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Determination of Pesticides Residues in Bee Products: An Overview of the Current Analytical Methods

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Abstract

The presence of undesirable compounds in honey and other bee products may modify their biological attributes. Such molecules may be present because of different human activities (i.e., pollutants, pesticides) or because of veterinary treatments designed to control and prevent diseases that affect bees. The use of pesticides in agricultural crops has been related with negative effects with and acute damages for bees. The widespread agricultural use of neonicotinoids is a common exposure pathway for bees, and it may be an important factor in declining bee health. In 2013, the European Union has forbidden the use of three pesticides belonging to the neonicotinoids: Imidacloprid, Thiamethoxam, and Clothianidin after the analysis of several scientific results of some studies where those pesticides were involved in an increased death of bees.

Keywords: honey, beehives, pesticides residues, good agricultural practices, analytical methods

1. Introduction

Honey has been described as a natural sweet mixture produced by honeybees from the nectar of flowers or from living parts of plants. Bees combine this mixture with substances of their own, and then it is deposited, dehydrated, and stored in the honeycomb for further uses [1]. Honey is the most characterized bee product due to its nutritional value as a natural. Honey is composed of several carbohydrates, mainly fructose and glucose (85–95% of total sugars). Glucose has a lower degree of solubility than fructose. The ratio of glucose to fructose determines the liquid state of a given honey. Other types of sugar are present due to the union of two or more molecules of fructose or glucose as polysaccharides. Additionally, certain substances are available in honey, such as organic acids, amino acids, proteins, enzymes, lipids, flavonoids, and vitamins that are responsible for its biological properties including antioxidant or antibiotic activities [2].

Additionally, certain substances are available in honey, such as organic acids, amino acids, proteins, enzymes, lipids, flavonoids, and vitamins that are responsible for its biological properties including antioxidant or antibiotic activities [3, 4].

Melissopalynological analysis is used to establish whether a honey is unifloral or not. Unifloral honey has a higher market price because at least 45% of the pollen

grains in its solids are from the same plant species. Therefore, the quality of a honey depends on the presence and concentration level of specific compounds and the botanical origin classification [5].

Honey can obtain the characteristics of plants whose pollen grains and nectar have been taken by bees. Thus, the biological properties are related to the plant species and its attributes [6]. Antioxidant activity is one of the observed biological properties of honey. The presence of enzymatic antioxidants (glucose oxidase, catalase) and non-enzymatic antioxidants (flavonoids, ascorbic acid, and phenolic acids) have been detected in many honeys [7, 8]. Several studies have looked to establish some relationship between phenolic compounds and the antioxidant properties of honey. An analysis of the phenolic compounds profile of unifloral *Rhododendron* honey produced in Turkey demonstrated that increased antioxidant activity was related to higher concentrations of those molecules. The same effect was observed for the antibacterial capabilities of honey samples [9, 10].

The identification of phenolic compounds includes many extraction techniques that permit the isolation of the phenolic fraction from the rest of the honey's components. Solid-Phase Extraction (SPE) procedures are recommended for cleaning the samples, followed by High-Performance Liquid Chromatography (HPLC) or Capillary Electrophoresis, CE [11]. Those techniques have been used to determine the chemical profiles of natural products from extracts obtained from complex organic matrices such as honey. Despite its high resolving power, high-performance liquid chromatography (HPLC) may present some limitations for the separation of molecules belonging to the same family, even when proper sample cleaning is performed to achieve better results. In the same way, capillary electrophoresis and the related technique, electrokinetic chromatography (EKC), in zone format (CZE) allow for the analysis of ionic and neutral compounds on the same column. The great advantage of this methodology is the amount of sample needed for each analysis; it requires only a few nanoliters of extract with a solvent waste of 1 mL–2 mL per assay [12]. Several research studies have focused on the identification of phenolic compounds in bee products. Samples of commercial propolis were studied using CE, and 15 polyphenols were separated with a buffer of sodium tetraborate 30 mM, pH 9.0, and under an applied voltage of 15 kV. Borate buffers form complexes with orthodihydroxyl groups on the flavonoid skeleton and facilitate separation. In the same study, three different extracts were produced (ethanolic, aqueous-ethanolic, and aqueous-glycolic extracts) to compare the levels of available analytes in each one. After this procedure, it was possible to establish a reproducible fingerprint of the polyphenolic profiles, the pattern of which depended on the nature of the extraction solvent [13].

By the way, the determination of the antioxidant capability of honey requires UV–Vis determinations. For instance, the colorimetric assays for the general quantification of phenolics is done by Folin–Ciocalteu reaction; assessment of radical scavenging using the reduction reaction of the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) is a very helpful method for this aim [3]; the assessment of antioxidant activity using the ferric reducing/antioxidant power assay (FRAP) [14, 15] and the oxygen radical absorbance capacity (ORAC) [16] has been frequently used.

2. Chilean honey

In 2020, according to figures obtained from Agricultural and Livestock Service of Chile, there are 8777 beekeepers who manage 1.241,504 beehives distributed throughout the country [17]. In that sense, 1991 tons were exported, and most of the honey was sent to European markets, with Germany being the main buyer,

followed by Belgium. Also, China and the United Arab Emirates have also emerged as important buyers of Chilean honey. Chilean bee products have interesting biological properties that improve their natural potential as an attractive exportable nutritional food. Chemical characterization is necessary for certifying their natural attributes. Furthermore, chemical content analysis enables the fulfillment of international regulations for healthy and safe foods because those markets are very strict in terms of the food safety issues. Several studies on the potential properties of native unifloral honey have been conducted. The Ulmo Honey (*Eucryphia cordifolia*) demonstrated the greatest antibacterial power among the selected Chilean unifloral honey samples. The main identified compounds are gallic, caffeic, coumaric, and chlorogenic acids [18, 19].

3. Undesirable residues

The presence of undesirable compounds in honey occurs when beehives or plants are exposed to pollutants caused by human activities; in this case, the final composition of honey is modified, and the effectiveness of its biological activity changes. Recently, it has been demonstrated that honey has a specific chemical profile of inorganic elements related to the place where it was produced [20]. This information enables the certification of the geographical origin of a honey [21]. Similarly, studies showed that the inorganic content is not dependent on the botanical origin, but rather on the composition of the soils and water in the areas surrounding beehives, and other environmental conditions play an important role in this case [10, 22]. Also, honeys that contained metals in their composition showed a decreased antioxidant activity compared with control samples [23]. The same trend was found in bee pollen samples obtained from the same beehives [24]. Furthermore, it was possible to observe that the chemical behavior of phenolic compounds was modified due to the presence of metals, based on analyses by capillary electrophoresis with diode array detector (CE-DAD) [12].

4. Good agricultural practices (GAP)

According to the Food and Agricultural Organization of the United Nations (FAO), the current definition of Good Agricultural Practices (GAP) includes codes, standards, and regulations that have been developed in recent years by the food industry and producers' organizations but also governments and nongovernmental organizations, aiming to codify agricultural practices at farm level for a range of commodities. Their purpose varies from fulfillment of trade and government regulatory requirements (in particular with regard to food safety and quality), to more specific requirements of specialty or niche markets. The objective of these GAP codes, standards, and regulations includes, to a varying degree: ensuring safety and quality of produce in the food chain; capturing new market advantages by modifying supply chain governance; improving natural resources.

Currently, despite those findings, the standard methods for the measurement of those compounds still include an analysis by HPLC with mass spectrometry (MS). The efficiency of mass spectrometry and the optimization of chromatographic procedures have helped to decrease the experimental time for each analysis. Moreover, several antibiotics can be detected in just one chromatogram. This was the case in a study that was performed by selecting 11 honey samples from different botanical origins produced in Granada, Spain. After HPLC coupled to electrospray ionization (ESI) time-of-flight (TOF) mass analyzer, the following antibiotics were quantified

with an average of MRL from 0.05 to 0.76 $\mu\text{g Kg}^{-1}$ and a run time of approximately 11 minutes: chlortetracycline, demeclocycline, doxycycline, methacycline, minocycline, oxytetracycline, tetracycline, and rolitetracycline [25].

5. Xenobiotics

In several areas where agricultural activities occur, the possibility of finding pesticide-free areas has decreased. Because of this, an increase in the density of apiaries has been observed with the systematic appearance of bee diseases. The continuous exposure of bees to xenobiotics (agricultural pesticides and veterinary products) is responsible for the presence of these compounds in recycled waxes [26]. Although the information available is just related to reports from a limited group of countries, there is enough evidence that the presence of these compounds in the surrounding areas of beehives, as well as in the composition of products obtained from those apiaries, may have a long-term effect on the reproductive health of beekeepers, even farmer workers, or consumers [27].

6. Residues of pesticides: analytical methods

The use of pesticides in agriculture is allowed under strict regulations, but nowadays there is enough evidence about the negative effects of those products over bee health. In that way, the presence of pesticides in the honey content may be detected owing to direct contamination from beekeeping practices or by indirect contamination from environmental sources [28]. Since the mid-1990s, several beekeepers from different parts of United States and Europe reported high colony losses caused by a phenomenon known as Colony Collapse Disorder (CCD) [29]. One of the key factors of this disorder has been the use of neonicotinoids, a class of neurotoxic pesticides. Some studies have showed its effects by killing bees after expositions of these compounds, and there is evidence of damages at sublethal doses of neonicotinoids over their nervous systems affecting foraging abilities, navigation, learning communication, and memory. Also, the suppression of the immune systems of bees may be caused by neonicotinoids [30, 31]. In 2013, the European Food Safety Authority (EFSA) identified several risks posed to bees by three neonicotinoid insecticides: Clothianidin, Imidacloprid, and Thiamethoxam as seed treatment or as granules, with particular regard to their acute and chronic effects on bee colony survival and development. For this reason, the use of those products has been restricted by European Union. In addition, these restrictions are extensive for treated seeds with those pesticides (EFSA Journal 2013; 3066; 3067; 3068) [32]. It has been described that neonicotinoids are not the only group of insecticides with negative effects. The organophosphate and organochlorine also cause damage to the bees [33].

Among the methods used for the detection and identification of both insecticides and pesticides, the most useful technique is HPLC with mass spectrometry [34]. As previously indicated for pesticides, the critical step in those determinations is related to the pretreatments of samples before chromatographic analysis. One alternative methodology was developed for 13 pesticides detected in 40 samples of honeys from Poland. In this case, all the samples were subjected to liquid-liquid extraction process on a diatomaceous earth support. The main difficulty in this methodology was the matrix effects over the percentages of recoveries of pesticides (63–117%), when the samples were fortified for assessing the success of this extraction process [35]. At the present time, the analyses include the QuEChERS method followed by dispersive solid-phase extraction (d-SPE). This is simple sample preparation technique

recommended for pesticides detection in a wide variety of food and agricultural products. Several studies have achieved satisfactory results in the determination of a long list of pesticides belonging to different classes such as organophosphates, triazoles, carbamates, dicarboximides, dinitroanilines, and neonicotinoids in honey-bee bodies, honey, and bee pollen. The advantage of QuEChERS is the improvement of precision of measurements and percentages of recovery of pesticides after analysis without a matrix effect of samples affecting the reliability of results. This permits better sensitivity and lower detection limits for each pesticide [36–38].

The group of compounds corresponding to agronomic pesticides and veterinary products is wide and growing day by day. Despite this, there is a consensus on the analytical methods available for their detection and quantification. **Table 1** presents a list of susceptible compounds that can be identified in bee products with their main methodologies for their extraction, detection, and/or quantification (adapted from [39]).

N°	Pesticide	DL	QL	Extraction method		Chromatography method	
		ng g ⁻¹	ng g ⁻¹	SPE	QuEChERS	LC-MS/MS	GC-MS
1	2,4 D	0.05	0.1	√		√	
2	ABAMECTIN	0.005	0.01	√		√	
3	ACEPHATE	0.005	0.01	√		√	
4	ACEQUINOCYL	0.005	0.01	√		√	
5	ACETAMIPRID	0.005	0.01	√		√	
6	ACETOCHLOR	0.005	0.01		√		√
7	ACRINATHRIN	0.005	0.01		√		√
8	ALACHLOR	0.005	0.01		√		√
9	ALDICARB	0.005	0.01	√		√	
10	ALDICARB SULFONE	0.005	0.01	√		√	
11	ALDICARB SULFOXIDO	0.005	0.01	√		√	
12	ALDRIN	0.005	0.01		√		√
13	AMINOMETHYLPHOSPHONIC ACID	0.005	0.01	√		√	
14	AMITRAZ	0.005	0.01	√		√	
15	ATRAZINE	0.005	0.01		√		√
16	AZADIRACTIN	0.005	0.01	√		√	
17	AZINPHOS ETHYL	0.005	0.01		√		√
18	AZINPHOS METHYL	0.005	0.01		√		√
19	AZOXYSTROBIN	0.05	0.1	√		√	
20	BAC C10 BENZALKONIUM CHLORIDE	0.005	0.01	√		√	
21	BAC C12 BENZALKONIUM CHLORIDE	0.005	0.01	√		√	
22	BAC C14 BENZALKONIUM CHLORIDE	0.005	0.01	√		√	
23	BENALAXYL	0.005	0.01	√		√	

N°	Pesticide	DL	QL	Extraction method		Chromatography method	
		ng g ⁻¹	ng g ⁻¹	SPE	QuEChERS	LC-MS/MS	GC-MS
24	BENOMYL/CARBENDAZIM	0.005	0.01	√		√	
25	BENTAZON	0.005	0.01	√		√	
26	BHC ALPHA	0.005	0.01		√		√
27	BHC BETA	0.005	0.01		√		√
28	BHC DELTA	0.005	0.01		√		√
29	BIFENAZATE	0.005	0.01		√		√
30	BIFENTHRIN	0.005	0.01		√		√
31	BITERTANOL	0.005	0.01		√		√
32	BOSCALID	0.005	0.01	√		√	
33	BRODIFACOUM	0.005	0.01	√		√	
34	BROMACIL	0.005	0.01	√		√	
35	BROMADIOLONE	0.005	0.01		√		√
36	BROMOPHOS ETHYL	0.005	0.01		√		√
37	BROMOPHOS METHYL	0.005	0.01		√		√
38	BROMOPROPYLATE	0.005	0.01		√		√
39	BUPROFEZIN	0.005	0.01		√		√
40	CADUSAFOS	0.005	0.01		√		√
41	CAPTAFOL	0.005	0.01		√		√
42	CAPTAN	0.005	0.01		√		√
43	CARBARYL	0.005	0.01	√		√	
44	CARBENDAZIM	0.005	0.01	√		√	
45	CARBOFURAN	0.005	0.01	√		√	
46	CARBOPHENOTHION	0.005	0.01		√		√
47	CARTAP HCL	0.005	0.01	√		√	
48	CHLOFENTEZINE	0.005	0.01	√		√	
49	CHLORANTRANILIPROLE	0.005	0.01	√		√	
50	CHLORDANE CIS	0.005	0.01		√		√
51	CHLORDANE TRANS	0.005	0.01		√		√
52	CHLORDENE	0.005	0.01		√		√
53	CHLORFENAPYR	0.005	0.01		√		√
54	CHLORFENSON	0.005	0.01		√		√
55	CHLORFENVINPHOS	0.005	0.01		√		√
56	CHLOROBENZILATE	0.005	0.01		√		√
57	CHLOROTHALONIL	0.005	0.01		√		√
58	CHLORPYRIFOS ETHYL	0.005	0.01		√		√
59	CHLORPYRIFOS METHYL	0.005	0.01		√		√
60	CYHEXATIN/AZOCICLOTIN	0.005	0.01	√		√	
61	CLETODIM (EYZ)	0.005	0.01		√		√

N°	Pesticide	DL	QL	Extraction method		Chromatography method	
		ng g ⁻¹	ng g ⁻¹	SPE	QuEChERS	LC-MS/MS	GC-MS
62	CLOTHIANIDIN	0.005	0.01	√		√	
63	COUMAPHOS	0.005	0.01	√		√	
64	CYANAZINE	0.005	0.01		√		√
65	CYFLUTHRIN (**)	0.005	0.01		√		√
66	CYFLUTHRIN BETA	0.005	0.01		√		√
67	CYHALOTHRIN GAMMA	0.005	0.01		√		√
68	CYHALOTHRIN L	0.005	0.01		√		√
69	CYPERMETHRIN	0.005	0.01		√		√
70	CYPROCONAZOLE	0.005	0.01	√		√	
71	CYPRODINIL	0.005	0.01	√		√	
72	CYROMAZINE	0.005	0.01	√		√	
73	DDAC - DIDECYLDIMETHYLAMMONIUM CHLORIDE	0.005	0.01	√		√	
74	DDD op	0.005	0.01		√		√
75	DDD pp	0.005	0.01		√		√
76	DDE op	0.005	0.01		√		√
77	DDE pp	0.005	0.01		√		√
78	DDT op	0.005	0.01		√		√
79	DDT pp	0.005	0.01		√		√
80	DELTAMETHRIN	0.005	0.01		√		√
81	DEMETON-S	0.005	0.01		√		√
82	DIAZINON	0.005	0.01		√		√
83	DICHOLOBENIL	0.005	0.01		√		√
84	DICHOFLUANID	0.005	0.01		√		√
85	DICHLORVOS	0.005	0.01		√		√
86	DICLORAN	0.005	0.01		√		√
87	DICOFOL op (**)	0.005	0.01		√		√
88	DICROTOPHOS (**)	0.005	0.01		√		√
89	DIELDRIN	0.005	0.01		√		√
90	DIFENOCONAZOLE	0.005	0.01	√		√	
91	DIFLUBENZURON	0.005	0.01	√		√	
92	DIMETHENAMID	0.005	0.01		√		√
93	DIMETHOATE	0.005	0.01	√		√	
94	DIMETHOMORF	0.005	0.01	√		√	
95	DIPHENYLAMINE	0.005	0.01		√		√
96	DISULFOTON	0.005	0.01		√		√
97	DODINE	0.005	0.01	√		√	
98	EMAMECTIN BENZOATE	0.005	0.01	√		√	

N°	Pesticide	DL	QL	Extraction method		Chromatography method	
		ng g ⁻¹	ng g ⁻¹	SPE	QuEChERS	LC-MS/MS	GC-MS
99	ENDOSULFAN I	0.005	0.01		√		√
100	ENDOSULFAN II	0.005	0.01		√		√
101	ENDOSULFAN SULFATE	0.005	0.01		√		√
102	ENDRIN	0.005	0.01		√		√
103	EPTC	0.005	0.01		√		√
104	ESFENVALERATE/FENVALERATE	0.005	0.01	√		√	
105	ETHION	0.005	0.01		√		√
106	ETHOPROFOS	0.005	0.01	√		√	
107	ETOFENPROX	0.005	0.01	√		√	
108	FENAMIPHOS	0.005	0.01		√		√
109	FENARIMOL	0.005	0.01		√		√
110	FENAZAQUIN	0.005	0.01	√		√	
111	FENBUCONAZOLE	0.005	0.01		√		√
112	FENCLORPHOS	0.005	0.01		√		√
113	FENHEXAMID	0.005	0.01		√		√
114	FENITROTHION	0.005	0.01		√		√
115	FENOXYCARB	0.005	0.01	√		√	
116	FENPROPATHRIN	0.005	0.01		√		√
117	FENPROPIMORF	0.005	0.01	√		√	
118	FENPYROXIMATE	0.005	0.01	√		√	
119	FENTHION	0.005	0.01		√		√
120	FERBAM	0.005	0.01		√		√
121	FIPRONIL	0.005	0.01		√		√
122	FLOCOUMAFEN	0.005	0.01	√		√	
123	FLUAZINAM	0.005	0.01	√		√	
124	FLUDIOXINIL	0.005	0.01	√		√	
125	FLUFENOXURON (**)	0.005	0.01	√		√	
126	FLUMETRALIN	0.005	0.01		√		√
127	FLUQUINCONAZOLE	0.005	0.01		√		√
128	FLUSILAZOLE	0.005	0.01		√		√
129	FLUTRIAFOL (**)	0.005	0.01		√		√
130	FLUTOLANIL	0.005	0.01	√		√	
131	FLUVALINATE (**)	0.005	0.01		√		√
132	FOLPET	0.005	0.01		√		√
133	FONOFOS	0.005	0.01		√		√
134	FORCHLORFENURON	0.005	0.01	√		√	
135	FORMETANATE	0.005	0.01	√		√	
136	FORMOTHION (**)	0.005	0.01		√		√

N°	Pesticide	DL	QL	Extraction method		Chromatography method	
		ng g ⁻¹	ng g ⁻¹	SPE	QuEChERS	LC-MS/MS	GC-MS
137	GLUFOSINATE AMONNIUM	0.005	0.01		√		√
138	GLYPHOSATE	0.005	0.01	√		√	
139	HALOXIFOP METHYL	0.005	0.01	√		√	
140	HEPTACHLOR	0.005	0.01		√		√
141	HEPTACHLOR EPOXIDE	0.005	0.01		√		√
142	HEPTENOPHOS	0.005	0.01		√		√
143	HEXACHLOROBENZENE	0.005	0.01		√		√
144	HEXAICONAZOLE	0.005	0.01		√		√
145	HEXAZINONE (**)	0.005	0.01		√		√
146	HEXYTIAZOX	0.005	0.01	√		√	
147	IMAZALIL	0.005	0.01	√		√	
148	IMIDACLOPRID	0.005	0.01	√		√	
149	INDOXACARB	0.005	0.01	√		√	
150	IPRODIONE	0.005	0.01		√		√
151	ISOFENPHOS	0.005	0.01		√		√
152	KRESOXIM METHYL	0.005	0.01		√		√
153	LENACIL	0.005	0.01		√		√
154	LINDANE	0.005	0.01		√		√
155	LINURON	0.005	0.01	√		√	
156	LUFENURON	0.005	0.01	√		√	
157	MALATHION	0.005	0.01		√		√
158	MANDIPROPAMID	0.005	0.01	√		√	
159	METALAXYL	0.005	0.01		√		√
160	METAMITRON	0.005	0.01	√		√	
161	METAFLUMIZOLE	0.005	0.01	√		√	
162	METHAMIDOPHOS	0.005	0.01	√		√	
163	METHIDATHION	0.005	0.01		√		√
164	METHIOCARB	0.005	0.01	√		√	
165	METHOXYCHLOR	0.005	0.01		√		√
166	METHOXYFENOZIDE	0.005	0.01		√		√
167	METOLACHLOR	0.005	0.01	√		√	
168	METOMYL	0.005	0.01	√		√	
169	METRAFENONA	0.005	0.01	√		√	
170	METRIBUZIN	0.005	0.01	√		√	
171	MEVINPHOS	0.005	0.01		√		√
172	MIREX	0.005	0.01		√		√
173	MONOCROTOPHOS	0.005	0.01	√		√	
174	MYCLOBUTANIL	0.005	0.01		√		√

N°	Pesticide	DL	QL	Extraction method		Chromatography method	
		ng g ⁻¹	ng g ⁻¹	SPE	QuEChERS	LC-MS/MS	GC-MS
175	NAPROPAMIDE	0.005	0.01		√		√
176	NOVALURON	0.005	0.01	√		√	
177	NUARIMOL	0.005	0.01		√		√
178	OMETHOATE	0.005	0.01	√		√	
179	OXADIAZON	0.005	0.01		√		√
180	OXAMYL	0.005	0.01	√		√	
181	OXYFLUORFEN	0.005	0.01		√		√
182	PACLOBUTRAZOL	0.005	0.01		√		√
183	PARATHION ETHYL	0.005	0.01		√		√
184	PARATHION METHYL	0.005	0.01		√		√
185	PENCONAZOLE	0.005	0.01	√		√	√
186	PENDIMETHALIN	0.005	0.01		√		√
187	PERMETHRIN	0.005	0.01		√		√
188	PHORATE	0.005	0.01		√		√
189	PHOSALONE	0.005	0.01		√		√
190	PHOSMET	0.005	0.01	√		√	√
191	PHOSPHAMIDON	0.005	0.01		√		√
192	PIRAZOPHOS	0.005	0.01		√		√
193	PIRIMETHANIL	0.005	0.01	√		√	√
194	PIRIMICARB	0.005	0.01		√		√
195	PIRIMIPHOS ETHYL	0.005	0.01		√		√
196	PIRIMIPHOS METHYL	0.005	0.01		√		√
197	PROCHLORAZ	0.005	0.01	√		√	√
198	PROCYMIDONE	0.005	0.01		√		√
199	PROFENOFOS	0.005	0.01		√		√
200	PROPAMOCARB	0.005	0.01	√		√	√
201	PROPARGITE	0.005	0.01		√		√
202	PROPICONAZOLE	0.005	0.01		√		√
203	PROPOXUR	0.005	0.01	√		√	
204	PROPYZAMIDE	0.005	0.01	√		√	
205	PROTIOCONAZOLE	0.005	0.01	√		√	
206	PYMETROZIN	0.005	0.01	√		√	
207	PYRACLOSTROBIN	0.005	0.01	√		√	
208	PYRIDABEN	0.005	0.01		√		√
209	PYRIPROXYFEN	0.005	0.01		√		√
210	QUINALPHOS	0.005	0.01		√		√
211	QUINOMETHIONATE	0.005	0.01		√		√
212	QUINOXIFENO	0.005	0.01		√		√

N°	Pesticide	DL	QL	Extraction method		Chromatography method	
		ng g ⁻¹	ng g ⁻¹	SPE	QuEChERS	LC-MS/MS	GC-MS
213	QUINTOZENE	0.005	0.01		√		√
214	ROTENONE	0.005	0.01		√		√
215	SIMAZINE	0.005	0.01		√		√
216	SPINETORAM	0.005	0.01	√		√	
217	SPINOSAD	0.005	0.01	√		√	
218	SPIRODICLOFEN	0.005	0.01	√		√	
219	SPIROTETRAMAT	0.005	0.01	√		√	
220	SULFUR (S8)	0.005	0.01		√		√
221	TEBUCONAZOLE	0.005	0.01	√	√		√
222	TEBUFENOZIDE	0.005	0.01	√		√	
223	TEFLUTHRIN	0.005	0.01	√		√	
224	TERBACIL	0.005	0.01		√		√
225	TETRACONAZOLE	0.005	0.01		√		√
226	TETRADIFON	0.005	0.01		√		√
227	THIABENDAZOLE	0.005	0.01	√		√	
228	THIACLOPRID	0.005	0.01	√		√	
229	THIAMETHOXAM	0.005	0.01	√		√	
230	THIDIAZURON (**)	0.005	0.01	√		√	
231	THIOCYCLAM HYDROGEN OXALATE	0.005	0.01	√		√	
232	THIOPHANATE METHYL	0.005	0.01	√		√	
233	TOLCLOFOS METHYL	0.005	0.01	√		√	
234	TOLYLFLUANID	0.005	0.01		√		√
235	TOXAPHENE (**)	0.005	0.01		√		√
236	TRIADIMEFON	0.005	0.01	√		√	
237	TRIADIMENOL	0.005	0.01	√		√	
238	TRIAZOPHOS	0.005	0.01	√		√	
239	TRICHLORFON (**)	0.005	0.01		√		√
240	TRIFLOXYSTROBIN	0.005	0.01		√		√
241	TRIFLUMIZOLE	0.005	0.01	√		√	
242	TRIFLUMORON	0.005	0.01	√		√	
243	TRIFLURALIN	0.005	0.01		√		√
244	TRIFORINE	0.005	0.01	√		√	
245	UNICONAZOLE	0.005	0.01	√		√	
246	VAMIDOTHION	0.005	0.01	√		√	
247	VINCLOZOLIN	0.005	0.01		√		√

Table 1.
List of pesticides analyzed in honey and beeswax samples. DL: Detection Limit; QL: Quantification Limit.

7. Conclusion

The identification and detection of pesticides in the final content of honey and beeswax could be useful for beekeepers for understanding one of the potential causes of bee death. In that term, this is helpful for making improvements in the regulations for beekeeping directed to take care and preserve bees and the production of honey.

Phenolic compounds are the main molecules involved in the biological activity of honey. These compounds and its biochemical properties may be affected due to the presences of residues such as pesticides. Likewise, a specific survey applied to beekeepers to obtain production data (tons per year, detection of decreased bee population, and presence of diseases such as nosema disease and varroa mite infestation) is useful for understanding the real impact of pesticides exposure for bees.

In that way, values for biological and or physicochemical activities, production data, and detection of pesticide joined to georeferentiation of selected study sites will allow us to build a map per region describing appropriate zones for apiculture development. Finally, it shall enhance the chances of beekeepers for increasing their production by protecting bees' health.

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Conflict of interest

The author declares no conflict of interest.

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