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Analytical Methods

Determination of pesticides in coconut (*Cocos nucifera* Linn.) water and pulp using modified QuEChERS and LC–MS/MS

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ABSTRACT

The use of pesticides is directly linked to improvements in productivity and to the preservation of coconut palms. However pesticide analysis is necessary to determine whether pesticide residues in the food products containing coconut are within the maximum residue limits (MRLs), ensuring the quality of these products. This work aimed to develop a method for multiresidue determination of ten pesticides in coconut water and pulp using QuEChERS and LC–MS/MS. The method was effective in terms of selectivity, linearity, matrix effect, accuracy and precision, providing LOD of 3 μg kg^{-1}, LOQ of 10 μg kg^{-1} and recoveries between 70 and 120% with RSD lower than 20%. The developed method was applied to 36 samples in which residues of carbendazim, carbofuran, cyproconazole and thiabendazole were found below the LOQ in coconut water and pulp.

1. Introduction

Millions of people consume food products containing coconut daily, especially coconut water, milk, oil and the flesh of the nut itself (Foale, 2003). Unfortunately the number of diseases and pests in coconuts (*Cocos nucifera* Linn.) is increasing throughout the world. There are several reports of symptons starting from the roots, stem (trunk) and leaves, besides pests and diseases in the fruits, which cause reductions in yield and size as well as malformations of the fruit, representing a big threat to the coconut industry (Ramjegathesh et al., 2012; Ranasinghe, Fernando, Zaneer, & Mubarak, 2003). Much research has been directed toward identifying resistant coconut varieties and biological control agents (Batugal, Benigno, & Oliver, 2005) as well as to the use of pesticides and technologies for their effective applications (Herath & Wijekoon, 2013).

Since the application of pesticides is essential to prevent the loss of production/productivity, it is important to determine the concentrations of pesticide residues in the coconut, to determine if the fruit is fit for human consumption and in accordance with established maximum residue limits (MRLs). In recent years, quantitative and qualitative pesticide analysis methods were developed and reported in the literature. Different strategies that included extraction techniques were employed: single drop microextraction (SDME) (Anjos & Andrade, 2014), liquid-liquid extraction (LLE) (Brito et al., 2002), solid phase extraction (SPE) (Brito et al., 2002; Deme, Azmeera, Kanjilal, Jonnalagadda, & Upadhyayula, 2013; Ogawa et al., 2006; Paranthaman & Kumaravel, 2013), matrix solid phase dispersion (MSPD) (Santos, Ferreira, Souza, & Navickiene, 2012; Silva, Aquino, Dórea, & Navickiene, 2008) and stir bar sorptive extraction (SBSE) (Pfannkoch, Stuff, & Whitecavage, 2012). The analytical techniques include gas chromatography-mass spectrometry (GC–MS) (Anjos & Andrade, 2014; Pfannkoch et al., 2012; Silva et al., 2008), gas chromatography with electron-capture detection (GC-ECD) (Anjos & Andrade, 2014), liquid chromatography with tandem mass spectrometry (LC–MS/MS) (Deme et al., 2013), liquid chromatography with ultraviolet detection (LC-UV) (Brito et al., 2002; Deme et al., 2013; Ogawa et al., 2006; Paranthaman & Kumaravel, 2013), liquid chromatography with photodiode array detection (LC-DAD) (Santos et al., 2012) and gas chromatography with thermionic sensitive detection (GC-TSD) (Brito et al., 2002). Experiments using

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bioassays were also reported for detection of pesticide in coconut (Elliott & Broschat, 2012).

In 2003, Anastassiades and coauthors (Anastassiades, Lehotay, Stajnbaher, & Schenck, 2003) developed an approach to the analysis of pesticide residues and named this method QuEChERS, which stands for Quick, Easy, Cheap, Effective, Rugged and Safe. Since then QuEChERS has undergone several modifications and has become well established for multiresidue analyses of pesticides in food and agricultural samples (Major, 2007). Among other beneficial features, the QuEChERS procedure uses acetonitrile, which permits extraction of polar analytes and has an elevated degree of selectivity and detectability and direct compatibility with both gas and liquid chromatography coupled with mass spectrometry (MS) (Lehotay et al., 2010). The QuEChERS method, when compared with other techniques mentioned above, minimizes the number of sample preparation steps since it only involves two steps, first extraction with acetonitrile and a mixture of salts by partition and then clean-up steps by dispersive solid phase extraction (d-SPE) using a sorbent comprising of primary and secondary amines (PSA). Other advantages of the QuEChERS method compared with other techniques are their excellent recoveries, less time for sample preparation and less solvent consumption (Zhang, Zhang, & Jiao, 2014). A modified QuEChERS method (Ferreira et al., 2015) was developed and applied to coconut tree trunk samples for determination of pesticide residues for evaluation of the acropetal translocation in endotherapic treatments. The results showed good analytical performance overcoming the difficulties of extracting pesticides from the fibers of the tree trunk.

Sample treatment is a crucial step when working with complex food matrixes, with high fat and protein contents, such as coconut water and pulp. Due to their lipid content and because the pesticides have different interactions and physico-chemical properties, as shown by their octanol-water partition coefficients (K_{ow}) and dissociation constants (pKa) at 25 °C, the analysis should be carried out separately for both matrices. The literature has reported an optional freezing out step prior to dispersive-SPE (d-SPE) as part of the clean-up in cereals, flax seeds, peanuts, doughs (Koesukwiwat, Lehotay, Mastovská, Dorweiler, & Leepipatpiboon, 2010), citrus extracts (Andraščíková, Hrouzková, & Cunha, 2013) and palm oil (*Elaeis guineensis*) (Sobhanzadeh, Bakar, Abas, & Nemati, 2012). Freezing induces most interferents in the samples to precipitate to the bottom of the tubes to be separated by simple decanting.

The objective of this work was to develop a method for multiresidue determination of pesticides in coconut water and pulp using a modified QuEChERS method and LC–MS/MS.

2. Materials and methods

2.1. Chemicals, reagents and apparatus

Certified standards of carbendazim, carbofuran, 3-hydroxy-carbofuran (3-OH-carbofuran), carbosulfan, cyproconazole, difenoconazole, spirodiclofen, imidacloprid, thiabendazole, thiamethoxam and thiophanate-methyl were acquired from Dr. Ehrenstorfer (Augsburg, Germany). All standards were of at least 95% purity as shown in Table 1, which also shows the class, chemical group, toxicological class, maximum residue limit (MRL) and chemical structure of each compound.

Anhydrous magnesium sulphate ($MgSO_4$) and anhydrous sodium acetate (NaOAc), both reagent grade, were purchased from Merck (Darmstadt, Germany). Bondesil C18 sorbent (particles of 40 μm) and primary secondary amine (PSA) were obtained from Agilent Technologies (Wilmington, USA). The solvents acetonitrile and methanol were from Mallinckrodt (Phillipsburg, USA) and glacial acetic acid was from J.T. Baker (Phillipsburg, USA). Ultrapure water was obtained from a Direct UV3® gradient system from Millipore (Molsheim, USA).

For the development of this work a PT 3100 Polytron Ultra Turrax (Luzern, Switzerland), a IKA® A11 basic analytical mill (Staufen, Germany), a QL-901 vortex and a NT 85 centrifuge mixer, all from Nova Técnica (São Paulo, Brazil) were used. A Sartorius CP-225 balance (Göttingen, Germany), a PT3100 Rotofix 46 centrifuge (Hettich, Germany) and polypropylene centrifuge tubes (15 and 50 mL) from Sarstedt (Nümbrecht, Germany) were also used.

2.2. Pesticide standard solutions

Stock standard solutions of individual compounds at the concentration of 1000 mg L^{-1} were prepared by exact weighing of the powder that was then dissolved in methanol or acetonitrile. A working standard mixture at the concentration of 10 mg L^{-1} was prepared in acetonitrile by appropriate dilutions of the stock solutions. All solutions were stored at −18 °C in the dark.

2.3. LC–MS/MS analysis

An Acquity UPLC™ system (Milford, USA) equipped with XEVO-TQ tandem quadrupole mass spectrometer from Waters (Manchester, UK) having an electrospray ionization interface (ESI) was used for the determination of the studied pesticides. The separations were achieved using an Acquity UPLC BEH C18 column (100 mm, 2.1 mm, 1.7 μm particle size) from Waters. The injection volume was 10 μL. The analytes were separated with a mobile phase consisting of eluent A: water: methanol (98:2, v/v) and eluent B: methanol, both with 0.1% formic acid and 5 mmol L^{-1} ammonium formate. A linear gradient program was used, with eluent B as follow: 5% at 0 min, 100% at 8.50 min, 5% at 8.51 min until 10.00 min. The flow rate was 0.225 mL min^{-1}.

The mass spectrometry detector was operated using the electrospray (ESI) source in the positive mode. ESI parameters were: capillary voltage 2.5 kV, source temperature 150 °C, desolvation temperature 500 °C, and nitrogen flow rates of 600 and 80 L h^{-1} for the cone and desolvation gases, respectively. Collision-induced dissociation was performed using argon as the collision gas at a pressure of 4×10^{-3} mbar with a flow rate of 0.15 mL min^{-1}. Optimization of the collision energy for each individual pesticide was done by direct-infusion into the MS using a Harvard syringe pump (Kent, UK). Data acquisition was performed using Mass Lynx 4.1 (Micromass, Manchester, UK) software.

2.4. Samples

The cultivar selected to validate the method was "green dwarf coconut", certified by the Brazilian Enterprise for Agricultural Research (EMBRAPA), without pesticides (blank sample), planted in the experimental field station at Itaporanga dÁjuda, Sergipe, Brazil. All the samples of coconut water and coconut pulp had between 8 and 10 months of maturity and were stored in a freezer at −17 °C until needed.

The samples were acquired from three different regions of Brazil. From the midwest region, in Goianésia-Goiás, the samples were purchased directly from the grower. From the northeast region, the samples were obtained from a farm located at Neópolis-Sergipe. The samples obtained from the Southeast region were purchased from a local store in Campinas, SP.

2.5. Sample preparation

The procedure used was the modified acetate QuEChERS method. Ten g (or mL) of sample were added to 10 mL of 1% acetic acid in acetonitrile, followed by vortexing for 1 min. Partition