OCCURRENCE OF PESTICIDE RESIDUES IN ITALIAN ORGANIC HONEY FROM DIFFERENT AREAS

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Introduction and Aim

Agricultural contamination with pesticides is a challenging problem that needs to be fully addressed. Bee products, such as honey, are widely consumed as food and their contamination may carry health hazards. The contamination of honey by pesticides may occur through direct contamination from beekeeping practices as well as indirect contamination from environmental sources (Kujawski, Pinteaux &, Namiesnik, 2012). Pesticide residues have been shown to cause mutations and cellular degradation. Besides being a risk to public health, the presence of pesticides in raw materials (nectar, pollen, plant exudates) or bee products (raw products, honey, royal jelly) decreases quality. The indirect contamination of honey from the environment is a result of pesticide utilization in agriculture or environmental contamination (Rissato, Galhiane, Knoll & Apon, 2004). European regulations establishing pesticide residue levels in food have prompted EU members to monitor OCPs (Regulation (EC) NO 396/2005). Insecticides used in an intensive agricultural production context like orchard areas in which a relatively high level of insecticides is generally applied may also contaminate honey. Few studies, however, have focused on pesticides used for crop protection introduced into hives by contaminated bees and wax. In this study different pesticides (organophosphorous - OPs and organochlorine - OCs) selected as representative of different contamination sources were measured in 72 organic honey samples using methods based on ASE extraction with clean up into the cell and GC-MS/MS detection (triple quadrupole - QqQ). Particular emphasis was given to the pesticides utilised in intensive orchards in order to elucidate and relate the honey contamination and its potential sources. This theme is relevant for honey bee products in which only a certification process procedure is regulated by law.

Materials e Methods

Honey samples

Sample no.	Origin information	Altitude (m a.s.l)	Area characteristic in relation to its potential pesticides sources
15	Market	-	Non-EU Produced (variable pesticides sources)
17	North Italy[a] (V.C.O.)	200	Industrialized area (OCPs source)
20	North Italy[b] (Trentino)	800	Intensive orchard (pesticides utilized in IPM* plan)
20	North Italy[c] (Valle Camonica)	1300	no presence of industries or agricultural intensive systems; absence of pesticides according to LOD of method

*IPM= integrated pest management; a=Verbano Cusio Ossola (north west);b=Trentino (north west); c=Valle Camonica (north east)

Table 1. Origins of 72 honey samples from different production areas and probable contaminant's sources

ASE - Extraction and clean-up in a single step of honey samples

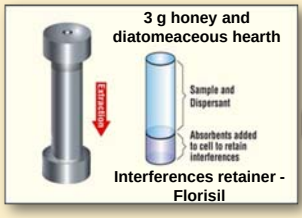
ACCELERATED SOLVENT EXTRACTION – ASE CONDITIONS

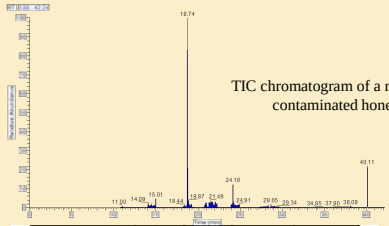
Extraction Solvent: n-Hexane : ethyl acetate (70:20, v/v)
Pressure: 1500 psi
Temperature: 80° C

Static Time:5 min
Static Cycles: 2
Flush:60%
Purge:90 s
Cell Size: 33 mL
Sorbent Clean-up: Florisil

GC-MS/MS parameters (QqQ)

Triple quadrupole mass spectrometry (QqQ) in electronic impact (EI) mode was employed for the simultaneous detection and quantification of POPs in honey samples. A GC Trace 1310 chromatograph coupled to a TSQ8000 triple quadrupole mass detector, (Thermo Fisher Scientific, Palo Alto, CA, USA), was used to confirm and quantify residues in fish samples by using a fused-silica capillary column Rt-5MS Crossbond-5% diphenyl 95% dimethylpolysiloxane (35 m x 0.25 mm i.d., 0.25 µm film thickness, Restek, Bellefonte, PA, USA). The oven temperature program was: initial temperature 80 ° C, hold 3 min, increased to 170 ° C at 10 ° C min^{-1}, then from 170 ° C to 190 ° C at 3 ° C min^{-1}, then raised to 240 ° C at 2 ° C min^{-1}, then ramped to 280 ° C at 3 ° C $min^{-1}$and finally from 280 ° C to 310 ° C at 10 ° C min^{-1} and held at this temperature for 5 min. The carrier gas (helium, purity higher than 99.999%) was in constant flow mode at 1.0 ml min^{-1}. A volume of 1 µL was injected using programmed temperature vaporizer injection (PTV) in splitless mode with a 1-min splitless period and the following inlet temperature programme: 80 ° C (0.05 min), 14.5 ° Cs^{-1} to 200 ° C (1 min) and 4.5 ° C s^{-1} to 320 ° C (12 min – cleaning phase). A baffle liner (2 mm × 2.75 mm × 120 mm, Siltek-deactivated; Thermo Fisher Scientific) was used. The transfer line was maintained at 270 ° C and the ion source at 250° C. The electron energy and the emission current were set to 70 eV and 50 µA, respectively. The scan time was 0.3 s and the peak width of both quadrupoles was 0.7 Da full width at half maximum. Argon was used as a collision cell gas at a pressure of 1.5 mTorr. The QqQ mass spectrometer was operated in selected reaction monitoring mode (SRM) detecting two-three transitions per analyte. Identification of pesticides was carried out by comparing sample peak relative retention times with those obtained for standards under the same conditions and the MS/MS fragmentation spectra obtained for each compound. The XcaliburTM processing and instrument control software program and Trace Finder 3.0 for data analysis and reporting (Thermo Fisher Scientific) were used.





TIC chromatogram of a naturally contaminated honey

Pesticides	LOD (ng g^{-1})	LOQ (ng g^{-1})	Recovery % (RSD) low spike (10 ng g^{-1})	Recovery % (RSD) high spike (50 ng g^{-1})
α-HCH	0.99	2.97	95 (4.5)	93 (7.8)
β-HCH	1.26	3.78	90 (6.54)	92 (5.4)
γ-HCH	1.17	3.51	85 (3.4)	80 (6.3)
δ-HCH	1.26	3.77	82 (7.7)	80 (3.2)
Aldrin	0.91	2.73	87 (5.2)	85 (1.2)
Heptachlor	0.95	2.84	93 (4.3)	90 (2.4)
Heptachlor epoxide	1.22	3.66	95 (4.8)	92 (3.3)
Endosulfan I	1.13	3.38	80 (5.6)	75 (1.8)
Endosulfan II	1.14	3.42	86 (8.8)	90 (5.3)
Dieldrin	1.00	3.02	87 (9.2)	92 (5.4)
Endrin	0.99	2.98	90 (4.3)	95 (3.0)
Endrin Aldehyde	1.34	4.02	92 (6.2)	90 (2.8)
p,p' DDE	0.85	2.55	95 (3.2)	96 (1.15)
p,p' DDD	0.91	2.74	90 (1.4)	98 (1.18)
p,p' DDT	0.94	2.83	92 (1.8)	90 (0.8)
Methoxychlor	1.07	3.22	87 (2.6)	85 (1.2)
Chlorpyriphos	1.21	3.63	88 (4.8)	90 (3.8)
Penconazol	0.75	2.25	85 (4.3)	82 (1.2)
Captan	1.25	3.75	92 (3.5)	95 (2.7)
Bupiramate	1.34	4.02	75 (2.6)	80 (1.15)
Quinoxyfen	0.79	2.39	98 (1.3)	98 (3.6)
Fluazinam	1.08	3.25	85 (2.1)	90 (2.2)
Trifloxystrobin	1.18	3.54	102 (0.9	100 (1.1)
Iprodion	1.07	3.22	94 (1.2)	98 (0.6)
Chloroantraniliprol	1.18	3.54	98 (1.3)	95 (0.5)
Spirodiclofen	1.14	3.42	87 (6.2)	90 (3.4)
Boscalid	1.48	4.44	92 (2.7)	95 (6.3)
Pyraclostrobin	0.85	2.55	90 (1.4)	96 (2.3)

Tab. 2 LOD, LOQ and recovery of studied analytes in organic honey samples

Results and Discussion

In the present study, the method developed was applied for the analysis of 72 organic honey samples produced in different geographic areas in order to screen and then relate the presence of pesticide residues to their potential contamination source confirming honey as a suitable indicator of environmental pollution as well as an indicator of the presence of pesticides utilised in crop protection management. Overall the results of detection frequency and concentration levels of pesticide residues found in all honey samples are presented in fig 1.

Residues of many pesticides were found in most of the samples investigated. The majority of honey samples (94%) contained at least one of the pesticides even if their concentrations were found to be lower than the MRL. The presence of many OCPs was detected in the honey samples produced in the industrialised area (V.C.O.) and in the industrial honeys. In the honey collected in Trentino, the geographical area characterized by intensive apple orchards, a great number of insecticides used for crop protection were found. In particular, chlorpyrifos was found in all samples analysed with frequency of 100% followed by quinoxyfen (90%), captan, trifloxystrobin, iprodion and boscalid detected with 75% prevalence in honey samples. Intensively cultivated apple plantations are subject to an extensive use of pesticides to control most agricultural pests even if the integrated pest management system is applied during the growing season contamination (Rissato, Galhiane, Knoll & Apon, 2004). Literature is scarce regarding the presence of insecticide residues used for crop protection in apple orchards in Italy especially in organic products as honey.

Conclusions

The optimised analytical method was applied to monitor the pesticide residues among samples produced in different areas characterised by different contamination sources. The method proved to be simple and rapid, requiring small sample sizes, minimizing solvent consumption. MS/MS detection provides both quantitative information and confirmation of pesticide residues in honey. The results of this study show that the pesticide contamination of honey is strictly related to the contamination source and could reflect the specific pollution of a given environment, confirming honey bee and beehive matrices as appropriate sentinels for monitoring contamination in the environment. This could represent an effective tool for beekeepers to select production areas especially for organic honey production.

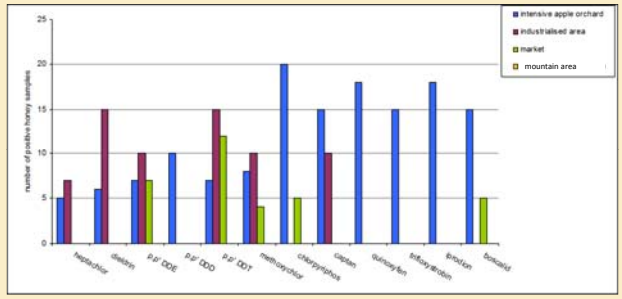


Fig. 1 Pesticides detection frequency in honey samples in relation to their sampling area

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