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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.