permitted, where any loss can cause statistically significant differences between classifying a honey as contaminated or uncontaminated by these elements.

5. Analytical methods

In recent years, much investigation has been focused on developing new methods for measuring metal concentrations in honey, with the aims of obtaining more reliable and exact values. Electrochemical techniques are one such option and have already shown more sensitive detection limits for some elements. One of these techniques is that samples are subjected to combined acid mineralization and microwave calcination before posterior analyses, with results evidencing good reproducibility for the quantification of copper, lead, cadmium, and zinc concentrations in eucalyptus honey [52]. Similarly, Buldini et al. [53] measured metal concentrations in various types of honey using hydrogen peroxide-mediated digestion and posterior analyte quantification using ionic chromatography or voltamperometry. The results from this method were satisfactory when compared against values obtained for the same samples by traditional methods. Nevertheless, the proposed method was determined only reliable for investigative ends as the large volumes of hydrogen peroxide needed to process each sample translate into a notable risk that would be difficult to implement and manage on an industrial scale. Moreover, higher sample quantities would also be required.

A distinct strategy for the analysis of metals through electrochemical techniques was proposed by Muñoz and Palmero [54]. Specifically, honey samples were diluted in hydrochloric acid, a solution to which gallium nitrate was then added to decrease any interferences that could complicate adequate zinc measurements. This method provided better results not only for zinc, but also for cadmium and lead in the assessed honey. The primary advantage of the technique proposed by Muñoz and Palmero [54] is that digestion through H_2O_2 was not used. Nevertheless, this technique was unable to measure elements such as copper, thus limiting its widespread application.

In general, metals are quantified through traditional methods such as AAS, ICP-OES, and ICP-MS due to high instrument sensitivities. While one-third of all honey mineral contents is potassium (K), elements frequently found in trace amounts include iron, copper, and manganese, among others [55, 56]. For the more predominant inorganic elements in honey, AAS is the most convenient measurement method [43, 57]. However, when the elements under study exist in lower concentrations, then the use of more highly sensitive techniques should be preferred, using ICP-MS as the primary option and ICP-OES as the secondary option [51, 58, 59]. In many cases, ICP-MS has been used for determining metals in other related products obtained from bees. The analysis of metal contents in honeybee venom showed that this equipment permits achieving very low levels for quantifying of As, Ba, Pb Cd, Sb and Cu. This tool is important when honeybee venom is a recommended treatment for certain diseases in medicine [60]. Whatever the analysis of honey, pollen, or any other product taken from beehives, it is important to note that the ICP-MS requires several steps to be considered before chemical analysis. In order to achieve reliable results, it is advisable the optimization of the

instrument including calibration with standard solutions, fortification of samples, and the use of a reference material. Also, for having a correct validating process for one analytical method, it is necessary to incorporate a confirmation method to obtain quality data. These last analyses may be performed using graphite furnace atomic absorption spectrometry [61].

6. Conclusions

Several regions in the world are suitable for honey production with different attributes due to the presence of melliferous species. However, in many cases, the apicultural activities, beehives, and the melliferous plants occur near of sources of pollution and it may produce certain changes in the composition of honey.

In the last years, the foreign trade has increased the demand for honey and beehive products without toxic residues for fulfillment of food safety policies.

Since honeybees are able to fly even 4 km per day as a maximum distance from the beehive to the floral source for collecting nectar, it is possible to detect certain undesirable compounds and/ or residual molecules from different human activities. These pollutants can be deposited onto the surfaces of melliferous plants and flowers, especially in the case of plants growing near industries, highways, or volcanoes. 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.

Metals are listed among the pollutant residues that can be detected in the final composition of honey, and besides, they are classified as the main group of inorganic pollutants. They can be toxic for human beings if found at levels above the permitted limits, due to damages to physiological functions of living systems and their persistence through the food chains.

One of the most remarkable problems in metals analysis is related to very low concentrations available in honey content. In addition, honey is a complex organic matrix and it has to be treated previously to chemical determination of metals by classic analytical methods for instance, inductively coupled plasma (ICP-OES) or atomic absorption spectroscopy (AAS). The chemical treatment of samples related to extraction methodologies of metals includes the calcination process and/or acid digestion. These last steps are mainly responsible for the quality of the obtained products because any loss of mass will be reflected in no reliable values.

In that way, it is very important to determine the presence of heavy metals in honey using analytical procedures to obtain reliable values. It must be considered that honey and/or another beehive product such as bee pollen, propolis, or beeswax are organic matrix and thus, a cleanup method of samples before chemical analysis for determining heavy metal content is essential for achieving optimal results.

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