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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

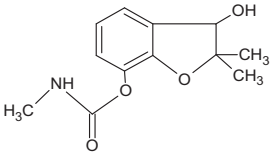
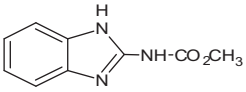
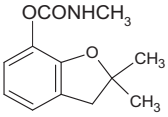
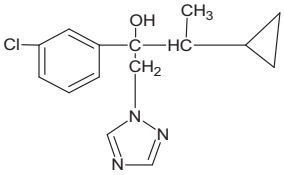
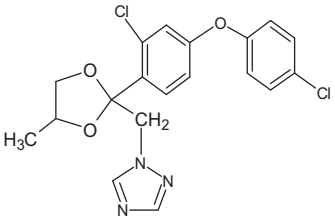
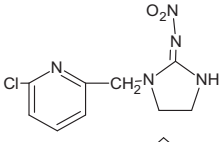
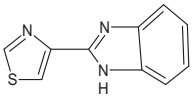
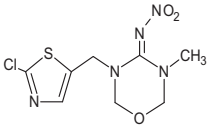
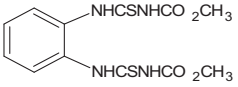
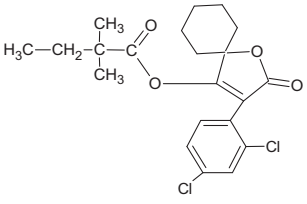
The cultivar selected to validate the method was "green dwarf coconut", certified by the Brazilian Enterprise for Agricultural Research (EMBRAPA), without pesticides (blank sample), planted in the experimental field station at Itaporanga dÁjuda, Sergipe, Brazil. All the samples of coconut water and coconut 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

Table 1
Description of the pesticides (IUPAC, 2015).

Pesticides	Class	Chemical group	Toxicological class	MRL* mg kg^{-1}	Chemical structure
3-OH-carbofuran	–	–	–	Expressed as carbofuran	
Carbendazim	Fungicide	Benzimidazole	Unlikely to present acute hazard in normal use	0.1	
Carbofuran	Insecticide, nematicide, acaricide	Carbamate	Highly hazardous	0.02	
Cyproconazole	Fungicide	Triazole	Moderately hazardous	0.05	
Difenoconazole	Fungicide	Triazole	Moderately hazardous	0.05	
Imidacloprid	Insecticide	Neonicotinoid	Moderately hazardous	0.05	
Thiabendazole	Fungicide	Benzimidazole	Slightly hazardous	0.1	
Thiamethoxam	Insecticide	Neonicotinoid	No information	0.05	
Thiophanate-methyl	Fungicide	Benzimidazole	Unlikely to present acute hazard in normal use	0.2	
Spirodiclofen	Acaricide, insecticide	Tetronic acid	No information	0.05	

* MRL – Maximum Residue Levels according to European Commission.

occurred using 4 g of $MgSO_4$ and 1.7 g of anhydrous sodium acetate, vortexing for 1 min and centrifuging at 3400 rpm for 8 min at 20 °C. An aliquot of 8 mL of the supernatant was transferred to a polypropylene tube then cooled in dry ice for 5 min. Then, the clean-up step was performed by removing 4 mL of the supernatant and adding 600 mg of $MgSO_4$, 500 mg of C18 and 100 mg PSA, followed by vortexing for 1 min and centrifugation at 3400 rpm for 8 min. The extracts were filtered with nylon filters (0.22 μm), diluted 5 times in ultra-pure water and submitted for analysis by LC–MS/MS. The injection volume was set at 10 μL.

2.6. Validation

Matrix effect (ME), linearity, selectivity, accuracy, inter-day and intra-day precision and limits of detection (LOD) and quantification (LOQ) were used to validate the procedure, according to SANCO No.12571/2013 (2013). The matrix effect was evaluated by the slope of the curves prepared in solvent (acetonitrile) and a matrix-matched blank extract. The signal of the pesticide in matrix at 20 μg kg^{-1} was compared to that in solvent at the corresponding concentration. ME (%) was calculated via the following equation (Stahnke, Kittlaus, Kempe, & Alder, 2012):

$$\mathrm{ME}\ (\%) = \left[\left(\frac{\text{peak area (fortified extract)}}{\text{peak area (solvent standard)}}\right) - 1\right] \times 100\%$$

The extraction/separation of matrix components extracted together with the pesticides during the QuEChERS method can be decreased using the appropriate dilution of sample extracts after the clean-up step and before injection in the LC–MS/MS. The extract was diluted in water 1:4 (v/v), then the dilution factor was 5-fold. This simple strategy offers the advantage of reduction of matrix concentration and reduces/eliminates the ME.

The linearity of the method was ensured using matrix-matched calibration, spiking blank extracts at seven concentration levels from 2.5 to 250.0 μg kg^{-1} (μg mL^{-1}). The selectivity of the compounds was evaluated by comparing injections of blank matrix extract and spiked blank extract. The LOQ of the method was set as the minimum concentration that can be quantified with acceptable accuracy and precision using the SANCO procedure. The accuracy was evaluated by recovery studies carried out at three concentration levels, 10, 20 and 50 μg kg^{-1} (μg L^{-1}), by spiking seven blank samples at each level. They were quantified using matrix-matched calibration. The intra-day precision (repeatability) was performed at the same concentration levels as the recovery studies. The intermediate precision (inter-day precision) was studied through spiking blank samples at 20 μg kg^{-1} (μg L^{-1}) on different days.

3. Results

The ten pesticides evaluated in this work (carbendazim, carbofuran, 3-OH-carbofuran, cyproconazole, difenoconazole, spirodiclofen, imidacloprid, thiabendazole, thiamethoxam and thiophanate-methyl) were selected because of their widespread use for coconut production in recent years in the northeast of Brazil. From these pesticides, the only ones authorized for coconut crop in Brazil are difenoconazole, thiabendazole and spirodiclofen. Because of possible illegal use, other pesticides were selected as analytes for monitoring in this work. The carbofuran (bannedin most countries) and 3-OH-carbofuran are degradation products of carbosulfan. However, carbosulfan is authorized and widely used in the coconut palms for foliar treatment (AGROFIT, 2016; Fontes, Ferreira, & Siqueira, 2002).

For the analysis of pesticides present in complex matrixes, the most common modes of ionization in LC–MS/MS include electrospray ionization with mass analyzers such as a triple-quadruple. In this work, a tandem quadrupole mass spectrometer was operated in selected reaction monitoring mode (SRM) with monitoring of two precursor/products ion transitions for each analyte. The target ion transition with highest intensity (primary ion transition) was used for quantitation, whereas the second target ion transition was used for confirmation, these ions being monitored under time-scheduled SRM conditions. The transitions and their optimization, as well as retention time and the molar masses for each compound, are shown in Table 2. The LC conditions were selected after optimization of MS parameters. The incorporation of formic acid and ammonium formate facilitated ionization of the analytes. Fig. 1 shows a chromatogram obtained from the selected ions in the SRM mode from solutions of analytes prepared in acetonitrile.

Coconut water and pulp are rich in sugars, amino acids, vitamins, organic acids, minerals, phytohormones and others substances (Priya & Ramaswamy, 2014). The fruit can be divided in two part: (a) one with more fatty content (coconut pulp), from which the oil can be extracted, (b) an other, more aqueous portion (coconut water). The pesticides may have affinities/interaction with both parts and these affinities or interactions of pesticides with the matrices depend on their physical chemical properties, such as acid dissociation constant (pKa), and n-octanol/water partition coefficient (K_{ow}) (Rachel, Jean-Louis, Alexia, Jean, & Ernest, 2010; Pfannkoch et al., 2012). In this paper, the two matrices, coconut water and pulp, were carefully separated before extraction. The determination of 10 pesticides in coconut water and pulp involved, initially, an extraction process with both the original and the acetate-buffered version of QuEChERS. These methods were based on extraction with acidified acetonitrile, followed by an induced liquid–liquid partition after the addition of salts. For the original QuEChERS, the salts were $MgSO_4$ and sodium chloride, while for the modified version $MgSO_4$ and sodium acetate were used. For the cleanup step, two sorbents, C18 and PSA were used. PSA is used for removing interferences such as sugar, fatty acids, organic acids, and anthocyanine pigments and C_{18} is used in order to remove lipids and nonpolar interferences (Pfannkoch et al., 2012; Jackson, Gordon, Wizzard, McCook, & Rolle, 2004; Chidambaram, Singaraja, Prasanna, Ganesan, & Sundararajan, 2013). For coconut pulp, before the clean-up step, matrix processing was performed with dry ice in order to prevent oxidation of the sample (Lee et al., 2011). Modified acetate QuEChERS was chosen

Table 2
Cone voltage, collision energy, precursor and product ions employed for determination of the analytes of interest.

Pesticides	t_R (min)	Molar mass (g mol^{-1})	Quantification transition (m/z)[a]	Voltage cone (V)	Confirmation transition (m/z)[a]
3-OH-carbofuran	3.94	237.2	238.0 > 163.0 (16)	25	238.0 > 181.0 (10)
Carbendazim	3.11	191.2	192.1 > 132.1(28)	24	192.1 > 160.1(18)
Carbofuran	5.14	221.3	222.1 > 123.0 (16)	25	222.1 > 165.1 (16)
Carbosulfan	8.33	380.5	381.3 > 118.0 (22)	31	381.3 > 76.0 (34)
Cyproconazole[b]	6.49	291.8	292.2 > 70.2 (18)	27	292.2 > 125.1 (24)
	6.64				
Difenoconazole	7.31	406.3	406.0 > 111.1 (60)	37	406.0 > 251.1 (25)
Imidacloprid	3.62	255.7	256.1 > 175.1 (20)	23	256.1 > 209.1 (15)
Thiabendazole	3.45	201.2	202.0 > 175.2 (25)	42	202.0 > 209.1 (30)
Thiamethoxam	3.14	291.7	292.2 > 132.0 (22)	19	292.2 > 211.2 (12)
Thiophanate-methyl	5.04	342.4	343.2 > 151.0 (21)	23	343.2 > 311.1 (11)
Spirodiclofen	7.98	411.2	411.2 > 71.2 (13)	22	411.2 > 313.0 (13)

[a] Collision energy (eV) is given in parentheses.
[b] Cyproconazole – Isomers.

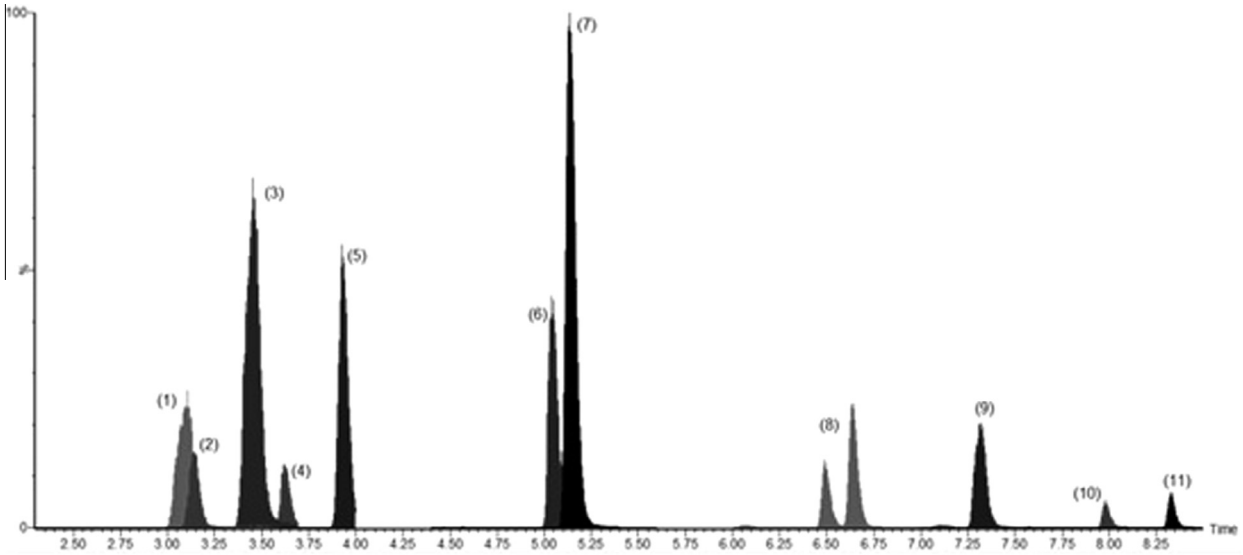


Fig. 1. The selected ion chromatogram obtained by LC–MS/MS prepared at final concentration of 20 μg L^{-1} in solvent. Identification of compounds: (1) carbendazim, (2) thiamethoxam, (3) thiabendazole, (4) imidacloprid, (5) 3-OH-carbofuran, (6) thiophanate-methyl, (7) carbofuran, (8) cyproconazole, (9) difenoconazole, (10) spirodiclofen, (11) carbosulfan.

in relation to the original because it reduced the amount of co-extractives in tests carried out by: (a) gravimetry, consisting in the evaporation of the extract and weighing the vials; and (b) recovery of the analytes by an analytical procedure.

3.1. Method validation

A validation protocol of the optimized procedure was carried out in order to establish the performance characteristics of the method. The methodology was validated in terms of analytical performance such as cleanliness of the extracts, efficiency of the extraction (recoveries) at different concentration levels, matrix effects, linearity, limits of detection and limits of quantification. The results of validation were investigated for each individual compound. The same methodology, under the same conditions, was separately developed and validated for the two matrices. The method was validated according to SANCO, 2013.

Studies that focus on method validation of QuEChERS and LC–MS/MS depend on the stability of the chromatographic system for quantitative analysis. The proposed method demonstrated its suitability for the detection and quantification of benzimidazoles, tetronic acid, neonicotinoids, triazoles and carbamate applied to coconut.

The matrix effect can be classified basically in three categories: (a) ±0–20% light effect; (b) ±20–50% medium effect and (c) above ±50% strong effect (Ferrer, Lozano, Agüera, Girón, & Fernández-Alba, 2011). The ME was evaluated based on comparison of the slope of the analytical curve prepared in matrix and in pure solvent, through signal enhancement, having values above those of the curve, or signal suppression, in the case of negative values, below those of the analytical curves. To avoid problems resulting from matrix components, the approach chosen to minimize ME was to dilute the final extract five times with ultrapure water before injection into the LC-ESI-MS/MS. Considering the

Table 3
Results of the analyses carried out to evaluate the matrix effect, accuracy and precision.

Compounds		Spiked level (μg kg^{-1}); R (%) ± RSD (%)				Matrix effect (%)	r^2	Linear regression
	Matrix[a]	10	20	50	20[b]			
3-OH-carbofuran	W	98 ± 3	103 ± 3	104 ± 2	100 ± 4	+4	0.9990	35116.5x + 871.44
	P	94 ± 11	98 ± 4	101 ± 2	108 ± 4	+2	0.9961	17116.3x + 2712.63
Carbendazim	W	**59 ± 3**	**62 ± 3**	**66 ± 2**	**65 ± 4**	+6	**0.9990**	321.834x – 231.379
	P	84 ± 8	87 ± 2	90 ± 3	86 ± 2	–13	0.9992	21048.1x + 1994.83
Carbofuran	W	115 ± 3	120 ± 3	120 ± 2	117 ± 4	+3	1.0000	55492.6x – 2015.05
	P	78 ± 10	82 ± 4	85 ± 3	90 ± 4	+32	0.9950	45516.5x + 5539.65
Cyproconazole	W	92 ± 3	96 ± 2	97 ± 1	94 ± 4	–1	1.000	22566.9x – 247.524
	P	80 ± 11	84 ± 7	89 ± 5	91 ± 2	–5	0.9983	13084.4x + 949.544
Difenoconazole	W	91 ± 2	89 ± 4	94 ± 4	91 ± 7	+3	0.9960	9631.95x – 722.551
	P	74 ± 14	75 ± 11	84 ± 9	87 ± 7	+18	0.9986	12211.2x + 643.526
Imidacloprid	W	97 ± 6	95 ± 2	99 ± 3	95 ± 4	+3	0.9990	7221.52x – 533.905
	P	87 ± 15	90 ± 6	93 ± 6	97 ± 5	0	0.9990	3563.9x + 16.0023
Thiabendazole	W	94 ± 3	97 ± 2	100 ± 3	95 ± 4	+5	0.9990	47039.6x – 2760.32
	P	84 ± 7	88 ± 4	92 ± 4	96 ± 1	–7	0.9984	33817.1x + 6026.21
Thiamethoxam	W	100 ± 4	104 ± 4	107 ± 3	105 ± 4	+6	0.9980	9838.83x + 463.367
	P	96 ± 7	94 ± 6	104 ± 1	115 ± 3	–11	0.9972	5699.25x + 817.233
Thiophanate-methyl	W	**172 ± 50**	**179 ± 4**	**176 ± 4**	**182 ± 5**	–	0.9971	–
	P	83 ± 15	83 ± 9	93 ± 7	88 ± 8	+7		401.155x – 78.9318
Spirodiclofen	W	91 ± 16	93 ± 4	93 ± 3	90 ± 8	+35	0.9960	1822.96x – 151.09
	P	–	–	–	–	–	–	–

[a] W = coconut water, P = coconut pulp.
[b] i.p. = intermediate precision.

results of ME, in order to avoid matrix effects as much as possible, the quantification was made by matrix-matched calibration curves.

Table 3 summarizes the results of ME and the results of validation performed for all targets as: (a) linearity estimated by spiking the samples with standards. The analytical curve was used to calculate the correlation coefficient (r^2), linear regression equation with slope and intercept values; (b) accuracy demonstrated by recovery; and (c) precision assessed by repeatability of responses after replicate injections (n = 3) and by intraday precision, both expressed as relative standard deviation (RSD, %).

Linearity was evaluated in the range of 2.5–250 μg L^{-1} (or μg kg^{-1}) by determination of the correlation coefficient and linear regression equations. All compounds presented good linearity, with $r^2 \geqslant 0.99$, as shown in Table 3. The selectivity/specificity of the method was ensured, since interfering peaks were not observed at the retention times of the studied compounds. The LOQ was 10 μg kg^{-1} and LOD was 3 μg kg^{-1} for all the compounds. The results of accuracy and precision are presented in Table 3. These results indicate that good recoveries were achieved from coconut water and pulp samples using the proposed method. The recovery values were satisfactory, in the range of 70–120% for all pesticides, except for thiophanate-methyl and its metabolite carbendazim for coconut water, and spirodiclofen for coconut pulp. Graphics of the recoveries can be seen in Fig. 2. The intra-day and inter-day precisions were evaluated in terms of relative standard deviations (RSD, %) and both presented values lower than 20%.

The determination of these pesticide residues showed significant differences in the behaviour of thiophanate-methyl, carbendazim and spirodiclofen in each matrix. This can occur because the extraction efficiency depends on several physicochemical characteristics, such as viscosity, and different concentrations of the electrolytes of pesticides as well as the pH of the matrices

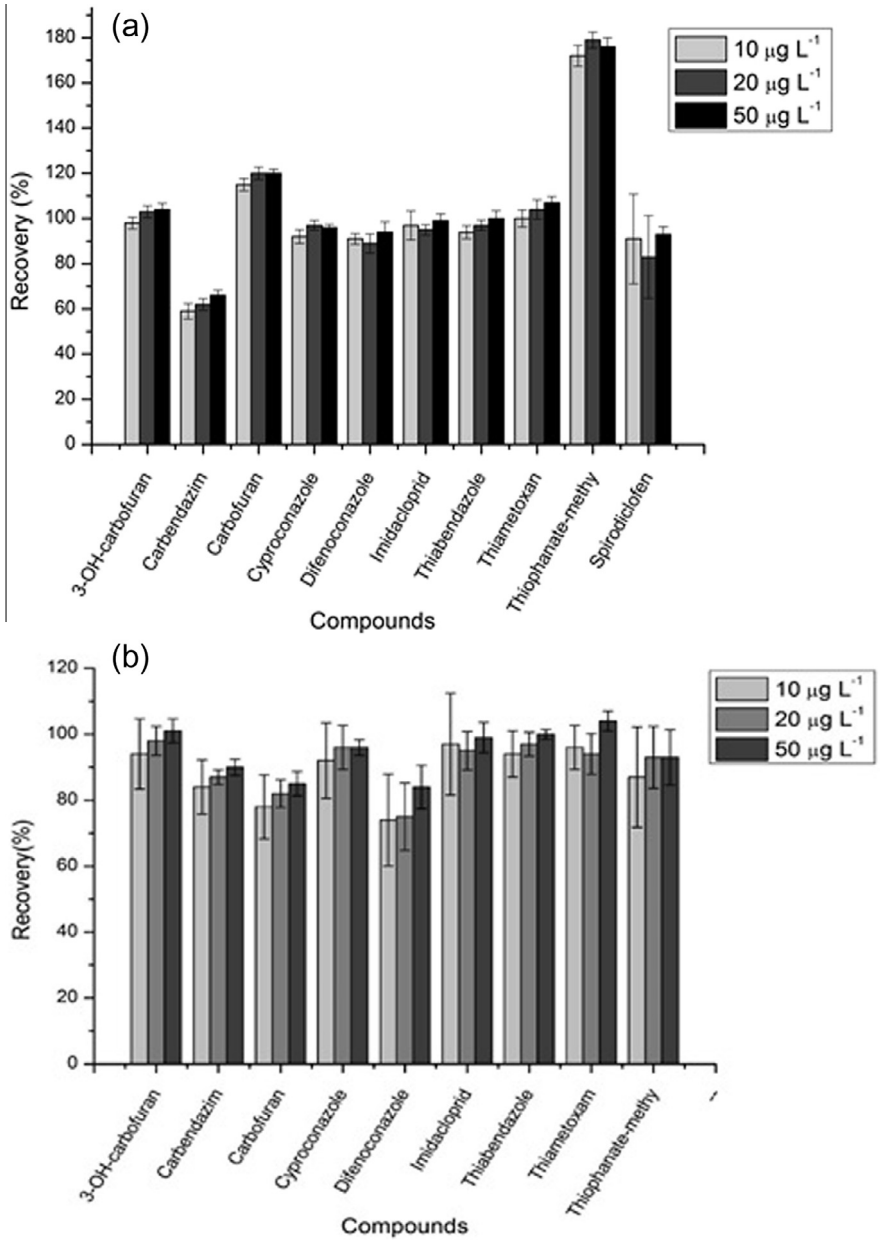


Fig. 2. Graphics of the recovery for the extraction of selected pesticides spiked at 10.0; 20.0 and 50 μg mL^{-1} in (a) coconut water and (b) coconut pulp matrices (n = 3). Error bars signify standard deviation.

and pKa of the pesticides. The recovery of thiophanate-methyl was above 120%, carbendazim had a recovery lower than 70% at the spiking levels of 10.0; 20.0 and 50.0 μg kg^{-1} in coconut water but had RSD < 4%. In the case of spirodiclofen it was not possible to extract it from the pulp. Both thiophanate-methyl and its metabolite carbendazim have different physicochemical properties. As an example, the solubilities of carbendazim and thiophanate-methyl in water are 8 mg L^{-1} and 20 mg L^{-1}, respectively. The pKa values for thiophanate-methyl is around 7.3 and carbendazim is 4.2. Both pesticides are considered as not significantly ionized in solution. The pH of the acetate buffer formed during QuEChERS could also influence the stability and ionization of analytes, besides the effects of the kind of matrix, because in coconut water, thiophanate-methyl and carbendazim had results of recoveries of the expected values of percentage, i.e., recoveries between 70 and 120% for these two pesticides, which did not occur in the pulp (Oshita & Jardim, 2015; IUPAC – Footprint Pesticides Properties Databases, 2015).

Although some studies suggest the solubility for spirodiclofen (parent) of log K_{ow} 5.83 in the fat matrix (Liu et al., 2014; FAO, 2015), the analyte was not recovered from coconut pulp. On the other hand, this analyte showed good results for coconut water, as shown in Fig. 2. According to Lehotay, Mastovská, & Yun 2005, the QuEChERS method is not likely to be applicable for extraction of lipophilic pesticides (log K_{ow} > 3.0) from high fat content samples. This can justify the difficulty to extract spirodiclofen from pulp that is highly fatty and the good recoveries obtained in coconut water, in which the fat content is lower.

Carbosulfan, with log K_{ow} 7.42 (IUPAC, 2015), was studied in these matrices because it is widely used on this crop by foliar application. However, when it was spiked into coconut water and pulp, this analyte was completely degraded to its metabolites, such as carbofuran and 3-hydroxy-carbofuran.

3.2. Method application to samples

The coconut production has great economic importance in Brazil. This palm is demanding in weather conditions such as temperature, atmospheric humidity, winds that stimulate transpiration and well-drained soil. When this does not occur, it results in plant disequilibrium in development, growth, physiology, morphology and disease control. A large portion of coconut palms produced in Brazil face high disease and insect pest pressures, which are commonly countered by pesticide application (Fontes, Ferreira, & Siqueira, 2002). The proposed method was applied to samples collected from different regions of Brazil. In order to demonstrate the applicability of the validated analytical method for the purpose of routine pesticide residue analysis in coconut

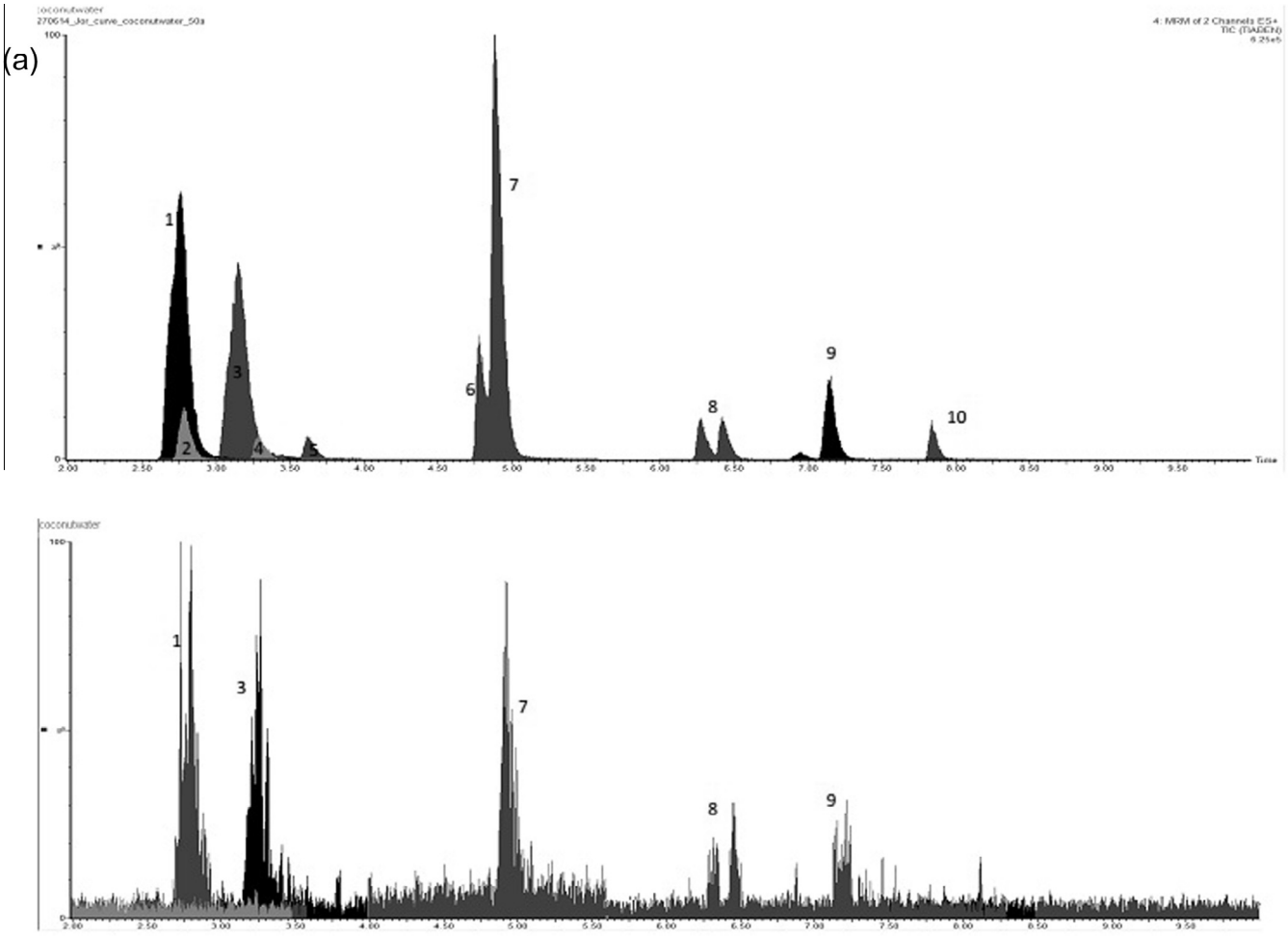


Fig. 3. (A) LC–MS/MS chromatogram for the extract of coconut water spiked at 50 μg kg^{-1} and below, LC–MS/MS chromatogram for the extract of coconut water of real samples from Neópolis, SE. Identification of compounds: (1) carbendazim, (2) thiamethoxam, (3) thiabendazole, (4) imidacloprid, (5) 3-OH-carbofuran, (6) thiophanate-methyl, (7) carbofuran, (8) cyproconazole, (9) difenoconazole, (10) spirodiclofen. (B) LC–MS/MS chromatogram for the extract of coconut pulp spiked at 50 μg kg^{-1} and below, LC–MS/MS chromatogram for the extract of coconut pulp of real samples from Neópolis, SE. Identification of compounds: (1) carbendazim, (2) thiamethoxam, (3) thiabendazole, (4) imidacloprid, (5) 3-OH-carbofuran, (6) thiophanate-methyl, (7) carbofuran, (8) cyproconazole, (9) difenoconazole, (10) spirodiclofen.

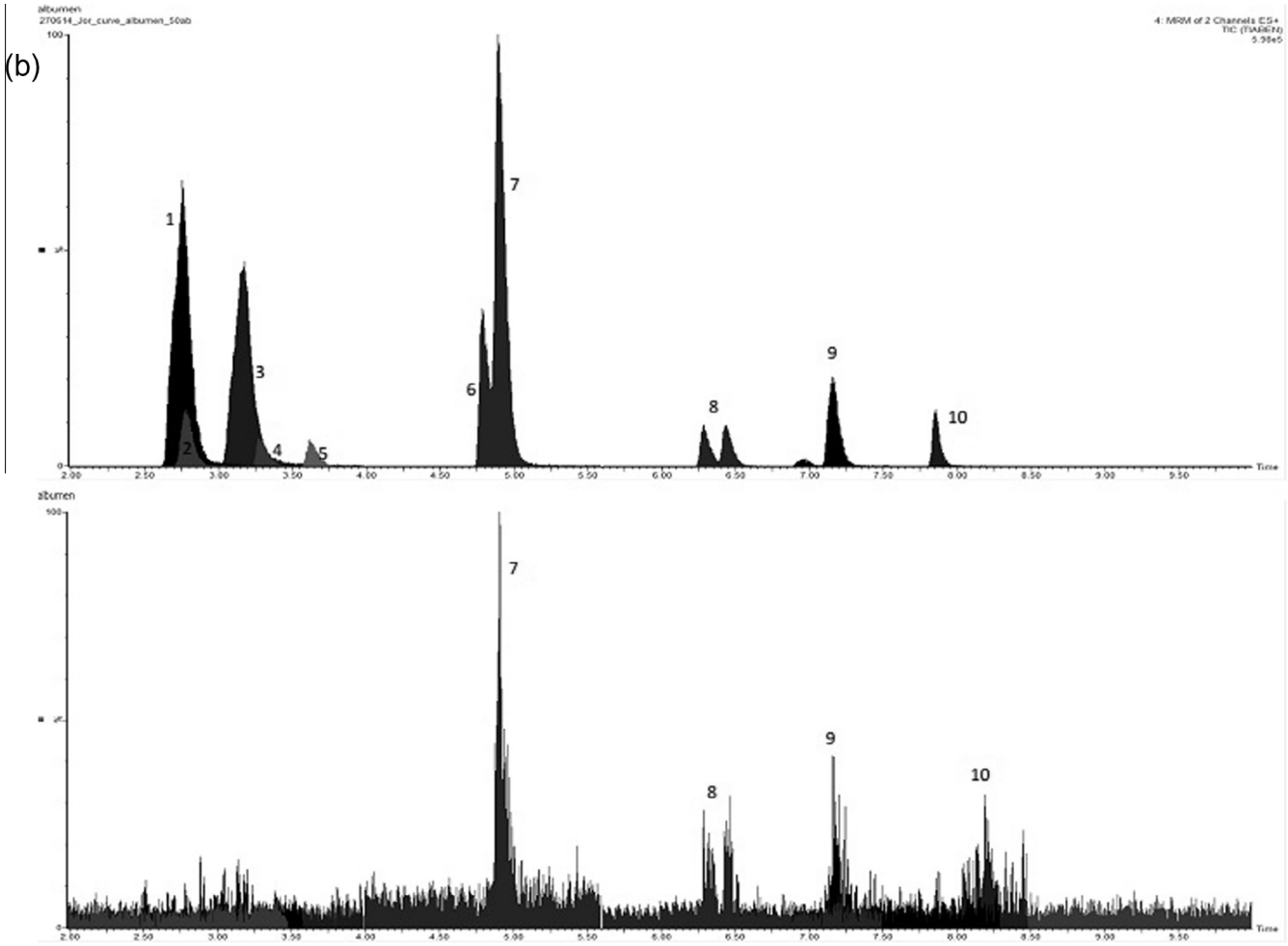


Fig. 3 (*continued*)

crops, 36 samples were monitored, being 18 samples of coconut water and 18 samples of pulp from different regions of Brazil, to check whether there was contamination of fruits with pesticides assessed in this study and if the fruits are suitable for consumption. Fig. 3 shows the chromatogram for extracts of coconut water and pulp spiked with the standard pesticides at 50 μg mL^{-1} and the samples from Neópolis, SE.

The results obtained show that all samples (both matrices) were contaminated with carbofuran. Coconut water samples from Goianésia-GO and Campinas-SP showed only difenoconazole. Most of the samples analyzed from Neópolis-SE had some pesticides detected, such as: carbendazim, thiabendazole, carbofuran, cyproconazole and difenoconazole. However, all samples showed levels below the limit of quantification (10 μg kg^{-1}) and maximum residue limits established by EU regulations (European Commission, 2015), ensuring the quality of these fruits.

4. Conclusions

The modified QuEChERS method combined with freeze out using dry ice before clean-up step represents a new approach for overcoming co-extractives (oils, waxes and sugar) in the extraction of pesticides in coconut water and pulp prior to LC-MS/MS analysis. The present method was successfully validated and its applicability proven for two matrices of the same fruit. It can be concluded, that this method can be used for the routine analysis of samples with satisfactory results, since it offers many advantages, such as, simplicity, ease of operation, relatively short analysis time, besides allowing inclusion of new pesticides, without need to modify the method as an "method add-on". The obtained pesticide residue values in both matrices in samples obtained from three Brazilian regions had MRLs values below those defined by the EU. This study showed the importance of doing the analyses in both matrices separately, considering the different interactions between the physicochemical characteristics of the matrices and the pesticides.

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