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Original Research Article

Multiresidue method for determination of pesticides in coconut (*Cocos nucifera* Linn.) endosperm by using GC–MS/MS and UHPLC–MS/MS analysis

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ABSTRACT

Several pesticides are used in the cultivation of coconut palm, hence evaluation of the residues from these substances must be analyzed in the fruit, to provide and ensure the food safety. Therefore, the development and validation of comprehensive and effective methods becomes crucial, given the complexity of the matrix. Thus, a novel method was developed for the determination of 10 different pesticides residues in coconut endosperm. QuEChERS showed to be an efficient method for sample preparation to overcome the complexity of the matrix. To extend the analytical scope, including a wide range of polarities, ultrahigh-performance liquid chromatography coupled mass spectrometry (UHPLC-MS/MS) and gas chromatography coupled mass spectrometry (GC–MS/MS) were employed. Triple quadrupole spectrometer was used in selected reaction monitoring (SRM) on both detectors, providing high sensitivity and selectivity. These approaches showed to be optimal, considering the parameters of linearity ($r \geq 0.99$), matrix effect (0–50 %), LOQ (5 μg kg^{-1}, except for thiamethoxam (20 μg kg^{-1})), specificity, trueness (60–118 %) and precision (repeatability and within-laboratory reproducibility) $\leq$ 20 %. The concentration levels obtained were higher than the MRL recommended for thiamethoxam (EU regulation <10 μg kg^{-1}), justifying the analysis of such residues in commercial coconut endosperm.

1. Introduction

In the recent years, there has been an increasing interest in the commercial use of coconut fruit (*Cocos nucifera* Linn.) in food (Ng et al., 2014), biodiesel (Qiu et al., 2016), pharmaceutical beauty and personal care industries (Ivić et al., 2017; Cortese et al., 2015), considering its flavor, nutritional values and low glycemic index (Cappelletti et al., 2015). The coconut endosperm is composed of liquid (coconut water) and solid albumen (coconut pulp, which emerges as the fruit matures). Several phytonutrients in the endosperm have been discovered, increasing the notoriety of the fruit (Krishnamurthy, 2015). Coconut water begins to accumulate in the fruit 30 days after fertilization, reaching its maximum volume after 180 days, when the pulp begins to thicken (Gomes and Prado, 2007). However, the practice of monoculture leads to a nutritional imbalance of the plant and/or soil, resulting in the appearance of pests and disease development (Altieri et al., 2012).

This has been causing serious damage to producers with reduced production/productivity, either by premature fall or bad formation of fruits. In this way, pests and/or diseases need to be identified so that adequate control and management measures are taken. One of the alternatives of pest and disease control and management is to use pesticides, either by traditional methods such as spraying (Lamichhane et al., 2016), or by alternative methods such as vegetal endotherapy (Montecchio, 2013).

Therefore, it is essential to assess whether the residue concentration in food is within the maximum residue limit (MRL), established by the regulatory agencies. In analyses of pesticides residues in coconut fruit, it is common to separate the endosperms by dividing the fruit into two matrices, coconut water and pulp. Therefore, there are more analytical methods reported for coconut water than for pulp. For coconut water the extraction methods commonly described are single drop microextraction (SDME) (Anjos and Andrade, 2014), liquid-liquid extraction (LLE) (Brito et al., 2002), solid phase extraction (SPE) (Brito et al., 2002; Deme et al., 2013; Ogawa et al., 2006; Paranthaman and Kumaravel, 2013), matrix solid phase dispersion (MSPD) (Santos et al., 2012), and Quick, Easy, Cheap, Effective, Rugged and Safe (QuEChERS) acetate modified (Ferreira et al., 2016). However, for coconut pulp only matrix

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solid phase dispersion (MSPD) is described (Silva et al., 2008). Following extraction, the samples are usually analyzed by gas chromatography coupled to mass spectrometry (GC–MS) (Anjos and Andrade, 2014; Silva et al., 2008), liquid chromatography coupled to mass spectrometry (LC–MS) (Deme et al., 2013) and ultrahigh-performance liquid chromatography coupled mass spectrometry (UHPLC–MS/MS) (Ferreira et al., 2016). In addition to mass spectrometry, other detectors can be used such as gas chromatography with electron-capture (GC-ECD), gas chromatography with thermionic sensitive detection (GC-TSD) (Brito et al., 2002), high performance liquid chromatography with ultraviolet detection (HPLC-UV) (Brito et al., 2002), high performance liquid chromatography with photodiode array detection (HPLC-DAD) (Santos et al., 2012). Among all these detectors, mass spectrometer in tandem has been preferred because they are more sensitive and selective as well as being meet the criteria recommended by international guidelines.

To facilitate the analysis of fungicides and insecticides residues in coconut endosperm a method combining coconut water and pulp in a single matrix was developed. Both GC–MS/MS and UHPLC-MS/MS techniques were employed due to the wide range of polarity, volatility, and thermal stability, of the pesticides residues. To the best of our knowledge, this is the first time that such an approach for this mixed matrix (endosperm) is described.

2. Materials and methods

2.1. Chemicals, reagents and apparatus

Certified standards (all > 98% purity) of carbofuran, difenoconazole, spirodiclofen, thiabendazole, thiamethoxam, pyraclostrobin, pyridaben, fluxapyroxad, fipronil, buprofezin and deltamethrin were acquired from Sigma Aldrich (Darmstadt, Germany). The molecular structures of these compounds are shown in Fig. 1 (PubChem, 2019).

C_{18} silica gel spherical sorbent (particles of 40−75 μm) from Sulpeco (Bellefont, USA) and primary secondary amine (PSA) from Agilent Technologies (Wilmington, USA). Magnesium sulfate anhydrous (≥ 99.5 %) and sodium acetate (≥ 99) from Sigma-Aldrich (St. Louis, USA). The solvents acetonitrile and methanol were purchased from Mallinckrodt (Phillipsburg, USA), glacial acetic acid was from J.T. Baker (Phillipsburg, USA), ethyl acetate and hexane from Scharlau (Barcelona, Spain). Ultrapure water was obtained from a Direct UV3® gradient system from Millipore (Molsheim, USA).

Shimadzu AY220 scale (Kyoto, Japan), Robot Coupe 3.7 L Blixer® 3 (Montceau-les-Mines, France) were used weigh and grind the coconut endosperm, respectively. To vortex the samples a Multi Reax from Heidolph (Schwabach, Germany), a Heraeus multifuge 3L-R centrifuge from Thermo Scientific (Langenselbold, Germany), an ultra-low temperature freezer VIP Plus MDF-C8V1 from Sanyo (Moriguchi City, Japan) were used. Polypropylene centrifuge tubes (50 mL) from Corning (Tewksbury, United States) and pipettes of different volumes from Transferpette® (Wertheim, Germany) were also used.

2.2. Pesticide standard solutions

Stock standard solutions of individual compounds (1000 mg L^{-1}) and working standard mixture (10 mg L^{-1}), followed by appropriate dilutions in acetonitrile for UHPLC-MS/MS and in ethyl acetate for GC–MS/MS were prepared. All solutions were stored at -18 °C protected from light until analysis.

2.3. Sample

For method development and validation, fruits from bunches 19–21, suitable for consumption were obtained from plants without use of pesticides. Coconut water and pulp were mixed using the processor until complete homogenization, and stored at −4 °C until processing. Ten fruits and one sample of coconut water were purchased from a grocery store in Jaguariúna, São Paulo, Brazil. After homogenization, each fruit were analyzed separately.

2.4. Optimization of sample preparation

Based on the number of pesticides and matrix composition, the samples were prepared following the steps: (1) coconut water and pulp were mixed in a blender until complete homogenization; (2) for extraction, 10 g of the mixture was vortexed for 2 min in 10 mL of 1% acetic acid in acetonitrile; (3) for partition, 7 mL of supernatant was vortexed using 1.7 g of anhydrous sodium acetate and 4 g $MgSO_4$ for 2 min, followed by centrifugation at 6500 rpm for 10 min, 10 °C; (4) prior the clean-up, the samples were placed in ultra-freezer at −80 °C for 20 min, followed by 2 min centrifugation at 6500 rpm; (5) for clean-up, a 4

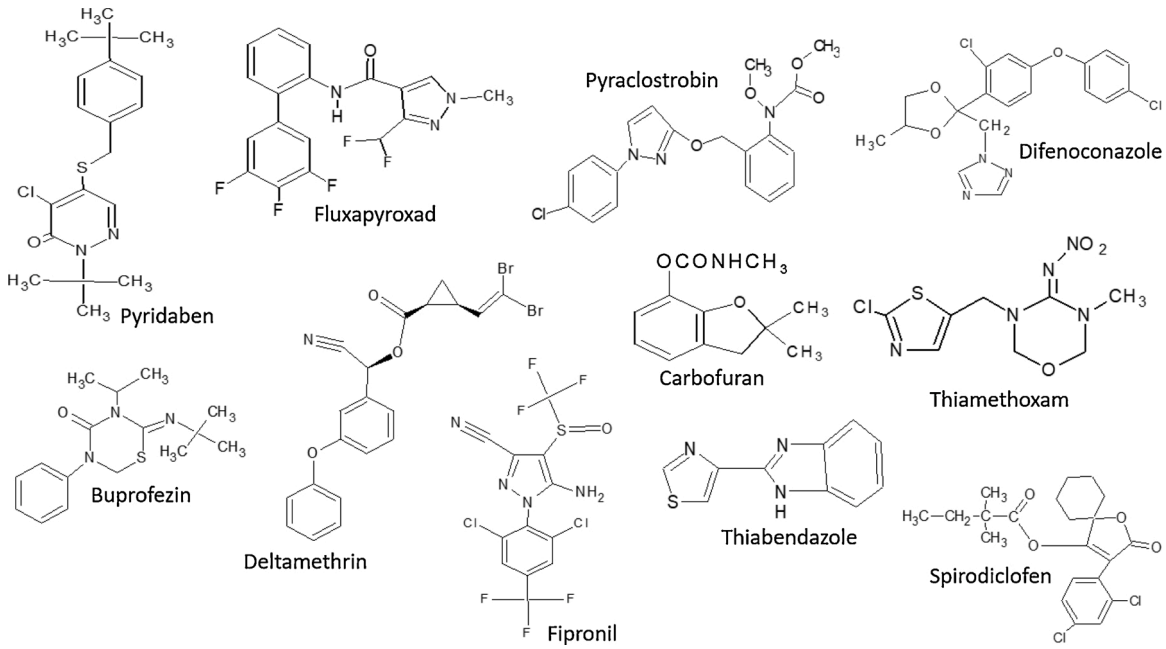

Fig. 1. Molecular structures of the selected pesticides (PubChem, 2019).

mL aliquot of the supernatant was transferred to another tube containing 100 mg PSA, 500 mg of C18 and 600 mg of $MgSO_4$, vortexed for 2 min and centrifuged at 6500 rpm for 10 min; (6) for analyses in GC–MS/MS, 2 mL aliquots were dried in concentrator using a low nitrogen flow and resuspended 0.5 mL in ethyl acetate; (7) for LC–MS analysis, 1 mL of the supernatant was evaporated identically to step 7, resuspended in 1 mL of eluent B (mobile phase), and diluted 5x in deionized water; (8) finally, the extracted samples were vortexed, following by filtration in 0.22 μm.

2.5. Gas chromatography coupled mass spectrometry

An Agilent 7890A system coupled to a Quattro micro triple quadrupole from Waters, equipped with CombPal CTC automatic injector was used with a J&W Scientific DB5-MS column (30 m x 0.25 i.d., 0.25 μm particle size). The helium gas (purity 99.9999 %) was used as carrier gas with a flow of 1.0 mL/min. The PTV (programmed temperature vaporizer) injector was used in solvent vent mode with initial temperature 50 °C maintained by 0.25 min with ramp of 12 °C/s until 280 °C and holding time of 20 min. The oven temperature started at 50 °C (for 1.5 min) followed by a temperature ramp from 25 °C/min to 150 °C, increasing from 5 °C/min to 280 °C, maintaining for 4 min with total run time of 36 min. The injection volume was 3 μL. The mass spectrometer was operated with an impact electron source (IE) at 70 eV, 200 °C and transferline at 280 °C.

2.6. Liquid chromatography coupled mass spectrometry

Ultra-high-performance liquid chromatograph (UHPLC) Acquity UPLC™ coupled to TQ Quattro Model XQ from Waters (Milford, USA), ionization source by electrospray operated in positive mode (ESI +) and mass analyzer in selected reaction monitoring (SRM) mode. Data acquisition was powered by Masslynx software version 4.1 from Waters (Milford, USA). Separation of the pesticides was performed using Phenomenex KINETEX Core-Shell Technology column (2.1 mm i.d. × 100 mm ×1.7 μm particle size).

The chromatographic conditions were 20 μL of injection volume and 12 min run time. Column oven temperature was set to 35 °C. Mobile phase was composed of Eluent A: water:methanol (98:2, v/v) and Eluent B: methanol, both with 0.1 % formic acid. The flow rate was 0.225 mL min^{-1} and a linear gradient with eluent B starting at 5% (0 min), 100 % at 8.50 min, 5% at 9.50 min until 12 min.

Optimization of the collision energy for each individual pesticide was performed by direct infusion into the MS using a Harvard syringe pump (Kent, UK). Collision-induced dissociation (CID) 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}. The conditions used for the mass spectrometer were: residence time (dwell time) 0.03 s, capillary voltage: 3 kV; desolvation temperature: 40 °C, desolvation gas flow and gas flow of the cone (N_2): 54 L h^{-1}, 500 L h^{-1}, respectively.

2.7. Method validation

In order to evaluate the method suitability for the pesticides determination in coconut endosperm, the following parameters of validation were evaluated: linearity, precision, trueness, limits of quantification (LOQs) and matrix effect (ME), according to SANTE/11813/2017 guidelines (European Commission, 2018).

3. Results and discussions

Treatments with fungicides and insecticides have become the most effective and important practice for ensuring control of insects and fungi (Abati et al., 2014). Thus, pesticides such as spirodiclofen, buprofezin, carbofuran, deltamethrin, difenoconazole, fipronil, fluxapyroxad, pyridaben, thiabendazole and thiamethoxam were chosen since they are widely used in coconut culture. These selected pesticides were separated and analyzed with GC-EI(+)-MS/MS and UHPLC-ESI(+)-MS/MS according to their physicochemical properties. In both techniques the selected reaction monitoring (SRM) was used, which presents high sensitivity and selectivity for the targeted quantitation of pesticides in coconut endosperm samples. MS conditions were studied individually for each pesticide as precursor ion and product ion, optimizing collision energies, retention time and cone voltage for LC, as shown in Table 1. The selected ions in the SRM mode chromatograms of spiked samples, after the QuEChERS procedure followed by LC–MS/MS and GC–MS/MS (Fig. 2).

Determination of residual pesticides in coconut endosperm is challenging since coconut water and pulp contain large amounts of lipids, proteins, nutrients, slightly acidic solution, sugars, salts, neutral fats, vitamins, as well as some phospholipids and phytohormones (Foale, 2003). The amino acid composition of coconut water is similar to milk as lysine, leucine, threonine, valine, isoleucine and phenylalanine. In countries with nutritional deficits, coconut water is used to replace vitamins and minerals. Additionally, is used as saline solution intravenously for rehydration and electrolytic replacement (Debmandal and Mandal, 2011; Carvalho et al., 2006; Ewansiha et al., 2012).

Due to the fact that this type of sample is rich in lipids and proteins, a freezing step prior to the clean-up, reduces the amount of co-extractives, improving significantly the selectivity and sensitivity (Ferreira et al., 2016; Anagnostopoulos and Miliadis, 2013). The freezing step was essential for both GC and LC analyzes, as it allowed successful extraction of compounds which had not been previously studied in the coconut endosperm, such as fipronil, buprofezin, pyridaben, deltamethrin and fluxapyroxad, where most of them were analyzed by GC. Additionally, a further clean-up was necessary using primary secondary amine (PSA) and C_{18}, to avoid retention of fatty acids, waxes, sugars, lipids, some pigments and organic acids which interfere with the identification/quantification of some pesticides during chromatographic analysis (Rejczak and Tuzimski, 2015). On the other hand, Walorczyk and Drożdżyński (2012) evaluated the possibility of applying the freezing step and increasing the amounts of PSA to dry animal feed and cereals. They concluded that depending on the type of matrix, this step only slightly reduces the amount of co-extractives. Also, the use of high amounts of PSA might remove more fatty acids co-extractives from cereals, however the recovery of some polar pesticides is decreased. In our studies, due to the high water and lipid content in coconut endosperm, the freezing step combined with the use of PSA and C18 was essential to the extraction and analysis of the selected pesticides.

Usually, the original QuEChERS method does not involve preconcentration, as it was developed to perform a light cleaning step in quantitative determination by gas (GC) or liquid (LC) chromatography coupled with mass spectrometry (MS) or in tandem (MS/MS), which are highly selective detectors (Anastassiades et al., 2003). However, using an extract preconcentration step is not uncommon to increase the signal of the analyte in mass analyzer, especially in the GC (Shamsipur et al., 2016).

After injection of the analytes from the extracts into the GC–MS/MS, due to the lower sensitivity, it was necessary to perform a preconcentration of the extracts prior to their analysis to maximize the analytical signal. When the solvent used to resuspend the extract was acetonitrile, lower detectability was observed in comparison to ethyl acetate, which is more appropriate for GC–MS analysis. For an initial volume of 2 mL and final volume of 0.5 mL, the calculated preconcentration factor was 4. This preconcentration step enhanced detectability, achieving an even lower limit of detection (LOD) and limit of quantification (LOQ). Otherwise, for LC–MS/MS, the samples had to be diluted to reduce interfering compounds, due to the high sensitivity of the equipment, as a strategy to overcome the matrix effect (Walorczyk, 2014). The final volume was 1.0 mL and initial volume, before dilution in deionized water, was 0.2 mL, with a dilution factor of 5.

Table 1
GC–MS/MS and LC–MS/MS parameters for determination of the pesticides.

Compounds	Equipment[b]	t_R (min)	Molar Mass (g mol^{-1})	Quantification Transition (m/z) (CE)[c]	Voltage cone (V)	Confirmation Transition (m/z) (CE)[c]
Buprofezin	GC	11.8	305.4	105.2 > 77.08 (15)	–	105.2 > 104.1 (15)
Carbofuran	LC	4.8	221.2	222.1 > 165.1 (16)	34	222.1 > 123 (16)
Deltamethrin	GC	27.6	505.2	253.0 > 93 (16)	–	253.0 > 172.0 (8)
Difenoconazole[a]	LC	6.5	406.3	406 > 251.1 (25)	46	406 > 111.1 (60)
	GC	26.6		265.1 > 202 (15)	–	323 > 202 (25)
		26.7				
Fipronil	GC	10.0	437.1	367.4 > 213.0 (22)	–	367.4 > 255.0 (20)
Fluxapyroxad	LC	5.8	381.3	159.2 > 111.1 (5)	25	159.2 > 139.1 (5)
Pyraclostrobin	LC	6.4	387.2	388 > 163 (29)	27	388 > 193.9 (12)
Pyridaben	LC	7.1	364.9	365 > 147 (24)	30	388 > 193.9 (12)
	GC	20.8		147.3 > 119.0 (5)	–	
Thiabendazole	LC	3.3	201.2	202 > 251. (25)	46	406 > 111.1 (60)
	GC	10.5		174.3 > 129.1 (5)	–	174.3 > 130.2 (5)
Thiamethoxam	LC	3.1	291.7	292 > 211 (12)	28	292 > 132 (22)

[a] Difenoconazole – Isomers for GC-MS/MS.
[b] GC-MS/MS – Gas chromatography coupled mass spectrometry triple quadrupole and LC-MS/MS – liquid chromatography coupled mass spectrometry triple quadrupole.
[c] CE - Collision energy (eV).

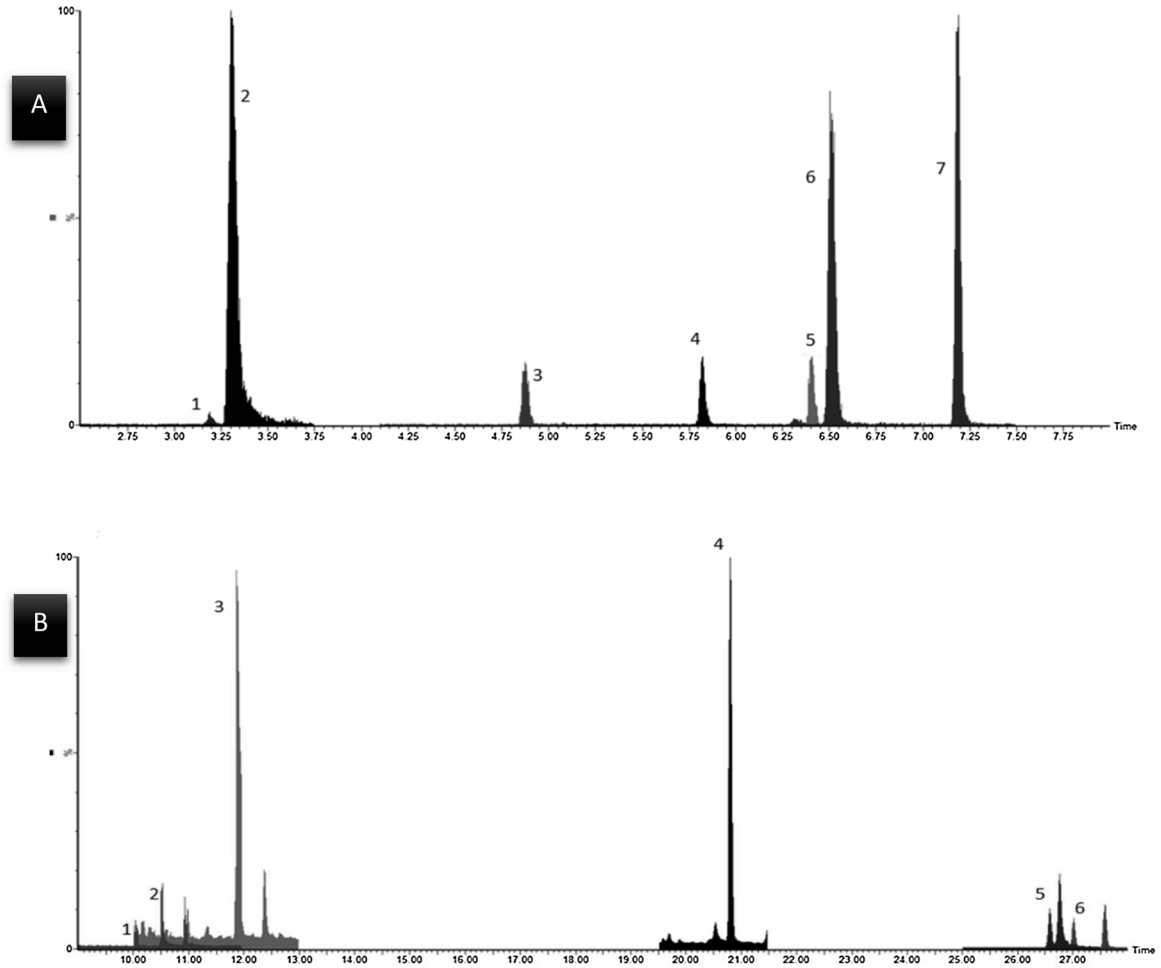

Fig. 2. (A) Total ion chromatogram of spiked sample at 20 μg kg^{-1} by UHPLC-MS/MS. Identification of compounds: Thiamethoxam; 2 – Thiabendazole; 3 – Carbofuran; 4 – Fluxapyroxad; 5 – Pyraclostrobin; 6 – Difenoconazole; 7 – Pyridaben. (B) Total ion chromatogram of spiked sample at 20 μg kg^{-1} by GC-MS/MS. Identification of compounds: 1 – Fipronil; 2 – Thiabendazole; 3 – Buprofezin; 4 – Pyridaben; 5 – Difenoconazole; 6 - Deltamethrin.

3.1. Method validation

The suitability of the method developed for routine analysis, was assessed by the parameters of specificity, linearity, precision, trueness, limits of quantification (LOQs) and matrix effect (ME), in agreement with the SANTE/11813/2017 guidelines (European Commission, 2018), presented in Table 2.

In the studies of specificity for GC and LC, the blank samples were analyzed and no interfering peaks from endogenous compounds at the retention times of any of the target analytes were observed. In the

Table 2
Results of the validation parameters linearity, matrix effect, trueness and precision.

Compounds	Equipment[a]	LOQ[b] μg kg^{-1}	Trueness and precision R(%)±RSDr(%)[c]			Precision R(%)±RSD$_{wR}$(%)[d]	Matrix Effect (%)[e]	r^2 [f]	Linear regression
			5 μg kg^{-1}	20 μg kg^{-1}	100 μg kg^{-1}	20 μg kg^{-1}			
Buprofezin	GC	5	74 ± 14	79 ± 17	100 ± 14	62 ± 5	–	0.9962	28.088x+614.938
Carbofuran	LC	5	92 ± 10	88 ± 1	88 ± 7	108 ± 6	+13	0.9967	276.492x+-4.8694
Deltamethrin	GC	5	85 ± 12	90 ± 19	99 ± 20	62 ± 9	–	0.9945	1.25416x+11.3779
Difenoconazole	LC	5	77 ± 8	66 ± 7	67 ± 15	97 ± 6	+20	0.9974	10.3983x+54.9017
	GC	5	87 ± 12	94 ± 11	107 ± 3	60 + 6	–	0.9960	2227.32x+412.462
Fipronil	GC	5	66 ± 9	118 ± 13	–	61 ± 3	–	0.9934	1.07531x+18.1127
Fluxapyroxad	LC	5	93 ± 8	84 ± 3	85 ± 7	110 ± 3	+8	0.9978	453.711x+-44.0661
Pyraclostrobin	LC	5	95 ± 9	76 ± 15	65 ± 10	100 ± 12	+24	0.9943	485.092x+-121.014
Pyridaben	LC	5	83 ± 13	106 ± 8	95 ± 19	119 ± 4	0	0.9927	37.7037x+185.852
	GC	5	62 ± 9	73 ± 12	87 ± 9	62 + 10	–	0.9933	1783.26x+1006.54
Thiabendazole	LC	5	80 ± 11	76 ± 4	81 ± 7	90 ± 20	+1	0.9978	3300x+-91.2008
	GC	5	109 ± 12	80 ± 11	116 ± 2	61 ± 9	–	0.9925	4.062x+26.0959
Thiamethoxam	LC	20	–	68 ± 20	100 ± 12	103 ± 9	+10	0.9909	67.4647x+134.036

[a] GC- gas chromatography coupled mass spectrometry triple quadrupole and LC – liquid chromatography coupled mass spectrometry triple quadrupole.
[b] LOQ – Limit of quantification.
[c] R(%) – recovery and RSD(%) – relative standard deviation.
[d] RSDwR(%) Within - laboratory reproducibility.
[e] CG curve was performed only in the matrix.
[f] r^2– correlation coefficient estimated linearity.

linearity studies, the correlation coefficient (r^2) was calculated for each calibration curve by linear regression analysis. The concentrations ranged from 2.5 to 250 μg L^{-1} (or μg kg^{-1}) for LC and from 2.5 to 450 μg L^{-1} (or μg kg^{-1}) for GC, with correlation coefficients ≥ 0.99.

In this study, the ME was calculated from the slopes of the calibration curves in solvent and matrix-matched solutions (Ferrer et al., 2011). The chromatographic approaches used for construction of the analytical curves differed in: (a) 5x dilution of the final extract before injecting into the LC; (b) 4x preconcentration of the final extracts prior injection into the GC. The results showed that for the LC, there was signal suppression with light effect (≤ 20 %) for carbofuran, pyridaben, difenoconazole, fluxapyroxad, pyraclostrobin, thiabendazole and thiamethoxam. Only pyraclostrobin displayed signal suppression of medium effect (20–50 %). For this reason, matrix-matched calibration solutions were prepared to assess the extent of matrix interference in the quantification of theses pesticides. For GC, the calibration curve in solvent was not obtained for any compound, whereas the matrix-matched calibration curve was successfully achieved. GC–MS did not exhibit larger matrix effects as in LC–MS/MS, but in both techniques (GC and LC) the validation was performed in the matrix. According to Kwon and collaborators (2012) some analytes such as pesticides when injected with the solvent react with the active sites of the deactivated liners, column, and detector due to polar groups such as phosphate, amine, and hydroxyl. Thus, a lesser amount of injected analyte is detected, in contrast, when to the injection of pesticides into the matrix, which may occur to induce an increase in the chromatographic response.

To demonstrate the trueness (bias) of methods through the average recovery percentage in endosperm without use of pesticides, the samples were spiked in three different concentration levels of 5, 20 and 100 μg kg^{-1}. Some of the results showed recovery lower than 70 %, for both GC and LC methods. However, due to the complexity of the sample, according to the requirements of EU guidelines (European Commission, 2018), the practical default range of 60–140 % with RSDs ± 20 % may be used for individual recoveries in routine analysis. Precision of the methods, expressed as relative standard deviation (RSD %), was tested in each spiked level. Part of the random experimental error, which affects both intra- and interday repeatability, was evaluated by injection of the spiked sample five times at different concentrations (low, medium, and high). For this purpose, the RSD ranged from 1 to 20 %. Therefore, within-laboratory reproducibility (RSD$_{wR}$) derived from on-going method validation was <20 % for each pesticide.

LOQ of analytical methods were calculated as the lowest concentration of the pesticides studied that has been validated with acceptable trueness and precision. LOQ for GC and LC was 5 μg kg^{-1}, except for thiamethoxam in LC, which was validated at the concentration 20 μg kg^{-1}.

Spirodiclofen is a non-systemic, lipophilic, insecticide with a log Kow of 5.83 (PubChem, 2019), which makes difficult its extraction from highly lipidic matrices. This is expected because the high amount of lipid impairs the spirodiclofen extraction by QuEChERS (Ferreira et al., 2016; Bretschneider et al., 2003). Furthermore, in our studies, during the method development for GC, it was not possible to establish the same linear range used for the other compounds for the fluxapyroxad with a log Kow of 3.08 (PubChem, 2019). As well as it did not recover at the concentrations established by MRL of EU pesticides database (40 μg kg^{-1}). For such reasons, it was not possible to validate fluxapyroxad using modified QuEChERS and GC–MS/MS in the coconut endosperm.

Table 3 presents the main remarks of the methods developed for coconut fruit based on the techniques of extraction and analysis. Compared with all the other methods developed, for the evaluation of pesticides in the coconut fruit, carbofuran is the most used pesticide when validating the methods by LC and GC. For carbofuran, Ogawa et al. (2006) developed the method for coconut water by LC, Silva et al. (2008) developed the method for the pulp by GC and Anjos and Andrade (2014) developed the method for coconut water by GC. In these studies, all LOQ were 10, 250 and 2.94 μg kg^{-1}, respectively. Ferreira et al. (2016) developed the method for carbofuran whose LOQ was reduced to 10 μg kg^{-1} for both matrices (coconut water and albumen) evaluated separately. Carbofuran was an insecticide widely used in the coconut culture. In Brazil and EU, both the use of carbofuran and carbosulfan are no longer authorized for the coconut palm tree. Carbofuran is an extremely toxic pesticide that can be found in the form of a carbosulfan degradation product or carbofuran itself. The metabolites of carbofuran are 3-hydroxycarbofuran, 3-ketocarbofuran and 2,3 dihydro-2, 2-diemethyl-7-benzofuranol.

Other studies have also evaluated the compounds investigated in this work using different extraction and analysis techniques, such as the two fungicides difenoconazole and thiabendazole for the pulp (Silva et al., 2008; Ferreira et al., 2016), and the insecticide thiamethoxam for both matrices (coconut water and pulp) analyzed separately (Ferreira et al., 2016). Silva et al. (2008) developed a method in which the LOQ was 250 μg kg^{-1}, with recoveries for difenoconazole being very low at all levels of spiked samples. Ferreira et al. (2016) developed the method for both matrices containing the three of the mentioned pesticides, in which the

Table 3
Main remarks of the methods developed for the extraction and analysis of pesticides in coconut fruit.

Matrix	Extraction Technique	Instrumental Technique	Pesticides	Validation parameters	Main remarks
Coconut water Brito et al. (2002)	**MSPD**[1]	HPLC-UV[7]	Captan Chlorothalonil Carbendazim Lufenuron Diafenthhiuron Endosulfan	**Trueness** 81−95% **RSD**[12] 1.6–12.5 % **LOQ**[13] 10–6000 (μg L^{-1})	**Pros:** The method covered a wide spectrum of polarity compounds (polar and non-polar). **Cons:** Extraction method varied according to the chemical group of the pesticide, being LLE for organochlorine and organophosphorus pesticides and SPE for benzimidazole, benzoylphenyl urea and thiourea pesticides increased time and analysis costs. It does not meet the requirements for identification and confirmation of results when using MS coupled to chromatography according to SANTE.
	LLE[2]	GC – TSD[8] GC-ECD[9]	Captan Tetradifon Trichlorfon Malathion Parathion-methyl Monocrotophos	**Linearity** 0.98−0.99 **Matrix Effect** not calculated	
Coconut water Ogawa et al. (2006)	**SPE**[3]	HPLC-UV[7]	Carbofuran 3-Hydroxycarbofuran	**Trueness** 75−104% **RSD**[12] 1.4–11.5% **LOQ**[13] 10 (μg L^{-1}) **Linearity** ≥ 0.99 **Matrix Effect** not evaluated	**Cons:** Limited number of compounds. It does not meet the requirements for identification and confirmation of results when using MS coupled to chromatography according to SANTE.
Coconut pulp Silva et al. (2008)	**MSPD**[1]	GC-MS[10]	Dimethoate Malathion Lufenuron Carbofuran 3-Hydroxycarbofuran Thiabendazole Difenoconazole Trichlorfon	**Trueness** 75−104% **RSD**[12] 1.4–11.5 % **LOQ**[13]150–250 (μg kg^{-1}) **Linearity** ≥ 0.99 **Matrix Effect** not calculated	**Cons:** The dispersion procedure of the sorbent with the sample during homogenization may be laborious, and sample can be lost in the process due to: (i) homogenization of the sample with the sorbent; (ii) filtering; (iii) the eluent was concentrated in a rotary evaporator followed by a nitrogen flow until the injection in the GC.
Lyophilized coconut water Santos et al. (2012)	**MSPD**[1]	HPLC-UV[7]	Lufenuron Bifenthrin Teflubenzuron	**Trueness** 74−116% **RSD**[12] 0.4–9.4% **LOQ**[13] 100 (μg kg^{-1}) **Linearity** ≥ 0.99 **Matrix Effect** 1.02−1.25	**Cons:** Lyophilization is a time-consuming procedure (48 h) and has been developed to make extraction accessible using MSPD. It does not meet the requirements for identification and confirmation of results when using MS coupled to chromatography according to SANTE.
Coconut water Deme et al. (2013)	**SPE**[3]	LC-MS/MS[11]	Acephate Monocrotophos Dimethoate Malaoxon Dichlorvos Malathion Phenthoate Parathion-ethyl Chlorfenvinfos Quinalphos Diazinon Phosalone Profenofos Ethion Chlorpyrifos Carbofuran Molinate Sulfotep	**Trueness** 86.8−107.6% **RSD**[12] ≤ 12 % **LOQ**[13]0.5–2 (μg L^{-1}) **Linearity** > 0.99 **Matrix Effect** 1.02−1.25	**Pros:** The method developed analyzed 15 pesticides at low concentrations (ng mL^{-1}) using an ESI-MS/MS detector. **Cons:** SPE was limited to extraction of liquid samples, such as coconut water. In addition to the SPE, the method used steps to remove traces of anhydrous sodium sulphate, drying in nitrogen for reconstitution in methanol/water containing formic acid. The method was used only for polar and non-volatile analytes. and analyzed by LC.
Coconut water Anjos and Andrade (2014)	**SDME**[4]	CG-MS[10]	Dimethoate Demeton-o Diazinon Dissulfoton Methyl parathion Fenitrothion Malathion Fenthion Dursban Parathion Endosulfan Ethion Bifenthrin Permethrin i Permethrin ii Azoxystrobin	**Trueness** 28.3−160% **RSD**[12] ≤37.9% **LOQ**[13] 100 (μg L^{-1}) **Linearity** > 0.99 **Matrix Effect** not evaluated	**Pros:** The method included the analysis of 19 pesticides, using few harmful solvents as toluene. **Cons:** Due to the complexity of the matrix, for some compounds, the recovery was below 50% as permethrin i and ii, endosulfan and molinate and above 120% as diazinon, methyl parathion, fenthion, and azoxystrobin. For carbofuran and fenitrothion, no repeatability value was achieved at low concentrations. In addition, the precision for carbofuran, dissulfoton, and azoxystrobin showed values above 20 %. The validation criteria in the protocol was not based on any recommended guidelines for food matrices.

(*continued on next page*)

Table 3 (*continued*)

Matrix	Extraction Technique	Instrumental Technique	Pesticides	Validation parameters	Main remarks
Coconut water Paranthaman and Kumarave (2013)	**SPE**[3]	HPLC-UV[7]	Monocrotophos Copperoxychloride Galaxy	**Trueness** – **RSD**[12]–**LOQ**[13] – **Linearity** = 0.99 for monocrotophos **Matrix Effect** not evaluated	**Pros:** The analyses were performed on commercial samples of coconut from different cultivars. Monocrotophos and copperoxychloride were detected in some samples, the monocrotophos being above the MRLs, shows the importance of evaluating coconut fruit. **Cons:** The method could have covered a greater number of compounds, in addition to evaluate in the pulp, but the SPE limited this evaluation. Also, the validation parameters were not clear, as well as the guide used was not mentioned. It showed only some results for monocrotophos. For copperoxycloride and galaxy, the validation results and protocol were not presented. It does not meet the requirements for identification and confirmation of results when using MS coupled to chromatography according to SANTE.
Coconut water and pulp, analyzed separately Ferreira et al. (2016)	**QuEChERS**[6]	LC-MS/MS[11]	3-Hydroxycarbofuran Carbofuran Carbendazin Carbosulfan Cyproconazole Difenoconazole Spirodicloflen Imidacloprid Thiabendazole Thiamethoxan Methyl thiophanate	**Trueness** 70−120% **RSD**[12] <20% **LOQ**[13] 10 (μg kg^{-1}) **Linearity** > 0.99 **Matrix Effect** −13% to +35 %	**Pros:** It was the first method developed using QuEChERS as an extraction method. **Cons:** The separated evaluation of the fruit, coconut water and pulp, became expensive, and time consuming. The method was used only for polar analytes.

1- Matrix Solid Phase Dispersion.
2- Liquid-Liquid Extraction.
3- Solid Phase Extraction.
4- Single Drop Microextraction.
5- Stir Bar Sorptive Extraction.
6- Quick, Easy, Cheap, Effective, Rugged and Safe.
7- High performance liquid chromatograph with an ultraviolet detector.
8- Gas chromatograph with thermionic sensitive detection.
9- Gas chromatograph with an electron-capture detector.
10- Gas chromatograph coupled mass spectrometry.
11- Liquid chromatography coupled mass spectrometry.
12- Relative Standard Deviation.
13- Limit of quantification.

LOQ for all these compounds was 10 μg kg^{-1}. Thiamethoxam was analyzed separately presenting good results even in low recoveries. However, in our work, when the two matrices were mixed, it was not possible to validate thiamethoxam at very low levels.

3.2. Method application to commercial samples

The analytical method developed was applied in commercial samples from grocery stores in State of São Paulo, Brazil. Ten fruits were

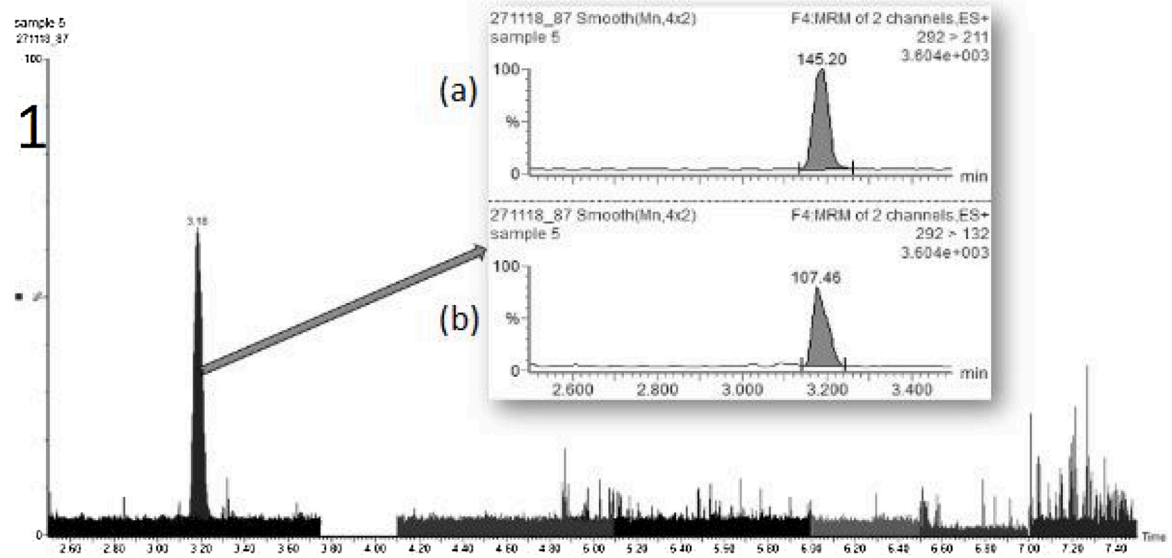

Fig. 3. Chromatograms obtained confirming the presence of thiamethoxam from monitoring of SRM: (a) ion precursor peak and (b) product ion peak in (1) sample.

analyzed. For some of the fruits the presence of thiamethoxam was identified, in which one of the fruits showed a level of 28.8 μg kg^{-1}, that is above the MRL established by the EU (Fig. 3).

Thus, some samples confirmed the presence of thiamethoxam from SRM mode of spectrometer and showed values above of the maximum residue limits set by EU (<10 ug kg^{-1}), and above of method LOQ (20 ug kg^{-1}). The concentrations of thiamethoxam found in commercial samples were confirmed considering: (i) the product ion with complete overlap to confirmation ion; (ii) ion ratio within ± 30 % (relative) of average of calibration standards; and (iii) retention time varying ±0.1 min.

4. Conclusions

The modified QuEChERS with preconcentration for GC–MS/MS and dilution for LC–MS/MS can be seen as the most sensitive and reliable approach to carry out such analysis, and was used in the development, validation and quantification studies. Use in selected reaction monitoring (SRM) mode was an effective and powerful tool for monitoring pesticides due to its high selectivity and sensitivity.

This study raises the concern of insecticides and fungicides residues in coconut endosperm samples and the importance of regular analysis for their detection. As thiamethoxam residue levels were above of MRL, the analysis also confirms an actual occurrence of a transfer of pesticides to coconut endosperm during the control and treatment of pest and diseases.

CRediT authorship contribution statement

Jordana Alves Ferreira: Formal analysis, Investigation, Resources, Visualization, Data curation, Writing - original draft, Conceptualization, Methodology, Validation. **Sonia C.N. Queiroz:** Investigation, Resources, Methodology, Validation, Conceptualization, Writing - review & editing, Supervision, Funding acquisition, Project administration.

Declaration of Competing Interest

The authors report no declarations of interest.

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