Chemosphere 154 (2016) 482–490

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

The occurrence of pesticides and persistent organic pollutants in Italian organic honeys from different productive areas in relation to potential environmental pollution

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HIGHLIGHTS

- The pesticide contamination of organic honey is strictly related to the contamination source.
- The study of contaminants is pivotal for beekeepers to select a production area dedicated to the organic honey production.
- Control of residues in foods is essential to assess human exposure to contaminants through the diet.
- High frequency of pesticides utilized in apple and citrus orchards were observed in organic honey samples.

ARTICLE INFO

Article history:
Received 4 February 2016
Received in revised form
31 March 2016
Accepted 2 April 2016

Handling Editor: Myrto Petreas

Keywords:
Pesticide residue analysis
Organic honey
Accelerated solvent extraction (ASE)
Triple quadrupole mass spectrometry (GC-MS/MS)
Contamination sources
Food safety

ABSTRACT

Bee products, such as honey, are widely consumed as food and consumer interest is currently oriented towards organic foods. Regarding this, the European Commission establishes that the qualification of organic honey and other beekeeping products as being from organic production is closely bound with the characteristics of hive treatments as well as the quality of the environment. Agricultural contamination with pesticides is a challenging problem that needs to be fully addressed, in particular in the field of organic production systems. In this study, the occurrence of different classes of contaminants selected as representative of potential contamination sources were investigated in 59 organic honeys: organochlorines, OCs; organophosphates, OPs; polychlorobiphenyls, PCBs and polybromodiphenylethers, PBDEs. A method based on Accelerated Solvent Extraction with "in line" clean-up and GC-MS/MS detection was developed to detect contaminants. Residues of many pesticides were found in most of the samples investigated. The majority of honey samples contained at least one of the pesticides, even if their concentrations were found to be lower than its MRL. Diazinon, Mevinphos, Coumaphos, Chlorpyrifos and Quinoxyfen were the residues frequently detected in samples coming from the apple and citrus orchard areas. Furthermore, the results of the present study show that the presence of the residue in organic honey may also be affected by the geographical area (e.g. the presence of an agricultural system) confirming honey bee and beehive matrices as appropriate sentinels for monitoring contamination in the environment. The optimised method proved to be simple and rapid, requiring small sample sizes and minimising solvent consumption, due to the ASE having an "in line" clean-up step.

1. Introduction

Honey is a natural food product, made of nectar, secretions of livings parts of plants or excretions of insects sucking on the living parts of plants, which *Apis millifera* bees collect, transform by combining with specific substances and deposit in honeycombs

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http://dx.doi.org/10.1016/j.chemosphere.2016.04.004

(Giorgi et al., 2011; Wilczynska and Przybylowski, 2007; Panseri et al., 2014). Honeybees (*Apis mellifera* L.) perform the vital task of pollinating agricultural crops and native species and are important for the commercial products of honey and beeswax. Honey composition mainly depends on the floral origin of nectar, climate conditions, bee physiology, honey harvesting and post-collection processing (Panseri et al., 2013). Today, consumer interest regarding honey and its derived products is oriented towards organic foods. Regarding this, the European Commission establishes that the qualification of organic honey and other beekeeping products as being from organic production is closely bound to the characteristics of hive treatments as well as the quality of the environment. This qualification also depends on the conditions of extraction, processing and storage of beekeeping products. The Council Regulation 1804/1999 EC is very restrictive with regard to the production of organic honey in terms of the origin of bees, siting of the apiaries, feed, disease prevention and veterinary treatments. In particular, it establishes that plants that can be foraged by bees, either biological or spontaneous, must be at least 3 km from any source of pollution and from any non agricultural production sources, possibly leading to contamination, such as industrial areas, urban centres or motorways. Also, the use of veterinary medicinal products in beekeeping is regulated by the European Council (EC, 1804/1999). Usually, beekeepers administered insecticides, fungicides, and acaricides to control some infestations such as *Varroa destructor*, *Acarapis woodi* and *Paenibacillus larvae* (López et al., 2014; Fell and Cobb, 2009; Genersch et al., 2010). According to the Council Regulation 1804/1999, the use of allopathic chemically-synthesised medicinal products for preventive treatments in organic beekeeping is prohibited, since these fat-soluble and non-volatile compounds can accumulate in the stored honey, where they are able to migrate from the wax comb (Panseri et al., 2014). In the cases of *Varroa* infestation, formic acid, acetic acid and oxalic acid can be used, as well as menthol, thymol, eucalyptol or camphor (Council Regulation, 1804/1999 EC). Therefore, in organic honey production, direct pollution by beekeeping practices as well as indirect contamination from the environment must be prevented. Many pollutants in the environment may contaminate bee matrices, comprising bee, honey and pollen. Environmental pollutants include pesticides (Chauzat et al., 2011), heavy metals (Tuzen et al., 2007), bacteria and radioactive materials (Al-Waili et al., 2012). Honeybees are able to cover a wide area and come into contact with contaminated food sources, such as pollen, nectar and water during foraging. Therefore, honeybees and beehive products are considered potential indicators for environmental biomonitoring (Malhat et al., 2015; Kasiotis et al., 2014). Lambert et al. described the use of bees, honey and pollen as sentinels for environmental chemical contaminants in France (Lambert et al., 2012). Porrini et al. described the use of honey bees and bee products as bioindicators of pesticide, heavy metal and radionucleotide pollution (Porrini et al., 2003); Panseri et al. (2014) demonstrated the high direct relation between the contaminant source and pesticide residues found in honey samples. Among the environmental contaminants, different studies have documented the occurrence of organochlorines (OCs), polychlorobiphenyls (PCBs), organophosphates (OPs) and polybromodiphenylethers (PBDEs) in honey. In particular organochlorine, and to a minor extent organophosphorous pesticides, are highly stable, minimally volatile, lipophilic and persistent organic pollutants. Due to these characteristics, the compounds tend to accumulate and bioaccumulate, representing important groups of dangerous organic contaminants, since they can contaminate foodstuffs if not directly treated (Panseri et al., 2014). Organophosphorus pesticides (OPs) represent important environmental and food contamination sources, as they are widely used in agriculture for the control and protection of crop-eating insects. In addition, OPs are acetylcholinesterase inhibitors leading to acute poisoning via food consumption (He et al., 2015). Recently EFSA (European Food Safety Authority) has realised scientific opinion on the risks to public health related to the presence of brominated flame retardants in food (EFSA, 2010). Thus, the Commission used the Recommendation of 3 March 2014 ask European countries to monitor traces of brominated flame retardants in food. Brominated flame retardants (BFRs), especially polybromodiphenyethers (PBDEs), are organobromine compounds applied to products in order to reduce their flammability (COMMISSION RECOMMENDATION, 2014). They contaminate the environment and food chain because of their persistent, lipophilic, bioaccumulative and toxic nature, and are suspected of causing neurobehavioral effects and endocrine disruption (Mohr et al., 2014). In general, the European Commission set the maximum residue levels values (MRLs) for feed as well as for food of animal origin (Commission Regulation 396/2005; Commission Regulation 839/2008).

Critical steps in the determination of contaminants residues in food are the extraction from matrices and the following sample clean-up (Rissato et al., 2007; LeDoux, 2011). Among the many extraction techniques, accelerated solvent extraction (ASE) is characterised by shorter extraction times and reduced solvent consumption. The accelerated solvent extraction utilises high temperatures combined with high pressure. A high temperature allows a higher rate of extraction due to a reduction of the viscosity and surface tension, and increases the solubility and diffusion rate into the sample. At the same time, high pressure prevents the solvents from reaching their boiling point and promotes penetration into the sample (Beyer and Biziuk, 2008). Recently, the ASE technique has also been tentatively used combining the clean-up step during the extraction process, generating an “in line” extraction-clean-up method in which the sample purification is directly performed in the ASE cell. Until now, only three studies reported the use of ASE for the extraction of pesticides from honey without “in line” clean-up (Kort et al., 2002; Wang et al., 2010; Lambert et al., 2012).

Considering the lack of information in the literature about the presence of pesticides and other contaminants in organic bee products, the aim of the present study was to investigate the presence of POPs in organic honeys arising from different Italian regions. Our attention was focused on the residues of pesticides used in citrus and apple orchards for crop protection [organochlorines (OCs) and organophosphates (OPs)] as well as other POPs present in the environment as a possible consequence of antrophic activities [polychlorobiphenyls (PCBs) and polybromodiphenylethers (PBDEs)]. Lastly, this paper presents a rapid, accurate and sensitive method to evaluate multiple residues by using the accelerated solvent extraction (ASE) sample preparation method with “in line” clean-up purification followed by GC–MS/MS (triple quadrupole – QqQ) analysis.

2. Material and methods

2.1. Chemicals and reagents

Mixtures of PCB congeners (PCB 28; PCB 52; PCB 101; PCB 138; PCB 153 and PCB 180) and PBDE congeners (PBDE 28; PBDE 33; PBDE 47; PBDE 99; PBDE 100; PBDE 153 and PBDE 154), PCB 209, internal standard (IS) for PCBs, and 3-fluoro-2,2,4,4,6-pentabromodiphenyl ether (FBDE), and IS for flame retardants, were purchased from AccuStandard (New Haven, USA). A mixture of 19 standard OCs (α-HCH; Hexachlorobenzene; β-BHC; Lindane; Heptachlor; Aldrin; Heptachlor epoxide; Trans Chlordane; 4,4′-DDE; Endosulphan I; 2,4′-DDT; Endrin; 4,4′-DDD; Endosulphan II;