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Occurrence of pesticide residues in candies containing bee products

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ABSTRACT

Pesticides can be found in bee products as they are usually employed to protect the beehive, but they can also reach the hive due to environmental contamination. These contaminants could be present in processed foods. One of the most common and consumed comfitures based in bee products are honey and propolis candies, for which no analytical method has yet been developed. This work presents the development of an ethyl acetate based extraction method followed by dispersive clean up using Primary and Secondary Amine (PSA) plus Graphitized Carbon Black (GCB) with Gas Chromatography coupled to Mass Spectrometry (GC–MS) determination for pesticide residue monitoring candies containing honey and propolis from the Mercosur region. Sixteen pesticides found in bee products as well as three pesticide metabolites of toxicological significance were evaluated including acaricides and insecticides (pyrethroids, organophosphates and some organochlorines). The method was validated, with a LOQ of 0.01 mg/kg for most analyzed pesticides, showing a recovery rate of 70–120% with <20% RSD. Real samples from the Mercosur countries were analyzed. Coumaphos and chlorpyrifos residues were detected in most of them. From these findings a preliminary toxicological evaluation of coumaphos via admissible daily intake (ADI) estimation was conducted.

1. Introduction

There are an increasing number of reports on the presence of pesticide residues in bee products (Bogdanov, 2006; Mullin et al., 2010; Wiest et al., 2011). Although these findings were primarily focused on understanding the bee disappearance phenomenon, they demonstrate the pesticides carry over from the field to honey, propolis and wax. Bee products can be consumed as such, but also they are added to a broad palette of processed foods.

Reports of pesticides occurrence in honey, propolis and beeswax are frequent in the literature (Pareja et al., 2011; Pérez-Parada et al., 2011; Serra-Bonvehí & Orantes-Bermejo, 2010). Bee products can be contaminated by pesticides due to environmental pollution or direct application of pesticides into the beehive to protect bees against acaroids like *Varroa destructor* (Adamczyk, Lázaro, Pérez-Arquillué, Bayarri, & Herrera, 2010; Niell et al. 2015; Serra-Bonvehí & Orantes-Bermejo, 2010). Many insecticides like organophosphates, pyrethroids, carbamates and even organochlorine compounds have been reported at low to high concentrations in honey and beeswax (Bargańska & Namieśnik, 2010; Mullin et al., 2010; Niell et al., 2015; Pareja et al., 2011; Serra-Bonvehí & Orantes-Bermejo, 2010; Zhu, Schmehl, Mullin, & Frazier, 2014). Other pesticide families like fungicides and herbicides had been found in honey and beeswax (Mullin et al., 2010; Zhu et al., 2014). But the most common contaminant type found in honey, propolis and wax are acaricides such as coumaphos, fluvalinate and chlorphenvinfos that are usually present at higher concentrations than those coming from environmental pollution (Chauzat & Faucon, 2007; Mullin et al., 2010; Serra-Bonvehí & Orantes-Bermejo, 2010).

Bee products (honey, royal jelly, beeswax and propolis) are used in the production of candies, soaps, cosmetic creams and ointments. They are included in baby foods and breakfast cereals that are also widely consumed by children. Many dietary supplements contain propolis due to its well-known antioxidant, antibacterial and nutritional properties (Burdock, 1998). Raw bee by-products are generally complex matrices that need special method development for the determination of pesticide residues in them and are not routinely investigated in control laboratories. Honey is a high sugar content matrix for which a number of protocols to determine pesticide residues in it had been reported. The advent of the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) methodology brought new developments for the pesticide residue

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analysis of bee products. QuEChERS methodology is a template based in a salting out step after extraction of the matrix with an organic solvent (acetonitrile, acetone or ethyl acetate) that causes phase separation, followed by a dispersive clean up. The resulting pesticides containing extract is analyzed through gas (GC) and liquid (LC) chromatography coupled to mass spectrometry (MS) detection and the residues determined (Anastassiades, Lehotay, Stajnbaher, & Schenck, 2003).

Depending on the nature of the matrix and the pesticides under study, different sorbents are currently employed in dispersive clean up step, such as primary and secondary amine (PSA) to eliminate acid compounds, graphitized carbon black (GCB) to sequestrate pigments and planar compounds, zirconia based sorbents that react with Lewis bases, C_{18} to absorb lipid compounds. (Li, Kelley, Anderson, & Lydy, 2015; Mullin et al., 2010; Niell et al., 2015; Paradis, Bérail, Bonmatin, & Belzunces, 2014). Different QuEChERS based approaches have been also reported for bees, honey, beeswax, and pollen (Niell et al., 2014; Niell et al., 2015).

For propolis there are only a few analytical procedures reported (Acosta-Tejada, Medina-Peralta, Miguel-Ordóñez, & Muñoz-Rodríguez, 2011; Chen et al., 2009; Medina-Dzul, Muñoz-Rodríguez, Moguel-Ordoñez, & Carrera-Figueiras, 2014; Pérez-Parada et al., 2011; Santana Dos Santos, Aquino, Dórea, & Navickiene, 2008). Propolis is a very complex analytical matrix, composed mainly by polyphenols and resin acids that have similar physicochemical properties to most of the pesticides that are commonly searched for. The load and nature of coextractives is significant in these extracts when conventional and solvent based extraction sample treatment procedures are used. Laborious protocols have been proposed for their removal, with the aim to not pollute the chromatographic system (Pérez-Parada et al., 2011).

Although pesticide residues are widely reported in unprocessed bee products, there has not been any assessment on the occurrence of pesticides in processed foods that includes some of these bee products. Moreover, the analysis of pesticide residues in candies makes the extraction more complex due to the candies formulation. In this work, a methodology for the pesticide residues analysis in candies containing honey and propolis has been developed and applied to the analysis of real samples purchased in the Mercosur region.

2. Materials and methods

2.1. Chemicals and materials

PSA, GCB and $MgSO_4$ were provided from Scharlau SL (Barcelona, Spain). PSA as bulk powder 40–60 μm and GCB 120–400 mesh. Sodium chloride was USP grade. Ethyl acetate (EtOAc) and acetonitrile (MeCN) of HPLC grade was purchased from Mallinckrodt Baker Inc. (Phillipsburg, USA).

Organic solvents were analytical grade, pesticide residues free and were purchased from Merck (Darmstadt, Germany). Pesticide standards and the internal standard were from Dr. Ehrenstorfer (Augsburg, Germany, >95%). Stock solutions were prepared from the standard substances at 2000 mg L^{-1} in ethyl acetate. Working standard mixtures were prepared by appropriately diluting the stock solutions with ethyl acetate. All solutions were stored at −4 °C.

2.2. Candy samples of honey and propolis

The candies analyzed in the study were purchased from the local and regional markets. They were all registered and labeled products to be sold over the counter, They were acquired in Montevideo (Uruguay) in November 2012, in Buenos Aires, Concordia and Entre Rios (Argentina) in June 2011 and Porto Alegre, Caxias do Sul, Rio Grande do Sul (Brazil), in March 2012. Candies were crushed in a mortar until a fine powder was obtained.

2.3. Instrumentation

Pesticides residues were analyzed using Gas Chromatography with Mass Selective detector (GC–MS). The equipment used had an HP 6890 GC coupled with a HP 5973 MS supported by reference libraries, equipped with HP-5 (5% diphenyl 95% dimethylsiloxane) bonded fused-silica capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness). Electron impact (EI) mass spectra was obtained at 70 eV and monitored from 50 to 550 *m/z* for full scan mode analysis. MS system was programmed in selected ion monitoring (SIM) mode for quantitative analysis. The working parameters were: injector temperature 290 °C; interface temperature 300 °C; carrier gas He at 38 cm/s. Oven conditions; from 60 °C initial (5 min hold), increased to 230 °C at a rate of 10 °C/min, then to 295 at 30 °C min (10 min hold), injection mode: split (ratio 12:1); injection volume: 1.0 μL. The identification of the compounds was confirmed by injection of solvent and matrix matched standards and comparison of their retention index and relevant MS ratios in accordance to international guidelines (SANCO, 2013). Identification parameters are shown in Table 1.

2.4. Sample preparation

10.0 g of crushed candies previously homogenized were weighed into a 40.0 mL PTFA centrifuge tube and 10.0 mL of distilled water were added 10.0 mL of ethyl acetate were poured into the tube and the mixture was shaken vigorously for one minute. Eight grams of anhydrous $MgSO_4$ and 1.5 g NaCl were added and agitated manually for 5 min, followed by 15 min of centrifugation at 3000 rpm.

2.4.1. Dispersive-SPE clean up

For the dispersive clean up, 5.0 mL of supernatant was transferred into a clean up tube containing 750 mg anhydrous $MgSO_4$, 150 mg PSA and 100 mg GCB. A vortex mixer shaked the mixture for 1 min and centrifuged 10 min at 3000 rpm.

Then, 4.0 mL were driven to dryness under reduced pressure and redissolved in 1.00 mL of a solution of TPP, internal standard, in AcOEt and directly analyzed by GC–MS.

2.4.2. Spiking procedure

10.0 g of blank crushed candies were weighed into a PTFA centrifuge tube. This sample was spiked by the addition of the appropriate mix of standard solution. Two concentration level of spiking were assayed (0.05 and 0.10 mg/kg).

2.4.3. Blank preparation

2.4.3.1. Honey candies. 100 g of honey candies purchased in the local market were used. They were crushed to a thin powder and checked for pesticide absence.

2.4.3.2. Propolis candies blanks. Blank propolis was obtained from organic producers and checked for the absence of pesticides residues with a protocol published elsewhere (Pérez-Parada et al., 2011). 100 g of crushed candies base were weighed in a round bottom flask. Using the geometric dilution procedure for mixtures, 80% ethanolic propolis tincture was added to achieve a final mixture containing a concentration of 1% propolis (typical concentration of propolis in candies). The sample was mixed for 30 min until an homogeneous mass was obtained.

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Table 1
Chromatographic optimization of the analytes using GC–MS. From left to right, the retention times in minutes (tR), selected m/z and quantitation ion (in bold) are shown. For each matrix (honey and propolis candies) the matrix matched calibration curve, correlation coefficient, the matrix effect and the limit of quantification (LOQ).

Compound	tR (min)	m/z	Honey candies				Propolis candies			
			Calibration curve (matrix)	r^2	Matrix effect (%)	LOQ (mg/kg)	Calibration curve (matrix)	r^2	Matrix effect (%)	LOQ (mg/kg)
2.4-DMA	11.39	106,121,120	y = 1.307 x − 0.016	0.998	–	0.03	y = 1.307 x − 0.016	0.998	–	0.03
DMPF	17.13	120,132,106	y = 0.975 x + 0.015	0.998	−40.1	0.01	y = 0.823 x − 0.011	0.998	−92.0	0.03
Vinclozolin	22.86	212,287,198	y = 0.414 x + 0.030	0.995	−4.3	0.01	y = 0.348 x + 0.060	0.997	−14.3	0.01
Malathion	23.76	125,158,173	y = 0.930 x + 0.036	0.999	+2.5	0.01	y = 0.934 x + 0.020	0.999	+3.0	0.01
Chlorpyrifos	24.00	258,197,314	y = 0.466 x − 0.028	0.999	+2.1	0.01	y = 0.398 x + 0.023	0.999	−12.9	0.01
Chlorfenvinphos	25.00	267,269,325	y = 1.310 x − 0.015	0.999	−22.1	0.01	y = 0.724 x − 0.018	0.997	−5.0	0.01
Fipronil	25.15	367,213,369	y = 0.722 x + 0.008	0.999	−9.7	0.01	y = 0.907 x + 0.021	0.999	+13.6	0.02
Dibromobenzophenone	26.56	185,340,183	y = 1.260 x + 0.021	0.999	+4.7	0.02	y = 1.088 x + 0.175	0.999	−13.1	0.01
Ethion	27.28	231,384,153	y = 1.448 x + 0.006	0.997	+18.2	0.01	y = 1.380 x + 0.021	0.999	+12.7	0.01
Bifenthrin	29.20	181,165,166	y = 5.040 x + 0.105	0.999	+11.0	0.01	y = 4.541 x − 0.108	0.999	−41.8	0.01
Bromopropylate	29.73	341,339,183	y = 1.069 x + 0.018	0.998	−1.7	0.01	y = 1.192 x + 0.014	0.999	+10.2	0.01
Tetradifon	29.75	356,159,229	y = 0.881 x + 0.026	0.999	+1.5	0.01	y = 0.682 x + 0.021	0.998	−21.4	0.01
Amitraz	30.28	121,293,132	y = 0.807 x − 0.011	0.998	−11.3	0.01	y = 1.122 x − 0.010	0.997	−18.0	0.04
λ − Chialothryn	30.45	181,197,208	y = 0.816 x − 0.0010	0.998	−19.2	0.01	y = 1.010 x − 0.004	0.998	+42.3	0.01
Coumaphos	31.59	334,362,364	y = 0.403 x + 0.004	0.998	−8.1	0.01	y = 0.612 x + 0.021	0.999	+39.4	0.01
Cipermethryn (sum)	32.40	163,165,181	y = 0.344 x − 0.013	0.999	−29.0	0.01	y = 0.772 x − 0.010	0.998	−12.8	0.10
Fenvalerate	33.51	125,209,181	y = 2.761 x + 0.029	0.998	−36.5	0.05	y = 0.757 x + 0.001	0.999	−40.5	0.01
τ-fluvalinate	33.83	250,252,181	y = 1.139 x − 0.008	0.999	−2.7	0.01	y = 0.769 x + 0.178	0.998	−32.7	0.01
Deltamethrin	42.00	181,251,253	y = 1.588 x +0.015	0.998	−56.5	0.05	y = 1.442 x + 0.002	0.999	−46.2	0.01

Matrix effect was calculated as: ME (%) = ((slope matrix/slope solvent) − 1) × 100. LOQs were determined using the graphical approach at a signal to noise ratio (S/N) of 10.

2.4.4. Calibration curves

Quantitation was performed through matrix-matched calibration. Matrix-matched standards were prepared by adding 1.00 mL of an appropriate working standard solution to yield the concentrations assayed including IS at 0.50 mg/kg to blank sample extracts.

2.4.5. Matrix effect calculation

The procedure we used to calculate the matrix effect was as ME (%) = (slope matrix/slope solvent − 1) × 100.

3. Results and discussion

3.1. Preliminary studies

Although some analytical methods for the determination of pesticide residues in bee products have been reported in the past (Acosta-Tejada et al., 2011; Chen et al., 2009; Li et al., 2015; Paradis et al., 2014; Pérez-Parada et al., 2011; Santana Dos Santos et al., 2008; Wallner, 1999; Wiest et al., 2011), the present work was focused on the development of a miniaturized, easy to perform and straightforward method for candies containing bee products. The sample preparation protocol is the direct extraction of crushed candies with ethyl acetate followed by a dispersive clean up step with PSA and GCB. The selection of ethyl acetate as extraction solvent was based on the lower solubility of sugars on this solvent (compared to acetonitrile used in QuEChERS method) and straightforward protocols to analyse GC amenable compounds. Multiresidue methods for GC–MS analysis of extracts without clean up has been published (Pihlström, Blomkvist, Friman, Pagard, & Österdahl, 2007) as well as with clean up using sorbents for dispersive SPE (Mol et al., 2007). The selected amounts of GCB and PSA gave clean extracts. Moreover, split injection was used to prevent contamination of the chromatographic system because the sample caramelized in the glass insert.

Given the pesticides that are commonly found in beehives, a simple method for the determination of 19 analytes was developed; 16 important pesticides and 3 of their metabolites at trace levels commonly used in beekeeping and crop protection activities, applicable to both comfiture types. Since the residue definition of amitraz also includes its metabolites N-2,4-dimethylphenyl-N-methyl-formamidine (DMPF), 2,4-dimethylformanilide (DMF), and 2,4-dimethylaniline (DMA), they must be targeted together. They are usually analyzed as a single residue by hydrolyzing amitraz to DMA (Hepperle, Mack, Sigalov, Schüler, & Anastassiades, 2015). This work tried to incorporate amitraz into a multirresidue method. However, DMF is not GC amenable and was not included in this study.

Despite the wide linear range where amitraz can be measured in matrix-matched calibration, it was not recovered from any of the studied matrices. Amitraz residues were not detected probably due to its hydrolysis during the extraction. On the other hand, DMA suffered strong coextractive coelution and interferences that did not allow its detection even in matrix matched calibration samples for both honey and propolis candies. Nevertheless, DMPF can be accurately determined in these matrices. DMPF was reported to be the main metabolite of amitraz in fruits and vegetables (Ferrer et al., 2010). For that reason, the present methodology may be useful for screening purposes of amitraz residues through the analysis of DMPF. Furthermore, dibromobenzophenone the main metabolite of bromopropylate, was also included in the scope since its relevance in European beehives (Bogdanov, 2006).

3.2. Method development

The developed methodology allowed the determination of the selected pesticides and metabolites in honey candies above 0.02 mg/kg except for amitraz. The recoveries at 0.05 and 0.10 mg/kg were between 70 and 120% range with RSD < 20% for most evaluated pesticides (see Table 2). Deltamethrin recovery was 126% at 0.10 mg/kg.

The matrix effect for honey candies was low for most of the studied pesticides. Linear response was obtained between 0.02 and 1.00 mg/kg with $r^2 > 0.995$ for these pesticides as seen in Table 2. In particular, deltamethrin showed high matrix effect (−56.5%). Matrix matched calibration was employed for quantitation purposes. LOQs were determined using the graphical approach at a signal to noise ratio (S/N) of 10. For honey candies LOQs were between 0.01 mg/kg for 15 of the evaluated pesticides and 0.05 mg/kg for deltamethrin.

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Table 2
Recoveries (%) and RSDs (%) for each analyte under study (n = 5) at 0.05 and 0.10 mg/kg using GC–MS. The RSD (%) values were obtained under repeatability conditions (same day).

Pesticides	Honey candies				Propolis candies			
	0.05 mg/kg		0.10 mg/kg		0.05 mg/kg		0.10 mg/kg	
	Rec (%)	RSD (%)	Rec (%)	RSD (%)	Rec (%)	RSD (%)	Rec (%)	RSD (%)
2.4-DMA	–	–	–	–	–	–	–	–
DMPF	72	14	115	13	73	13	75	14
Vinclozolin	84	10	106	19	108	15	108	6
Malathion	79	9	80	9	83	3	115	7
Chlorpyrifos	109	15	103	17	94	12	83	13
Chlorfenvinphos	110	8	111	15	101	7	91	20
Fipronil	113	20	109	16	74	10	107	13
Dibromobenzophenone	74	12	73	3	118	10	94	9
Ethion	119	8	113	14	89	5	101	11
Bifenthrin	113	9	116	15	78	4	114	11
Bromopropylate	97	16	115	14	115	7	121	8
Tetradifon	114	9	114	8	101	9	102	9
Amitraz	–	–	–	–	–	–	–	–
λ-Chialothryn	114	14	82	10	111	10	109	4
Coumaphos	77	16	75	12	70	10	115	6
Cypermethrin	ND	–	85	12	ND	–	82	13
Fenvalerate	93	16	110	17	92	9	118	2
τ-fluvalinate	75	8	94	11	85	3	84	3
Deltamethrin	116	12	126	15	111	10	104	8

ND: not determined.

For propolis candies, despite the increase in matrix complexity due to the inclusion of the propolis tincture, the same method allowed the determination of the evaluated pesticides showing a similar performance to honey candies. All analytes were recovered between the 70–120% range (except bromopropylate with 121% at 0.10 mg/kg) with good reproducibility (<20% RSD). The matrix effect for propolis candies varied from low (−5% for chlorfenvinphos) to strong (−92% for DMPF).

The calculated LOQs for propolis candies ranged from 0.01 mg/kg for 14 of the studied pesticides to 0.10 mg/kg for cypermethrin. Fig. 1 shows a comparison of chromatogram of targeted analysis of pesticides in SIM mode. As expected, honey candies extracts were cleaner than propolis ones.

It should be emphasized that the developed method was performed for candies that contain bee products. Candies are a manufactured matrix while honey and propolis are natural ones. In candies, bee products are diluted/dispersed into a solid sugar environment. Candies composition is not similar to honey itself since candies are sucrose based while honey is a fructose and glucose based product.

The performance of these protocols cannot be compared with other analytical methodologies specifically developed for “pure” bee products, which are much more complex matrices. This means that this is the first record of an analytical method to analyze selected pesticide residues in candies containing bee products with GC–MS determination. Ethyl acetate is a suitable solvent to extract, from the two types of candies, the pesticides under study. Several authors recently reported analytical methods to analyze pesticide residues in a sugar based matrix such as honey using ethyl acetate as extraction solvent (Louca Christodoulou et al., 2015; Nakajima

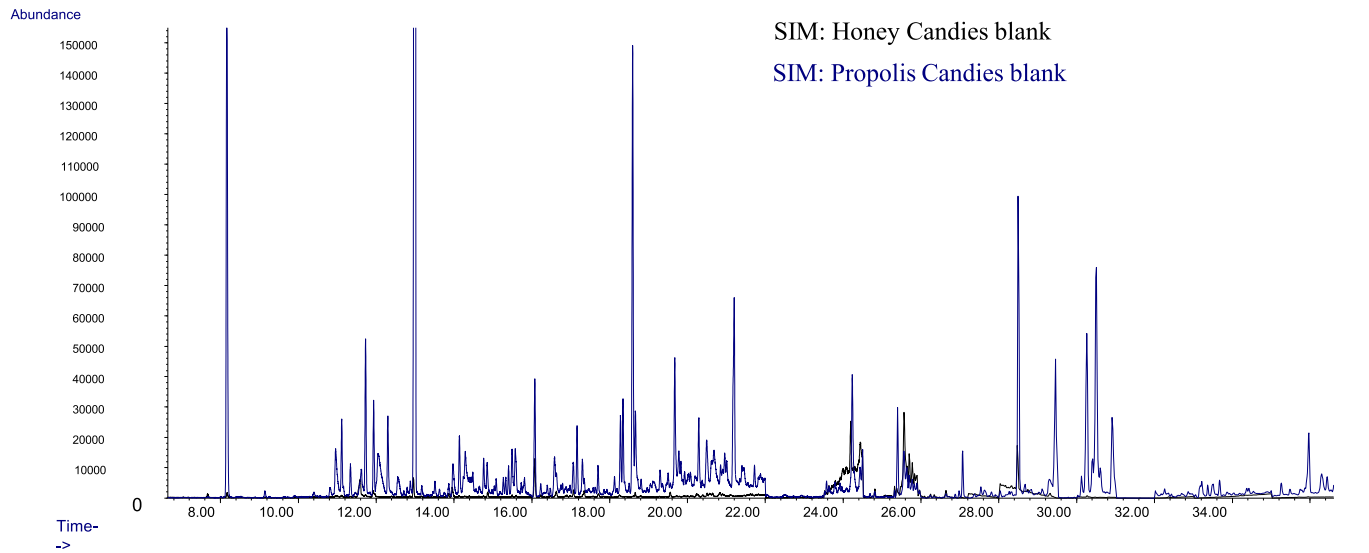

Fig. 1. Overlay of SIM chromatograms for the two matrixes. In black line honey candy extract) and in blue line propolis candy extract. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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Table 3
Pesticide residues in real samples of propolis candies expressed in μg/kg. The result was expressed as the confidence interval at 95% of the calculated average ($n = 3$). LOD for chlorpyrifos: 4.0 μg/kg; LOD for coumaphos: 3.0 μg/kg.

Candies samples								
Compound	Uruguay				Argentina		Brazil	
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 1	Sample 2	Sample 1	Sample 2
Chlorpyrifos	12.2 ± 1.4	14.1 ± 1.7	10.2 ± 1.5	21.4 ± 2.0	ND	ND	11.4 ± 1.5	10.7 ± 1.2
Coumaphos	11.9 ± 1.6	27.9 ± 1.4	ND	4.1 ± 0.7	36.1 ± 1.4	21.7 ± 2.9	ND	ND

et al., 2015; Ozcan & Aycan, 2013) with acceptable analytical performance.

3.3. Pesticide residues in real samples

The analytical procedure thus developed was applied to real samples obtained in different Mercosur countries. Pesticide residues were not detected in honey candies samples but the propolis ones showed the occurrence of coumaphos and chlorpyrifos at quantifiable levels as seen in Table 3.

Chlorpyrifos was detected in samples of different brands of Argentina, Brazil and Uruguay whereas coumaphos was only detected in Argentinean and Uruguayan ones. The highest concentration of coumaphos was found for an Argentinean propolis candy sample (0.36 mg/kg) whereas chlorpyrifos was detected at 0.02 mg/kg in one Uruguayan sample. The confirmation of these pesticides in real samples is shown in Figs. 2 and 3 by meeting the relative ion abundance (RIA) criteria stated in SANCO document (SANCO, 2013).

Whereas chlorpyrifos was detected at lower amounts, showing the environmental fate of this contamination, as it is one of the most applied agricultural pesticides in the Mercosur region and is not employed in beekeeping activities. However, coumaphos, an acaricide widely employed worldwide to protect bees against *Varroa destructor*, was present at higher concentrations. These results are in accordance with Pérez-Parada et al., 2011 findings in Uruguayan propolis samples. The amounts of coumaphos detected in propolis candies, show that the occurrence of pesticides residues in them may depend on the origin of the sample and the apicultural practices performed. The method presented here shows its applicability for testing the main acaricides used in beekeeping all over the world (Bogdanov, 2006). These preliminary findings suggest that acaricides are the main source of pesticide residues in propolis candies. However, environmental pollutants could also play a role in the occurrence of pesticide residues in these commodities as shown for chlorpyrifos which is not used in beekeeping due to its high toxicity to bees (0.36 μg/bee) (Tomlin, 2011). As new pesticides could be used for plant protection in extensive plantations in the future, the scope of the method could be extended.

3.4. Preliminary toxicological assessment

Considering that high levels of coumaphos were found in propolis candies samples, we estimate the toxicological relevance for this commodity. Taken into account that propolis candies are usually swallowed when people are suffering colds or some disorder of the respiratory tract, usually in winter, an estimation of the number of propolis candies that a person could ingest daily was conducted. Coumaphos has an ADI of 0.0003 mg/kg of body weight per day (Canadian Pest Management Regulatory Agency, 2003).

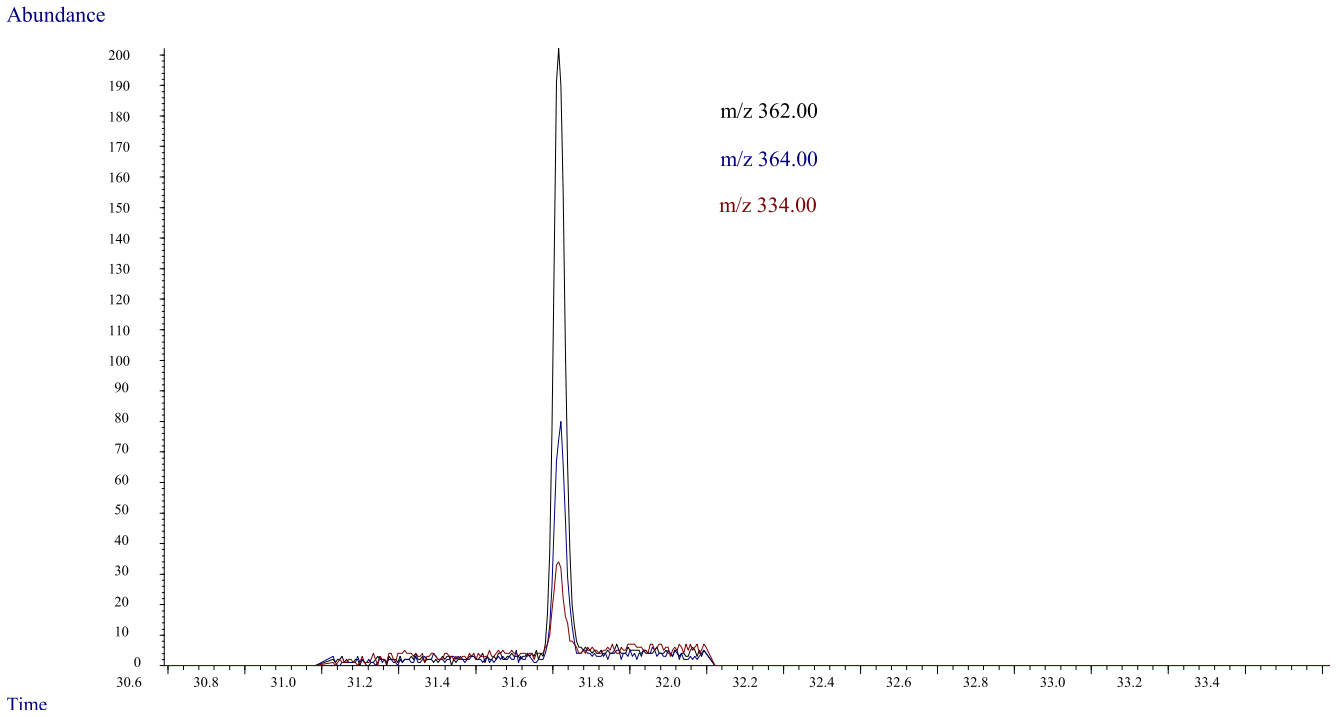

Fig. 2. GC–MS confirmation of coumaphos in a propolis candy sample.

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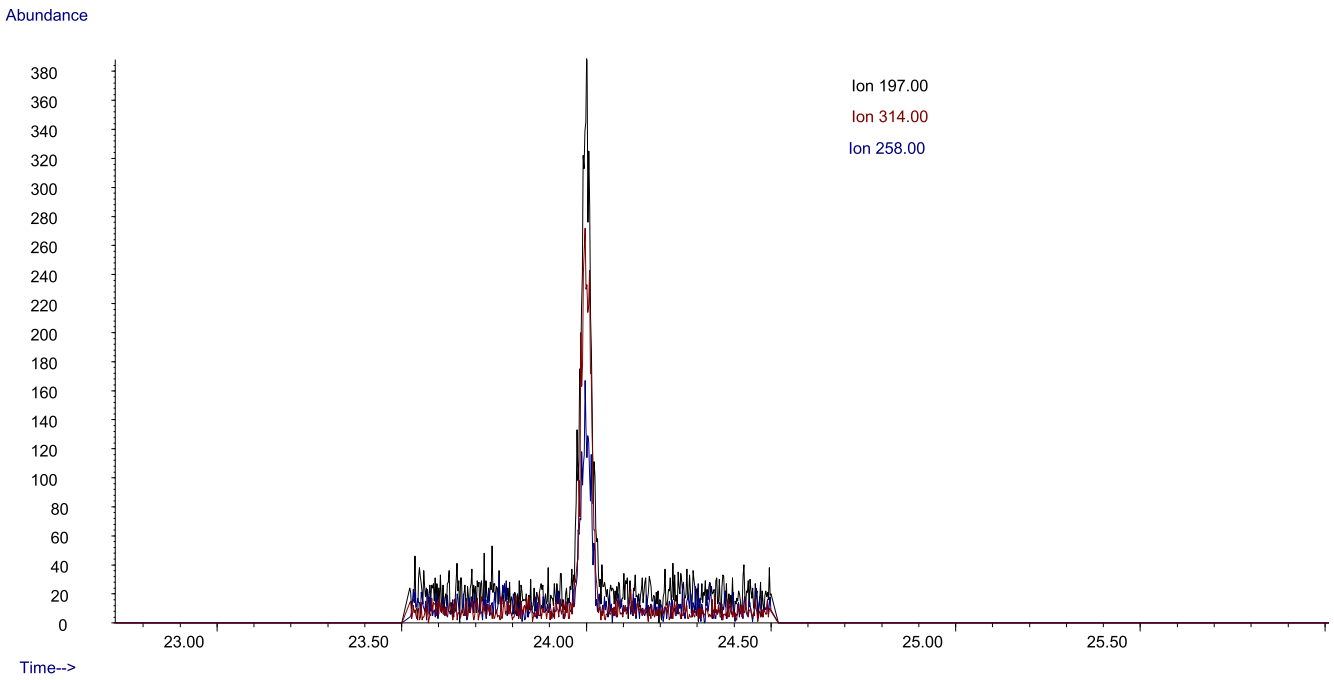

Fig. 3. GC–MS confirmation of chlorpyrifos in a propolis candy sample.

Therefore for an adult of 60 kg body weight, 18 μg will complete the tolerable daily doses for coumaphos. Considering that an average candy weighs 5 g and assuming the worst case scenario (0.36 mg/kg), it contains ~1.8 μg of pesticide; therefore the ingestion of 10 candies will be enough to reach the ADI for coumaphos. This rough estimation highlights the importance of performing routine controls on the pesticide residue content of foods that contain bee products as well as the need of specific regulations.

4. Conclusions

In this study a novel analytical methodology for trace quantitative determination of pesticide residues in candies containing bee products was presented. The proposed method was sensitive, reliable and successfully applied to the analysis of real samples. These developments allowed the detection of pesticide residues in candies for the first time in literature. Coumaphos and chlorpyrifos residues were found in propolis candies from Mercosur region whereas residues were not found in honey candies. More knowledge on the occurrence of pesticide residues in candies should be gathered in order to obtain a clearer picture on pesticides residues in manufactured bee products. The issue is particularly relevant for infant and baby foods with those bees' products and where pesticide residues must be avoided.

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References

Acosta-Tejada, G. M., Medina-Peralta, S., Miguel-Ordóñez, Y. B., & Muñoz-Rodríguez, D. (2011). Matrix solid-phase dispersion extraction of organophosphorus pesticides from propolis extracts and recovery evaluation by GC/MS. *Analytical and Bioanalytical Chemistry, 400*(3), 885–891.

Adamczyk, S., Lázaro, R., Pérez-Arquillué, C., Bayarri, S., & Herrera, A. (2010). Impact of the use of fluvalinate on different types of beeswax from Spanish hives. *Archives of Environmental Contamination and Toxicology, 58*(3), 733–739.

Anastassiades, M., Lehotay, S. J., Stajnbaher, D., & Schenck, F. J. (2003). Fast and easy multiresidue method employing acetonitrile extraction/partitioning and "dispersive solid-phase extraction" for the determination of pesticide residues in produce. *Journal of AOAC International, 86*(2), 412–431.

Bargańska, Z., & Namieśnik, J. (2010). Pesticide analysis of bee and bee product samples. *Critical Reviews in Analytical Chemistry, 40*(3), 159–171.

Bogdanov, S. (2006). Contaminants of bee products. *Apidologie, 37*(1), 1–18.

Burdock, G. A. (1998). Review of the biological properties and toxicity of bee propolis (propolis). *Food and Chemical Toxicology, 36*(4), 347–363.

Chauzat, M. P., & Faucon, J. P. (2007). Pesticide residues in beeswax samples collected from honey bee colonies (*Apis mellifera* L.) in France. *Pest Management Science, 63*(11), 1100–1106.

Chen, F., Chen, L., Wang, Q., Zhou, J., Xue, X., & Zhao, J. (2009). Determination of organochlorine pesticides in propolis by gas chromatography-electron capture detection using double column series solid-phase extraction. *Analytical and Bioanalytical Chemistry, 393*(3), 1073–1079.

Ferrer, C., Aguera, A., Mezcua, M., Fernandez-Alba, A. R., Mack, D., Anastassiades, M., et al. (2010). Efficiency evaluation of the main multiresidue methods used in Europe for the analysis of amitraz and its major metabolites. *Journal of AOAC International, 93*(2), 380–388.

Hepperle, J., Mack, D., Sigalov, I., Schüler, S., & Anastassiades, M. (2015). Analysis of "amitraz (sum)" in pears with incurred residues - comparison of the approach covering the individual metabolites via LC-MS/MS with the approach involving cleavage to 2,4-dimethylaniline. *Food Chemistry, 166*, 240–247.

Li, Y., Kelley, R. A., Anderson, T. D., & Lydy, M. J. (2015). Development and comparison of two multi-residue methods for the analysis of select pesticides in honey bees, pollen, and wax by gas chromatography-quadrupole mass spectrometry. *Talanta, 140*, 81–87.

Louca Christodoulou, D., Kanari, P., Kourouzidou, O., Constantinou, M., Hadjiloizou, P., Kika, K., et al. (2015). Pesticide residues analysis in honey using ethyl acetate extraction method: validation and pilot survey in real samples. *International Journal of Environmental Analytical Chemistry, 95*(10), 894–910.

Medina-Dzul, K., Muñoz-Rodríguez, D., Moguel-Ordoñez, Y., & Carrera-Figueiras, C. (2014). Application of mixed solvents for elution of organophosphate pesticides extracted from raw propolis by matrix solid-phase dispersion and analysis by GC-MS. *Chemical Papers, 68*(11), 1474–1481.

Mol, H. G. J., Rooseboom, A., van Dam, R., Roding, M., Arondeus, K., & Sunarto, S. (Nov 2007). Modification and re-validation of the ethyl acetate-based multi-residue method for pesticides in produce. *Analytical and Bioanalytical Chemistry, 389*(6), 1715–1754.

Mullin, C. A., Frazier, M., Frazier, J. L., Ashcraft, S., Simonds, R., vanEngelsdorp, D., et al. (2010). High levels of miticides and agrochemicals in north american apiaries: implications for honey bee health. *PLoS One, 5*(3), e9754.

Nakajima, T., Tsuruoka, Y., Kanda, M., Hayashi, H., Hashimoto, T., Matsushima, Y., et al. (2015). Determination and surveillance of nine acaricides and one

metabolite in honey by liquid chromatography-tandem mass spectrometry. *Food Additives and Contaminants – Part A Chemistry, Analysis, Control, Exposure and Risk Assessment, 32*(7), 1099–1104.

Niell, S., Cesio, V., Hepperle, J., Doerk, D., Kirsch, L., Kolberg, D., et al. (2014). QuEChERS-based method for the multiresidue analysis of pesticides in beeswax by LC-MS/MS and GC×GC-TOF. *Journal of Agricultural and Food Chemistry, 62*(17), 3675–3683.

Niell, S., Jesús, F., Pérez, C., Mendoza, Y., Díaz, R., Franco, J., et al. (2015). QuEChERS adaptability for the analysis of pesticide residues in beehive products seeking the development of an agroecosystem sustainability monitor. *Journal of Agricultural and Food Chemistry, 63*(18), 4484–4492.

Ozcan, N., & Aycan, O. (2013). Determination of chloramphenicol in honey, milk, and egg by liquid chromatography/mass spectrometry: single-laboratory validation. *Journal of AOAC International, 96*(5), 1158–1163.

Paradis, D., Bérail, G., Bonmatin, J. M., & Belzunces, L. P. (2014). Sensitive analytical methods for 22 relevant insecticides of 3 chemical families in honey by GC-MS/MS and LC-MS/MS. *Analytical and Bioanalytical Chemistry, 406*(2), 621–633.

Pareja, L., Colazzo, M., Pérez-Parada, A., Niell, S., Carrasco-Letelier, L., Besil, N., et al. (2011). Detection of pesticides in active and depopulated beehives in Uruguay. *International Journal of Environmental Research and Public Health, 8*(10), 3844–3858.

Pérez-Parada, A., Colazzo, M., Besil, N., Geis-Asteggiante, L., Rey, F., & Heinzen, H. (2011). Determination of coumaphos, chlorpyrifos and ethion residues in propolis tinctures by matrix solid-phase dispersion and gas chromatography coupled to flame photometric and mass spectrometric detection. *Journal of Chromatography A, 1218*(34), 5852–5857.

Pest Management Regulatory Agency, Health Canada. (2003). *Proposed acceptability for continuing registration*. Re-evaluation of coumaphos. Document PACR2003-04.

Pihlström, T., Blomkvist, G., Friman, P., Pagard, U., & Österdahl, B.-G. (2007). Analysis of pesticide residues in fruit and vegetables with ethyl acetate extraction using gas and liquid chromatography with tandem mass spectrometric detection. *Analytical and Bioanalytical Chemistry, 389*, 1773–1789.

SANCO. (2013). *DG SANCO: Quality control procedures for pesticide residues analysis*. SANCO/12571/2013.

Santana Dos Santos, T. F., Aquino, A., Dórea, H. S., & Navickiene, S. (2008). MSPD procedure for determining buprofezin, tetradifon, vinclozolin, and bifenthrin residues in propolis by gas chromatography-mass spectrometry. *Analytical and Bioanalytical Chemistry, 390*(5), 1425–1430.

Serra-Bonvehí, J., & Orantes-Bermejo, J. (2010). Acaricides and their residues in Spanish commercial beeswax. *Pest Management Science, 66*(11), 1230–1235.

Tomlin, C. D. S. (2011). *The pesticide manual: A world compendium* (15th ed.).

Wallner, K. (1999). Varroacides and their residues in bee products. *Apidologie, 30*(2–3), 235–248.

Wiest, L., Buleté, A., Giroud, B., Fratta, C., Amic, S., Lambert, O., et al. (2011). Multi-residue analysis of 80 environmental contaminants in honeys, honeybees and pollens by one extraction procedure followed by liquid and gas chromatography coupled with mass spectrometric detection. *Journal of Chromatography A, 1218*(34), 5743–5756.

Zhu, W., Schmehl, D. R., Mullin, C. A., & Frazier, J. L. (2014). Four common pesticides, their mixtures and a formulation solvent in the hive environment have high oral toxicity to honey bee larvae. *PLoS One, 9*(1).

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