

ORIGINAL ARTICLE

Honey Safety Hazards and Public HealthRazzagh Mahmoudi^{*1}, Ameneh Ghojoghi², Peyman Ghajarbeygi¹¹Health Products Safety Research Center, Qazvin University of Medical Sciences, Qazvin, Iran²Department of Food Hygiene & Aquatics, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran

(Received: 4 April 2016 Accepted: 7 June 2016)

KEYWORDSHoney;
Chemical
contamination;
Methods; Hazards;
Adulteration

ABSTRACT: Honey is the oldest natural food produced by honeybee and comprises wide variety of valuable ingredients including carbohydrate, proteins, minerals, vitamins, organic acids, polyphenols and flavonoids that contribute to well-known therapeutic properties. This review provide available scientific information on different ways of honey adulteration and chemical contamination with the certain focus on the variety of methods for analyzing the residue levels in honey samples. For data collection, different scientific databases including Science Direct, Springer, PubMed and Magiran were searched. Honey such as other food products at risk to various types of contaminations and adulterations. Microbial and chemical hazards have been reported in various honey samples all over the world. Therefore, its use without knowing the source and its safety may be significant health risks. Honey labeling according to qualitative analysis is very necessary confirmed that health care. Health officials in all countries have to introduce firm regulation and laws that control and regulate honey production, handling, and analysis to ascertain its safety. Obviously, investigation of sensitivity of methods in order to detect the chemical residue levels for preventing the disruptive impacts on consumer's health is momentous and all reasonable efforts should be taken for having adequate control over honey production and standardizing the maximum residue levels of chemicals to minimize possible contaminations.

INTRODUCTION

Honey is a unique gift of nature with medical, cosmetic, nutritional properties and is the oldest natural food that human has been utilized. Honey is defined as “a thick, sweet, syrupy substance which bees make as food from

the nectar of flowers and store in honeycombs.” After collecting nectars by honey bee, its ripening undergoes through dehumidification, adding invertase enzyme (digestion of carbohydrates), thickening and moisture

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evaporation up to 13-18% [1]. The utilization of honey returns to ancient times since 4000 BC [2]. In addition, medical properties of honey have been recorded on Egyptian papyrus about 3,500 years ago [3].

Annual world honey production is estimated at about 1.4 million tones. Asia is the largest producer of honey, accounting for about 40% of the global production [4]. Such a nutritious bioproduct is of various valuable compositions. The fundamental constituent of honey is carbohydrate (95-97% of dry matter). Proteins, amino acids, minerals, vitamins, organic acids, polyphenols, alkaloids, anthraquinone glycosides, cardiac glycosides, flavonoids, reducing compounds, and volatile compounds are remnant compositions of honey that has been discovered [1, 3].

According to the source of botanical extract, honey is of various valuable compounds with well-proved therapeutic features. Different investigations have found honey as an antioxidant agent, anti-inflammatory factor, capacity of plasma glucose and blood lipid Regulation, immunomodulatory effect as well as memory enhancing agent [1, 3-6].

The therapeutic properties of oral administration of honey include treatment of laryngitis, osteoporosis, gastrointestinal ulcers, anorexia, insomnia and constipation, liver, cardiovascular and gastrointestinal problems. Topical application of honey is useful in mucocutaneous injuries like eczema, lip sores, sterile and infected wounds, genital lesions, burns, surgery scars, and athlete's foot problem treatments [1, 3-9].

Nowadays, honey is produced in an environment polluted by different sources of contamination. The contamination sources can be environmental and apicultural ones [2]. Environmental contaminants are pesticides, heavy metals, bacteria, and radioactivity. These contaminants are present in air, water, soil, and plants and are transported to beehives by bees. On the other hand, contaminants from beekeeping practices comprise acaricides used for parasitic mites (mainly

Varroa) control, bee repellents used at honey harvest, pesticides for wax moth and small hive beetle control, and the antibiotics. Aside from the extensive and increasing consumption of honey in all over the world, safety of this product is threatened by different ways of honey adulteration and chemical hazards like heavy metal, aflatoxins, antibiotics, and pesticides. These chemical substances lead to bioaccumulation in human body and with long half-life of the residues variety of nutritional and organoleptic effects would be expected [4, 5]. Impacts of exposure to these hazardous chemicals range from allergic reactions to metabolic, respiratory, nervous disorders and haemopoietic system disability as well as induction of resistant strains of bacteria [4]. Moreover, this chemical hazard application in honey is accomplished by serious economic loss due to decreasing products quality and making the marketing much more difficult [5].

MATERIALS AND METHODS

For data collection, different scientific databases including Science Direct, Springer, Pub med and Magiran over the past two decades were searched.

Honey and its beneficial effects

Therapeutic effect of honey has a valued place both in modern and ancient medicine. Different compounds of honey are associated with wide range of nutritional and health benefits. Polyphenol compounds of honey act as a cellular antioxidant agent and confront with oxidative stresses through hydrogen donation, removal of free radicals, inhibition of enzymatic reactions that take place in metallic ion chelation and being as free radical substrates especially ROS [6]. Polyphenols are also involved in memory enhancing activity in molecular level as well as antidepressant, antinociceptive and anxiolytic effects [1]. In recent studies, the intrinsic antioxidant effect of honey is due to its natural compounds like peptides, Millard reaction proteins, ascorbic acid, tocopherols, catalase (CAT), superoxide

dismutase (SOD), reduced glutathione (GSH), flavonoides (such as apigenin, pinocembrin, kaempferol, quercetin, galangin, chrysin and hesperetin) and phenolic acids (such as ellagic, caffeic, p-coumaric and ferulic acids). Honey has a valued place as an anti-inflammatory factor, while in simulation model of colitis it showed same efficacy as prednisolone [3].

Regulation of plasma glucose and blood lipid levels and C reactive protein involved in inflammatory reactions, antibacterial, antifungal properties and immune system enhancing feature are among other benefits of honey. Immuno-modulatory potential of honey takes place through proliferation of blood B cells and T lymphocytes. Moreover monocytic cell line culture represented increasing release of inflammatory cytokines, such as tumor necrotic factor-alpha (TNF- α), interleukin, the enhancing phagocytosis activity by changing oxidative burst process with inhibition of phagocytic myeloperoxidase function. Honey also affects releasing of antibodies against thymus dependent antigens through primary and secondary immune responses in mice [6]. Likewise, apoptotic potential of honey polyphenols mainly by attenuating microglia-induced neuro inflammation has been approved [3].

Several studies denote the blood cholesterol and glucose regulation of honey, which makes it as a nutritional supplement for diabetic, impaired glucose tolerance and hyperlipidemic individuals. Reducing fasting glucose levels in long-term consumption of honey, leads to dietary supplementation for healthy and diabetic individuals [6]. Honey exhibit cardiovascular protection effect by inhibition of ROS induced LDL oxidation in an in vitro study [3]. Also in some countries, honey is considered as the first line treatment of superficial wounds as well as deep lesions like abscess [1].

Antimicrobial activity of honey is a major focus of research and such a property has been proved since Aristotle era [3]. Presence of hydrogen peroxide, inherent acidic feature of honey (pH=3-4.5), high

viscosity and carbohydrate concentration (~ 80% w/w), having various organic acids like gluconic acid which leads to creating acidic nature of honey and finally non-peroxidic components like polyphenols, makes honey as a diverse antimicrobial agent [6]. Honey has inhibitory effect on nearly 60 species of bacteria, yeast, fungi, some viruses and leishmania and promotes its antibacterial effect (*Escherichia coli*, *Salmonella*, *Shigella* and *Helicobacter pylori*) through bacteriostatic or bactericidal activity [3]. Besides, growth inhibition of *Aspergillus flavus*, reduces aflatoxin B1 and B2 production. An antifungal property of honey is well documented which demonstrates growth inhibition of the yeast *Candida albicans* and most species of *Aspergillus baumannii* and *Penicillium chrysogenum* and all the common dermatophytes [7].

Honey and pesticide contamination

There is a global concern about widespread use of pesticides in agricultural and beekeeping industry. More than 150 different pesticides have been recorded in colony samples. The highest rate of contamination belongs to varroacides, which has accumulation effect in bee breeds, beeswax and pollen. Amitraz, flumethrin, bromopropylate, coumaphos, and fluvalinate are most often detected varroacides, used extremely for *Varroa jacobsoni* treatment. Insecticides, fungicides, bactericides and herbicides, organic acids are among other pesticides [8].

Uncontrolled and worldwide administration of pesticides to combat honey bee mites and agronomical pests, directly involved in unavoidable impression on contamination of honey, well documented human health hazard, even residues possess carcinogenic and genetic mutation effect as well as cellular degradation. Otherwise, there is a serious adverse consequence of pesticide residues on consumer's health and chronic toxicities. Pesticide health concern varies from mild skin irritation to birth defects, endocrine disorders, nervous

malfunction, even coma and death. Some pesticides are considered as persistent organic pollutants (POPs). Effect of exposure to POPs ranges from Immune system, reproductive, endocrine disorders to neurobehavioral disorder, carcinogenic, infertility and mutagenic effects during chronic exposure. The most important POPs are Aldrin, heptachlor, toxaphene, chlordane, DDT, endrin, dihedron, hexachlorobenzene, mirex and toxaphene [8].

There are two major sources of honey bee pesticide contaminations:

1- Direct application of pesticides in bee hives especially for varroa treatment

2- Environmental contamination which consist of four different pathways: 1) Direct contact with crop protection pesticide used in plants and soils or encounter with direct pesticide spray drift. 2) Consumption of contaminated pollen and nectar. 3) Picking contaminants through tainted water. 4) Inhalation of pesticides during daily out hive flight [9].

Based on European Union regulations, honey should have no chemical contaminations, including pesticides. Maximum concentration level of pesticides residues in honey samples have been legislated by different national regulations. However, lack of identical agreement leads to many problems in international trade and marketing. The MRLs value (maximum concentrations of pesticide residues) has been considered by European Union legislation and US Environmental Protection Agency for common pesticides used in apiculture (Table 1) [8]. According to the North America and European regulations, organochlorine pesticides have been banned in agricultural purposes since 1978, because of their extreme insolubility and persistency in the environment [10].

Although, such pesticides still apply without approval protocols in pest control and frequently found in soil, a potential source of atmosphere contamination through evaporation to the air, implication of water and plants, ultimately lead to animal and human bioaccumulation followed by acute and chronic toxicity [10].

Table 1. Pesticide MRLs values by US and European Union legislation

Pesticides	MRLs value by European Union legislation	MRLs value by US Environmental Protection agency
Amitraz	0.2 ppm	1mg•kg ⁻¹
Coumaphus	0.1 ppm	0.1mg•kg ⁻¹
Cyamizole	1 mg.kg ⁻¹	-
Fluvalinate	0.02 ppm	0.05 mg•kg ⁻¹

Genome sequencing of honey bee approved potential effect of pesticides in genome disorders and honey bee sensitivity to the pesticides. Furthermore, similar to other insects, there is a deficiency in some detoxification enzyme genes in honey bee; consequently there is more sensitivity of honey bee to the environmental chemicals [8]. Aberrantly, wide spread use of pesticides is followed by honey bee poisoning. The symptoms of poisoning depend on the kind of pesticides applied and

duration of exposure, besides, developmental stage of honey bee. The primary effect of chemicals involves worker bees and the most sensitive stage to the pesticide poisoning is larval stage. House bees pick contaminants through pollen collection in the field and store it in honey combs in hive. Population decrement is result of house bee poisoning and killing followed by lesser brood care [8]. In most instants, field bees are directly contaminated by pesticides in the field, but other worker

bees in the hive are trained with the contaminated nectar and pollen that field bees has been collected. The adverse effect of field bee mortality is that young bees are forced to establish the field bee's roles earlier than normal; therefore, colony disrupting would occur soon [11].

There are several international reports which suggest pesticide application without approved protocols is possibly responsible for CCD disease (colony collapse disorder) which cause approximately 30% losses in total bee populations in some areas of mid-2000 [8].

A study was carried out in India to assess acute toxicity of pesticides in the laboratory conditions, evaluation through pesticide spraying on flowering plants of mustard and directly exposure of honey bees (*Apis cerana* and *A. mellifera*). Direct administration of the insecticides created more mortality rather than indirect filter paper contamination assays. Some insecticides (chlorpyrifos, deltamethrin, malathion, monocrotophos and dichlorvos and profenofos) showed approximately 100% mortality of the bees with direct or indirect exposure at their field recommended doses in 48 hours. In addition, insecticides like flubendiamide, imidacloprid, thiamethoxam and methyl demeton caused very high mortality through direct pesticide spraying but lesser mortality through filter paper contamination method has been detected. The study revealed that monocrotophos is considered as the most toxic insecticide with 100% mortality after 1 hour spraying at the field recommended doses, followed by thiamethoxam, dichlorvos, profenofos and chlorpyrifos, respectively. Thus, they are not recommended to be used in apiarian practices. Among all fungicides experimented, chlorothalonil, carbendazim, propiconazole, mancozeb, and insecticides such as acetamiprid and endosulfan showed any repellent effect in both direct and indirect methods [12].

A series of experiments were carried out in France to analyze 80 different environmental contaminants,

pesticides and veterinary drugs from vast chemical classes detected in 142 samples of honeys, 145 samples of honeybees and 130 samples of pollens. This study was aimed to determine contamination of three matrices of honey, honey bee and pollen. It was found that the majority of samples (honey, honey bee and pollen) were not only considered to be contaminated with pesticides used in varroa treatment but also by fungicides like carbendazim. Overall, 36 type of contaminants were detected and only 10 compounds were identified in all matrices. Pesticides that are recommended to use in beekeeping industry are amitraz, carbendazim, thiophanatemethyl, coumaphos, flusilazole, triphenylphosphate (a biquitous contaminant of water and air), phosmet and tau-fluvalinate. Concentration of carbendazim, flusilazole and carbaryl detected in pollen were significantly higher in comparison to other matrices. So detection of contaminants in the shortest time (nearly 3 days) is one valuable capacities of pollen matrix and provide possibly explanation for the fact that pollen matrix is preferred for evaluation of acute contaminations. In addition, study revealed that honey is considered as the most frequently detected matrix in the lowest concentration. The least frequently detected matrix belongs to pollen in the highest concentration. Honey bee is proposed as intermediate matrix [13].

A similar study was conducted in Egypt in order to obtain global view of the presence of 14 organophosphorous insecticides (OPs) in honey bee (*Apis mellifera*) and hive matrices (honey and pollen) during spring and summer of 2013 from 5 different provinces of middle Egypt. The most frequently detected pesticides were profenofos, chlorpyrifos, malathion and diazinon. LC/MS–MS was used for determination of samples contamination with OPs by use of modified QuEChERS assay. Among all three matrices pollen possess the most levels of contaminations to OPs. Study showed that toxic levels of OPs accumulated in honey, pollen and bees through

consuming the food does not exceed levels of concern. Moreover, Hazard quotients of bee lethality through direct exposure of honey bees to OPs is lesser than levels of threat in Egypt. This study suggests that direct or indirect exposure of honey and pollen to OPs create minority concern due to lethality of bees in Egypt [8].

Fifty samples of honey collected from different markets of Portugal and Spain during 2002 were analyzed for 42 kinds of organochlorine, carbamate, and organophosphorus pesticide contaminations. The main detected pesticides in honey samples were organochlorines. Among them, γ -HCH was the most frequently detected followed by HCB and the other isomers of HCH (R-HCH and α -HCH). The only organophosphorus pesticides identified in the tests were heptenophos, methidathion and parathion methyl in 2% of honey samples. It was concluded that Portuguese honeys were more contaminated than Spanish ones. However, levels of pesticide residues found in honeys of both countries do not exceed levels of concern [14].

To determine potential exposure of bees to chemical hazards, pesticides and other management practices of local beekeepers and farmers, 61 honey samples were collected and analyzed during 2011 from four different regions of Colombia. An analytical procedure based on multiresidue method, using gas chromatography with nitrogen phosphorous detector/micro electron capture detector has been developed for selected insecticides, fungicides and acaricides assessments. In this study, pesticide residues were found in 32 samples with the most frequently levels of organochlorine and organophosphorus residues. The main detected compounds were chlorpyrifos (36.1% incidence), followed by profenofos (16.4%), DDT (6.6%), HCB, γ -HCH (4.9%) and fenitrothion (1.6%) respectively. Only 4.9% of the samples exceeded the MRLs levels established in Regulation (EC) No 396/2005 by European department [15].

In Turkey, different honey samples were collected from markets of Konya in order to assess pesticide residues. In fact, all honey samples were contaminated with Aldrin, cis-chlordane, trans-chlordane and oxy-chlordane. In 55 out of 109 samples tested, concentration of organochlorine pesticide residues of oxy-chlordane were relatively higher than the country codex MRLs. Results indicated that all honey samples were considered to be contaminated and in some extent pose a threat to consumer's health [9].

A research has been conducted in the US for determining presence of fluvalinate and coumaphos residues in both hive honey samples and bottled ones. Majority of samples analyzed from US had no coumaphos or fluvalinate residues above levels of concern except for trace levels of coumaphos founded in three samples from hives and trace levels of fluvalinate identified in one hive sample. Moreover, no pesticide residue from bottled honey samples was reported [9].

Honey and aflatoxin contamination

Yeasts, molds and spore-forming bacteria are microbial contaminants of honey which pose human health hazard due to wide range of adverse effects. These microorganisms are contributed to different activities such as spoilage of provisions, inhibition of other existing microorganisms, production of antibiotics, enzymes, mycotoxin and growth factors (vitamin and antibiotics), metabolic conversion of different substances. Microbiological characteristics of honey are particularly associated with its inherent safety and quality [19]. Therefore, in order to exert enough control over the contamination of honey having adequate knowledge of the moisture and temperature conditions influencing growth of microorganisms in honey is needed [16].

Certain kind of fungi with ability to grow on food such as cereal, legumes, dried food produces health-threatening mycotoxins. One of the most commonly

observed mycotoxins is called aflatoxins (B1, B2 and G1 & G2) [17]. Among them aflatoxin B1 and B2 are mainly produced by *A. flavus* and *A. parasiticus* and aflatoxin G1 and G2 are direct metabolite of *A. parasiticus* [18]. Aflatoxin B1 is the most toxic one among all four aflatoxins and is mainly carcinogenic which is associated with human liver cancer through DNA mutation [6]. The mold, *Aspergillus* might result from the intestinal contents of honey bee, hive and the field that bees forage. In addition, *Aspergillus* has been detected from intestines of honey bee larvae [16]. To some extent pollen, appear to be as the original source of honey microbial contaminations. Honey bees are considered to be contaminated through pollen consumption as well as exchanging the food in the hive [19].

The contamination of food by these pathogenic species and the resultant toxin production is considered as inevitable infestation by the US Food and Drug Administration (FDA) [19]. FDA has set the levels of aflatoxin contamination to 20 ppb limit in all foods in order to control exceeding levels of threat (Food and Drug Administration). The upper contamination limits of European Union regulation is much more stringent with the limit of 4-8 ppb corresponding to all foods [18]. The intrinsic properties of aflatoxin have been reported to affect human health due to mutagenic, carcinogenic, toxigenic, neurotoxic, immunosuppressive, cytotoxic, nephrotoxic and oestrogenic effects beside economical loss by product damaging plus well documented hazard to human health [17,19]. Aflatoxins can contribute to acute and chronic toxicity of consumers through interfering with tissue damaging, gene expression alteration and potential effect of cell apoptosis. Some studies related to aflatoxin effects have provided information about co-occurrence of Hepatitis B virus (HBV) with aflatoxin contamination of food, which is attributed, with increased risk of hepatocellular carcinoma [18].

A preliminary study on honey samples in Portugal revealed the low level of analyzed samples with *Bacillus cereus* and fungi: yeasts, *Mucor* sp, *Penicillium* spp and several species of *Aspergillus*, particularly *A. flavus*, *A. candidus*, *A. Fumigatus* and *A. niger* [19].

In Argentina, a study was carried out to explore natural mycobiota occurring in bee pollen with special attention to incidence of fungal species that are potentially mycotoxin producer. The most often detected fungi were yeasts and *Penicillium* spp, which possibly have a different range of mycotoxin producing actions, *Penicillium verrucosum*, *A. niger* aggregate, *A. carbonarius*, *A. ochraceus*, *A. flavus*, *A. parasiticus* and *Alternaria* spp. The later genus was identified very frequently. It was found that 28.6% of the isolates from *A. flavus* and *A. parasiticus* showed the ability of producing aflatoxin B1. Aflatoxin B2 was only identified in 10% of the sample cultures and no trace levels of Aflatoxins G1 and G2 were detected from the cultures under the experimented conditions [6].

In Portugal 80 samples of honey were analyzed from retail market concerning to contamination with Bacillaceae spores (*Clostridium perfringens*, *Bacillus cereus*), fungi and aflatoxins. The potential ability for aflatoxin production is studied by high performance liquid chromatography assay (HPLC) and conventional microbiological methods. Yeasts and molds were isolated from 88.8% of samples whereas three different species of molds (*Aspergillus*, *Penicillium* and *Mucor*) and two species of yeasts (*Candida* and *Saccharomyces*) were present. The main detected *Aspergillus* was *A. flavus* (57.5%), followed by *A. niger* (51.3%), *A. fumigatus* (45.0%) and *A. candidus* (28.7%), respectively. *Penicillium* spp. and *Mucor* sp. were isolated from 38.8 and 31.3 % of the samples. *Saccharomyces* sp. and *Candida humicola* were highly detected (88.8 and 75.0% respectively). Results indicated that none of samples was contaminated with aflatoxins [19].

A series of studies carried out to evaluate aflatoxins (B1, B2, G1, and G2) and carbamate pesticide contamination of 44 honey samples in Egypt and other countries (Kuwait, Saudi Arabia, China, Turkey, Ukraine, Libya, Ethiopia, Italy and USA) during 2012–2013. The study showed that none of samples revealed to be contaminated with aflatoxins. In minority of collected samples promocarb, pirimicarb and aldicarb residues were detected which were under maximum residue limit [17].

Twenty-one samples of honey were analyzed in different regions of Palestine to determine aflatoxin residues in honey samples. Variable amounts of aflatoxin residues ($0.5\text{--}22\ \mu\text{g kg}^{-1}$, mean $12.1\ \mu\text{g kg}^{-1}$) were detected. The most prevalent contaminated samples were from humid hot semi-coastal regions. Majority of honey samples were contaminated with aflatoxin residues. It was found that residue concentration exceeded levels of concern and created threat for consumer's health [20].

In Pakistan, both branded and unbranded honey samples were considered for aflatoxins (B1, B2, G1, and G2) and heavy metals (cadmium, manganese, lead, mercury, nickel and cobalt) contamination analysis. Minimum level of aflatoxins was identified in both branded and unbranded honey sample, which were under health threatening levels [21].

Honey and antibiotic contamination

Antibiotics are vital components of treatment and elimination of disease in human, animals and plants [4].

Antibiotic contamination in honey can be a result of

improper treatment of hives to combat various diseases such as American foulbrood (AFB), European foulbrood (EFB) and nosemosis, a parasitic disease affecting adult bees. Antibiotics can also enter the honey supply because of antibiotic spray on fruit trees for treatment of fire blight [22].

Both European and American foulbrood diseases, caused by *Paenibacillus (Bacillus)* larvae and *Streptococcus pluton* bacteria, are commonly treated by oxytetracycline. There are some other antibiotics, which are currently used in beekeeping such as erythromycin, lincomycin, monensin, streptomycin, and enrofloxacin. Chloramphenicol, macrolides, tetracycline, sulfonamide, streptomycin, and nitrofurans residues have been commonly found in honey [4, 22]. However, the use of antibiotics in beekeeping is illegal in some EU countries. Extensive use of antibiotics leads to an accumulation of residues in honey decreasing its quality and making the marketing much more difficult. While antibiotic residues show to have a relatively long half-life, they may directly affect consumer's causing allergic reactions in hypersensitive individuals, the haemopoietic system disorder and induction of resistant strains of bacteria [4].

Honey is one of many foods that are monitored for antibiotic residues worldwide. Honey producers, importers, exporters and regulators need simple, fast and effective ways to test honey for antibiotics, ensuring compliance with the maximum residue limits (MRLs) and minimum required performance limits (MRPLs) established for relevant countries (Table 2) [22].

Table 2. Maximum Residue Limits for antibiotics in honey by USA and Canada [23]

Antibiotic	USA,ppb	Canada,ppb
Tetracycline	10	75
Oxytetracycline	10	300
chlortetracycline	none	30
Sulfanamide	none	30
Tylosin	60	60
Erythromycin	none	30

There are several international reports of antibiotic residues in honey samples. In the period 2000–2001, 248 samples of locally produced and imported honey were monitored for the presence of residues of veterinary drugs. Streptomycin was detected in 4 out of 248, tetracycline in 2 out of 72, and sulfonamides in 3 out of 72 samples. No residues of β -lactam antibiotics and chloramphenicol were found. In imported honey samples, streptomycin was detected in 51 out of 102 samples, tetracyclines in 29 out of 98 samples, sulfonamides in 31 out of 98 samples, and chloramphenicol 40 out of 85 samples. For the streptomycin and tetracycline contamination, most cases involved the beekeeper admitting to having added foreign honey to his production [24].

Of the 75 honey samples obtained commercially in Switzerland, 34 samples, which originated from Asian countries, 13 samples (17%) contained chloramphenicol residues. The concentration of chloramphenicol in honey was between 0.4 and 6.0 $\mu\text{g}/\text{kg}$, with six samples containing approximately 0.8–0.9 $\mu\text{g}/\text{kg}$ (just below the Swiss limit) and two containing approximately 5 $\mu\text{g}/\text{kg}$ [25].

In another study, 251 honey samples produced across Greece were analyzed by liquid chromatography to detect tetracycline-derived residues. Twenty-nine percent of the samples had tetracycline residues. Majority of samples contained residues from 0.018–0.055 mg/kg of honey while some others had residues in excess of 0.100 mg/kg [26].

Centre for Food Safety (CFS) found that two of the 19 samples of honey collected for examination for antibiotics contained trace amounts of chloramphenicol, one brand of honey produced in Jiangxi and another brand produced in Zhuhai. Other antibiotics found in trace amount of honey samples, namely streptomycin, sulfamethoxazole (a kind of sulfonamides) and ciprofloxacin (a kind of quinolone), they can normally be used in food animals [27].

In China, five antibiotic compounds, tetracycline, oxytetracycline, doxycycline, chlortetracycline, and chloramphenicol, were successfully separated and determined in honey samples. The detection limits were 10 $\mu\text{g}/\text{L}$ for chloramphenicol, 20 $\mu\text{g}/\text{L}$ for tetracycline, oxytetracycline, and doxycycline, and 40 $\mu\text{g}/\text{L}$ for chloramphenicol [28].

In India, high levels of antibiotics in honey exported from India to EU and US have been reported by Agricultural Processed Food Product Export Development Agency from 2005 onwards [29].

In 2006, about 14% samples were contaminated with tetracycline and in 2007–2008 about 28% samples were contaminated with same antibiotics. In 2009–2010, 2% of samples from 362-tested honey samples had more than prescribed limit of antibiotics. In 2000–2001, streptomycin was detected in 4/248, tetracycline in 2/72, and sulfonamides in 1/72 samples. Nectar and honey samples collected from bee hives during the peak flowering seasons of rubber (March to April) and banana (December to January) plantation crops in southern part of Tamil Nadu were analyzed for antibiotic residues. These samples showed 4–17 and 11–29 ng/kg of streptomycin, 2–29 and 3–44 ng/kg of ampicillin, and 17–34 and 26–48 ng/kg of kanamycin, respectively [30].

Mahmoudi et al. investigated the occurrence of oxytetracycline residue in 145 honey samples (collected from Ardabil provinces, Northwest region of Iran) by using ELISA and HPLC methods. The ELISA assay showed that 34 samples out of 145 samples were positive for oxytetracycline residue. ELISA analyses demonstrated that the minimum and maximum levels of oxytetracycline residue were 5.32 and 369.1 ng/g, respectively. HPLC analyses confirmed the ELISA findings, although the level of oxytetracycline detected in honey samples using HPLC method was remarkably ($P < 0.05$) lower than that detected by ELISA. Considering the relatively high contamination level of

foods of animal origin with oxytetracycline and their high levels of consumption, it is likely that consumers experience a high risk of exposure to drug residues, especially through honey bees [4].

Out of the 3855 honey samples tested, 1.7% samples were non-compliant with EU4 standards, and the range of antibiotics detected in the honey samples were: streptomycin 3–10.8 µg/kg, sulfonamides 5–4.6 µg/kg, tetracyclines 5–2.1 µg/kg, chloramphenicol 0.1–169 µg/kg, nitrofurans 0.3–24.7 µg/kg, tylosine 2–18 µg/kg, and quinolones < 1–504 µg/kg [31].

Fifty honey samples comprised of chestnut, pine, linden, and multiflower honeys collected from the hives in Southern Marmara region of Turkey were analyzed for erythromycin residues by liquid chromatography-mass spectrometry using electrospray ionization in the positive ion mode (LCESI-MS). Four of the honey samples were contaminated with erythromycin residues at the concentrations ranging from 50 to 1776 ng/g. An erythromycin-fortified cake-feeding assay was also performed in a defined hive to test the transfer of erythromycin residue to the honey matrix. In this hive test, the residue levels in the honey three months after dosing were approximately 28 ng/g [32].

Another study aimed to assess oxytetracycline (OTC) residue levels in honey after treatment of honeybee colonies with two methods of application (in liquid sucrose and in powdered icing sugar). The samples of honey were extracted up to 12 weeks after treatment and following metal chelation and analyzed by HPLC, which showed that the current method of application of oxyteracyclin (terramycin) in liquid form results in very high residue levels in honey with residues of 3.7 mg/kg, eight weeks after application [33].

Recently researchers have developed a method to detect simultaneously the presence of 17 antibiotics (macrolides, tetracyclines, quinolones, and sulfonamides) in honey samples taken from supermarkets while five were collected from various

private beekeepers throughout Granada and Almeria. The results of the study show that one of the commercial honey samples contained 8.6µg/kg, while another contained trace levels of of sarafloxacin. In addition, residues of tylosin, sulfadimidine and sulfachlorpyridazine were found in the honey from one beekeeping farm [34].

A total of 57 real royal jelly samples collected from beekeepers and supermarkets were analyzed for seven fluoroquinolones used in beekeeping, viz. ciprofloxacin, norfloxacin, ofloxacin, pefloxacin, danofloxacin, enrofloxacin, and difloxacin, which were analyzed by high performance liquid chromatography with fluorescence detection. Ofloxacin, ciprofloxacin, and norfloxacin were detected in concentrations ranging from 11.9 to 55.6 ng/g in some royal jelly samples, and difloxacin was found at concentration of about 46.8 ng/g in one sample though it is rarely used in beekeeping [35].

The result of antibiotic residue investigation (including enrofloxacin, penicilin, chloramphenicol, gentamicin, tylosin, tetracycline, and sulfonamide) in 135 honey samples collected randomly from Qazvin Province (Iran) showed that the range of antibiotic residues value was 0.0–72.1 ng/g, besides, the highest percentage of antibiotic residues in honey samples was the enrofloxacin (20.7%). The highest mean contamination (ng/g) was enrofloxacin (10.8 ± 1.6) followed by penicillin (4.4 ± 2.9), and the lowest was chloramphenicol (0.1 ± 0.1). The highest level of antibiotic residues (71.85%) was found in honey samples collected during the autumn season [2].

Honey and heavy metal contamination

The best possible definition for heavy metal presented by is “the trace minerals with inorganic and metallic sources which have at least 5 time specific gravity of water as well as toxic effects on human health” [35].

According to Nielsen 1984 reports, the most significant heavy metals are Pb, Cd.

The most significant heavy metals are Pb, Cd, Hg, Cr, Cu, Mn, Ni, Zn, and Ag. Heavy metals tend to act as toxic substance even at low concentrations due to accumulation in human body organs and lack of biodegradability effect [36, 37].

Contamination of honey with these toxic metals is a challenging problem that needs to be fully addressed because of public health concern [36]. The intrinsic properties of heavy metals have been reported to create wide range of health problems such as metabolic and respiratory disorders, headaches, nausea, and vomiting. In addition, the disruptive effect of Pb on brain, kidney, nervous system, and red blood cells is well-documented [37]. In addition, presence of lead and arsenic in food is strongly banned because of the extreme toxicity [38].

There is great deal of evidence that heavy metals does not only contribute to nutritional adverse effects but also some of their beneficial role on human health is known. However, the major contaminant of food supply is cadmium, lead and mercury, some metals including

iron, zinc and copper are necessary for human body metabolism and well doing. Hg, Cu, Mn, Zn, Ag which are considered as heavy metals have significant benefit in the form of environmental contaminants bio-indicator such as heavy metals, pesticides and environmental radioactivity [37].

The acidic nature of Honey makes possible the transmission of heavy metals from containers and processing equipment [39]. The mineral and heavy metal composition of honey is deeply influenced by the soil constituents, transmitted to the floral plants as well as nectar and ultimately create the honey mineral compositions. Likewise the beekeeping practices, environmental pollution, honey processing , atmospheric precipitation, tainted water, application of insecticides , pesticides and fertilizers are proposed to be possible sources of trace mineral contamination of honey samples [36,37].

For the heavy metals such as cadmium, lead and mercury, maximum residue levels in certain foods have been established [23].

Table 3. Some heavy metals maximum levels in certain foods

Metal	EFSA in foodstuffs, Less than mg/Kg	EFSA, Less than	FDA, Less than
Lead	0.020	20 ppb	50 ppb
Cadmium	0.050	50 ppb	
Mercury	0.5	500 ppb	

There are several studies about heavy metal contamination of honey samples from different regions

of the world, some mentioned in Table 4 [35].

Table 4. Heavy metal contamination of honey samples from different parts of the world.

No.	Region	Heavy metal	Level contamination	Reference
1	Kahramanmaras, City, Turkey	Cd, Mn, Fe and Mg	0.32, 0.03, 0.36 and 10.45 (ppm)	[40]
2	Middle Anatolia, Turkey	Mn, Pb and Cd	0.32-4.56 ppm, 8.4-105 ppb and 0.9-17.9 ppb	[41]
3	Southeastern Anatolia, Turkey	Mn	1.0 ppm	[42]
4	Different regions in Turkey	Mn	0.49 ppm	[43]
5	Different regions in Turkey	Pb, Cd and Mn	55.2 ppb, 4.53 ppb and 0.31 ppm	[44]
6	Kayseri	cadmium, copper, zinc, nickel, lead	0.11-0.18 ppm, for 0.15-0.66 ppm, 2.2-11 ppm, 0.2-0.8 ppm and 0.1-0.85 ppm	[45]
7	-	Pb, Cd, Cu, Fe and Mn	0.02±0.03, 0.01±0.01, 0.91±0.66, 3.13±1.44, 1.26±1.29 ppm,	[46]
8	Argentina	Cd and Pb	0.2 to 1.37 ng/g and from 4.6 to 30.5 ng/g-1	[47]
9	Italia	Pb, Cd, Cu, Fe and Zn	0.10005, 0.25005, 5.00005 and 3.105 ppm	[48]
10	France	Pb, Cd, Cu, Fe, Mn and Zn	were 0.793 ppm, 0.152 ppm, 0.305 ppm, 11.03 ppm, 3.685 ppm and 1.343 ppm	[49]
11	Nigerian	Cu, Fe, Mn and Zn	21 ppm, 220,6 ppm, 3 ppm and 63.4 ppm	[50]

In Iran, Saghaei et al. investigated the level of some heavy metals in honey samples collected from different regions of Urmia. Accordingly, the mean contaminations of Pb, Cr, Zn, as and Ni were 0.04 ± 0.1 ppm, 7.09 ± 9.4 ppm, 9.99 ± 26.5 ppm, 0.0008 ± 0.0011 ppm, 0.003 ± 0.005 ppm, respectively. Based on their results, the Pb level was lower than the maximum residue limits (EU ML). Other metal levels were within the acceptable levels [51].

Akbaria et al. assessed metal concentration of 10 different honey brands and results showed that the average amount of metal trace levels (including Se, Cu, Cd, Pb, As and Mn) were measured less than 0.5 mg/kg, lead content had the lowest level concentration (0.11 mg/kg) [18]. In the current study, Pb and Zn levels were 0.08 ± 0.04 and 4.41 ± 3.40 ppm respectively. Determined levels of Pb and Zn in the present study were lower than the levels of these metals in honey samples collected

from different regions of the world specially Turkey and Iran [52].

Study was carried at by Mahmoudi et al with the aim of evaluating the quality of Iranian honey (from northwest regions including West Azerbaijan, East Azerbaijan and Ardabil provinces) in term of some heavy metals (Pb, Zn and As) contamination. Estimated amounts of metals in honey samples collected from northwest of Iran was lower than permitted levels. Among the examined elements, Pb residues in honey possess great concern. According to WHO reports, the average recommended daily intake of Pb is 210 µg/d for a 60 kg adult person. In a theoretical food basket, an ordinary person should take 20 gram honey daily. Based on this study results the mean Pb content of honey samples was 0.08 ppm. Therefore, consuming 20 g per day of honey provides 1.6 µg Pb intakes in a day. The results revealed that the lead intake levels in Iranian people with an average

weight of 60 kg, is lower than the recommended limit. The average recommended daily intake of As with FAO/WHO recommendation is 130 $\mu\text{g}/\text{day}$ for a 60-kg adult person and although the average level of As in the honey samples (0.11 ppm) was lower than similar reports and interestingly it was less than recommended dose. The amounts of Zn residues in honey samples were higher than levels of compared studies. The mean value of Zn intake from honey consumption was 4.41 ppm, which is less than the recommended amount by PMTDI as 60 mg/day. Based on the results of this study and comparison with recommended daily intake levels, the heavy metal content of honey samples from northwest of Iran were not harmful and is unlikely to cause any intoxication following consumption. However, it is recommended to conduct beekeeping practices far from industrial areas with high pollution of heavy metals [36]. Reports indicate that honey samples collected from industrial regions have higher levels of heavy metal (Cd, Pb, Hg, Zn, Cu, Ni and Cr) than those from non-industrial areas [37].

Honey adulteration

Adulteration of various foods is well documented throughout the history but expensive ones, which produce under wide weather fluctuation, and hard harvesting conditions are significantly worthwhile.

Honey is known as possessing this controversial potential. With the emergence of high fructose corn syrup by the industry, Honey adulteration has been discovered in the world market in 1970s. Honey authentication has recently become a major consumer concern through inevitable economic loss and nutritional and organoleptic effects. Potential use of honey as authenticated product does not only create human health threat but also marketing deduction through consumer confidence losing would be the main concern [5].

Based on the Codex Alimentarius regulations and other international honey standards, honey is mentioned to do not contain any food ingredient additive nor should any particular contents of honey be removed from it [5]. Detailed characteristic of natural honey and its constituents limit is described in table 5 [23].

Some known pathways leading to honey adulteration is adding inexpensive sweeteners such as corn syrups (CS), high fructose corn syrups (HFCS), high fructose insulin syrups (HFIS) or invert syrups (IS). The latter method is considered difficulty detecting by direct sugar analysis due to similarity of physical features of adulterated honey with natural one. Likewise, Invert sugar or syrup components are the same as natural honey constituents [5].

Table 5. Limits of natural honey constituents

Constituent Content	Limits
Moisture (water)	Not more than 20% (< 20%)
Sum of Fructose and Glucose	Not less than 60 g/100 g (> 60%)
Sucrose	Not more than 5g/100 g (< 5%)
Water insoluble solids	Not more than 0.1 g/100 g (< 0.1%)
Free acidity	Not more than 50 milliequivalents per 1000g; i.e., not more than (MW x 50)/1000
Diastase activity	Not less than 8 Schade units (see)
Hydroxymethylfurfural (HMF)	Not more than 40 mg/Kg Not more than 80 mg/Kg for the honey from tropical ambient temperatures
Electrical conductivity	Not more than 0.8 mS/cm ⁻¹

Direct adulteration of honey is accomplished by directly addition of external substances to the honey. Indirect adulteration is attributed with manual feeding of bees with artificial sugar at the stage of brood emerging naturally. Indirect honey fraud has difficulty showing detectability [37]. The herbal sources of honey adulteration are classified as C3 or C4 plants, which refer to their carbon metabolism. Most of honey adulteration contributed plants like rice, wheat and beet are supposed to be C3 plants although maize and sugarcane are C4 plants. For instance, sugars with the source of C3 plants are mostly used for adulteration of honey in Czech Republic [38].

This syrup utilization in the form of honey adulteration brings many difficulties in trace level detecting because of artificially resembling of their chemical Features to the natural one. Aberrantly, feeding honey bees with industrial sugar has recently become a major human concern [37].

Using worthless substances in honey constituents as well as health threatening cocktail of chemicals such as antibiotics, colourings and hydroxymethyl furfural (HMF) is among other disadvantages of honey fraud problem. It is well documented that approximately half of honey existed in Czech market is considered as adulterated product of the country [38].

Setting up various techniques to distinguish natural honey is a great focus of research. Each detection method is considered applicable based on the type of adulteration procedure. It is noticeable that in order to obtain acceptable honey characterization, contribution of different methods concomitantly is required [5].

Surveys of detecting indirect adulteration

Cordella et al. (2005) used high performance anion exchange chromatography with Pulsed Amperometric Detection (HPAEC-PAD) method in order to evaluate natural and adulterated honey. The study was conducted with the honey samples from France containing 10-40%

unnatural sugar syrups as the supporting feeding of honey bees. It was found that external honey bee feeding with artificial syrup in improper protective measures affect the final sugar composition of honey [38]. HPAEC-PAD method is also contributed for determining honey botanical extract with the advantages of lesser time consuming and inexpensive than other methods [5].

A similar study was carried by Ruiz-Matute et al. to investigate sugar composition of high-fructose corn syrup (HFCS) and its effect on final sugar composition of honey by gas chromatography coupled with mass spectrometry (GC-MS) method. HFCS was used for feeding of the bees. Sucrose syrups were defined as control substance. Study revealed that HFCS included fructosyl-fructose and some unknown sugars suspected to be fructosyl-glucose. Fructosylfructose was detected in the artificial feeding bee's honey. This substance was similarly identified at lower concentration in the honey of free-flying bees and the bees, which consumed sucrose syrups [37].

A research was carried out by Guler et al. with the aim of carbon isotope ratio analysis. Sensitivity of methods was developed to analyze 100 samples of pure natural honey plus honey produced by artificial sugar syrups feeding bees. The syrups administered with the amount of 5, 20 and 100 liters per colony, which contained different amount of high fructose-85 (HFC-85%), with moderate fructose-55 (HFC-55%), sucrose syrups (SS) glucose syrups (GMS) and bee-feeding syrups (BFS). All the analysis was based on detection of D13C quantity for honey sugars and proteins, the difference in the D13C values of the proteins and sugars (Dd13C) and the concentration of C4 sugars.

Bertelli et al. investigated useful method to distinguish indirect adulteration of honey by sugar syrup administration in 2010. The effective method consist one-dimensional (1D) and two-dimensional (2D) nuclear magnetic resonance (NMR) coupled with

Multivariate statistical analyses. Study involved analysis of 63 natural honey samples and 63 adulterated honey samples by seven different sugar syrups. 1D-spectra and a cross verification analysis showed the acceptable detection capacity as well as 2D NMR assay with noticeable results [37].

Methods of detecting direct adulteration

In order to discriminate direct adulteration of honey, usual and traditional analysis of physical profile plus chemical composition is considered. However presence of problems in this analytical method such as time-consuming and difficulty of preparation procedures and complicated analytical assessment has impelled the need for new effective methods of detection [37].

The most commonly used methods for honey authentication is carbon-isotope ratios detection, nuclear magnetic resonance (NMR), gas chromatography (GC) and liquid chromatography (LC) assay. GC-LC method with the effective ability for sugar identification has received a great attention among other analytical methods [38]. Also is considered as replacement for isotope analysis due to some limitation it possess [5]

Some other Analytical methods for honey adulteration detection

Near Infrared Transflectance Spectroscopy (NIR)

A method which is commonly used for quality evaluation of honey with the features of rapid testing ability, inexpensive and non-destructive.

Fourier Transform Infrared (FTIR) spectroscopy with Attenuated Total Reflectance (ATR)

In comparison to time-consuming carbon isotope ratio procedure, this method can be administered in pleasant time.

Protein analysis

Molecular weights of honey proteins are deeply influenced by the honeybee species. Therefore, to determine the species of honey bee which produces honey, honey protein characterization can be applicable.

Liquid Chromatography Coupled to Isotope Ratio Mass Spectrometry (HPLC-IRMS)

This new identified method is considered the first isotopic Spectrometry procedure with the ability to diagnose indirect beet sugar feeding adulteration. This effective procedure has many benefits in the form of easy preparation procedure, reduced reagent consumption and good sensitivity.

Calorimetric methods (Application of DSC)

An analyzing method based on DSC application; create range of advantages such as determining thermal properties of honey and the effect of honey authentication on its physicochemical profile and structural features. Application of glass transition temperature to distinguish honey and syrup is among other advantages.

Stable Carbon Isotope Ratio Analysis (SCIRA)

SCIRA method is subjected for detecting honey adulteration by the $^{13}\text{C}/^{12}\text{C}$ isotope ratio. Such ratio shows different values between C4 or CAM plants and C3 plants.

Fourier Transform (FT) Raman spectroscopy

This spectroscopy procedure is effectively capable to discriminate beet and cane invert syrups as well as types of adulteration substances aside from their botanical extract.

Microscopic analysis

Microscopic detection is a worthwhile method for detection of cane sugar in adulterated honey with demonstrating its microscopic structures such as parenchyma cells, single ring vessels and epidermal cells. [5]

CONCLUSIONS

Honey has found a valued place in global trade due to wide range of nutritional, cosmetic, modern and traditional therapeutic features. The exponentially growth of universal honey trade compels the need for a certain and international standards for marketing and consuming the honey.

The floral sources of natural honey create a significant influence on the safety and contaminants of the samples. Diversified natural xenobiotics found to be present in honey due to both environmental and apiarian practice pollution. According to the obtained data from studied literature uncontrolled administration of Antibiotics, heavy metal, aflatoxin, pesticide residues in apiarian practices and different ways of product authentication, provide possible explanation for the need of sensitivity of new methods for honey analysis regarding its origin, composition, adulteration and trace levels of chemical residues. The scientific literature indicates that setting up an international regulation for honey contamination and maximum residue levels of toxic chemicals will impose a great effect for dealing with problem constructively.

ACKNOWLEDGEMENTS

This study was supported by the Qazvin University of Medical Sciences and research affairs of University of Tabriz, Iran. The authors declare that there is no conflict of interest.

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