



Pesticide residues in cashew apple, guava, kaki and peach: GC- μ ECD, GC-FPD and LC-MS/MS multiresidue method validation, analysis and cumulative acute risk assessment



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ARTICLE INFO

Article history:

Received 15 March 2014
Received in revised form 6 May 2014
Accepted 11 May 2014
Available online 15 May 2014

Keywords:

Organophosphorus
Pyrethroids
Cashew apple
Guava
Kaki
Gas chromatography
LC-MS/MS
Cumulative acute exposure assessment

ABSTRACT

A multiresidue method for the determination of 46 pesticides in fruits was validated. Samples were extracted with acidified ethyl acetate, MgSO_4 and CH_3COONa and cleaned up by dispersive SPE with PSA. The compounds were analysed by GC-FPD, GC- μ ECD or LC-MS/MS, with LOQs from 1 to 8 $\mu\text{g}/\text{kg}$. The method was used to analyse 238 kaki, cashew apple, guava, and peach fruit and pulp samples, which were also analysed for dithiocarbamates (DTCs) using a spectrophotometric method. Over 70% of the samples were positive, with DTC present in 46.5%, λ -cyhalothrin in 37.1%, and omethoate in 21.8% of the positive samples. GC-MS/MS confirmed the identities of the compounds detected by GC. None of the pesticides found in kaki, cashew apple and guava was authorised for these crops in Brazil. The risk assessment has shown that the cumulative acute intake of organophosphorus or pyrethroid compounds from the consumption of these fruits is unlikely to pose a health risk to consumers.

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1. Introduction

The use of pesticides in agriculture is still necessary to guarantee the worldwide food supply. However, the presence of residues in treated food with possible health risk to consumers is a global concern. Results from Brazilian pesticide monitoring programs have shown that almost half of the 13,556 samples of 22 different crops analysed tested positive for at least one pesticide (Jardim & Caldas, 2012). Dithiocarbamates, organophosphorus, pyrethroids and *N*-methyl carbamates were among the most detected pesticides. Organophosphorus and *N*-methyl carbamate insecticides, the most acute toxic pesticides used currently worldwide, are neurotoxic and can inhibit the enzyme acetylcholinesterase (Ecobichon, 2001). Pyrethroids are also neurotoxic, by blocking voltage-dependent sodium, chlorine and calcium channels (Soderlund et al., 2002). The metabolites produced by certain dithiocarbamate fungicides have been shown to be carcinogenic in rats (USEPA, 2001).

Over the last 40 years, various methods have been developed to investigate multiresidues of pesticides in food, given the variety of products applied to crops. In the QuEChERS method (Quick, Easy, Cheap, Effective, Rugged and Safe) samples are extracted with acetonitrile, magnesium sulfate (MgSO_4) and sodium chloride (NaCl), followed by clean-up using dispersive solid-phase extraction with primary and secondary amine (PSA) (Anastassiades, Lehotay, Štajnbaher, & Schenck, 2003; Melo et al., 2012). Gas or liquid chromatography coupled with mass spectrometry is normally used to quantify the residues. In addition to acetonitrile, other solvents can be used for pesticide extraction, including ethyl acetate (Aysal, Ambrus, Lehotay, & Cannavan, 2007; Banerjee et al., 2007; Berrada et al., 2010; Mol et al., 2007), which is more suitable for GC analysis than acetonitrile, as it is less polar and provides a smaller liquid-to-gas expansion volume (Mastovska & Lehotay, 2004). These methods are very flexible and have high selectivity and sensitivity.

Gas chromatographs coupled to flame photometric (FPD) and electron capture (ECD) detectors are also widely used, mainly for the detection of organophosphorus and pyrethroid compounds, respectively (Gowda & Somashekar, 2012; Hunter, Riederer, & Ryan, 2010; Liu, Mitrevski, Li, Li, & Marriott, 2013; Wang, Zhang,

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Wang, & Guo, 2013; Zhao et al., 2011). FPD and ECD are cheaper and have lower maintenance costs, hence are more readily available in some countries than mass spectrometers. Most importantly, they are very sensitive for those groups of pesticides, which is essential if the data are used for dietary exposure assessment.

Brazil is the third largest fruit producer worldwide and an important exporter of tropical and subtropical fresh and processed fruits, mainly to European countries and the United States (Carvalho & Miranda, 2009). Pesticide residue data for kaki and guava fruits are non-existent in the country and scarce worldwide, and we could not find in the literature any residue data for cashew apple. This study aimed at fully validating a multiresidue method to analyse 46 pesticides and metabolites in kaki, cashew apple, guava and peach by GC- μ ECD, GC-FPD and LC-MS/MS, and determine the pesticides in Brazilian commercial fruit samples. The fruits analysed in this study have an edible peel, and consumers may ingest surface residues. Additionally, as seasonal fruits, they are largely consumed during certain periods of the year, which may lead to a high acute exposure to the pesticides present. Hence, we also conducted a deterministic cumulative acute dietary exposure assessment of organophosphorus and pyrethroid pesticides to evaluate the risk to consumers.

2. Materials and methods

2.1. Chemicals and reagents

Ethyl acetate and anhydrous magnesium sulfate ($\geq 99.5\%$) were obtained from Sigma-Aldrich (St. Louis, MO), methanol and acetonitrile from Merck KGaA (Darmstadt, Germany), acetic acid, toluene and anhydrous sodium acetate (99.5%) from J. T. Baker (Phillipsburg, NJ), PSA (primary-secondary amine) from Sulpelco (Bellefonte, PA), and ammonium formate from Fluka (Buchs, Switzerland). The other chemicals were from Vetec (Rio de Janeiro, Brazil). All organic solvents used in the multiresidue method were HPLC grade. Analytical standards (purity from 78% to 99.5%) of pesticides and metabolites were purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany), except for thiram (99.9% purity) and CS₂ (PA), which were obtained from Sigma-Aldrich and Vetec, respectively.

Stock solutions of individual pesticide standards (1 mg/mL) were prepared in 10 mL (certified “A” class volumetric flasks) of toluene, methanol or acetone according to their solubility and stability. Intermediate stock standard mixtures of organophosphorus, pyrethroids, chlorothalonil, pendimethalin and trifluralin were prepared in ethyl acetate (30 ng/ μ L), and of *N*-methyl carbamates in methanol (10 ng/ μ L). Working standard mixtures were prepared by dilutions of the intermediate stock standard solutions, and the solutions stored in amber vials at -15°C . A thiram stock solution (2.37 mg/mL) was prepared in acetone and the working standards in ethanol. The stock and working solutions of CS₂ were prepared in ethanol.

2.2. Sample processing

A total of 238 samples (at least 1 kg) of whole fruit (67 kaki, 32 cashew apple, 44 guava and 67 peach), and frozen fruit pulp (11 cashew apple, 14 guava and 3 peach) were purchased at local supermarkets between January 2010 and February 2012. Control (blank) fruit samples were provided by a local organic farmer or purchased on the market (labelled as organic), and checked for absence of pesticides before use in the validation studies. All samples were kept frozen (-15°C) until analysis, when they were left at room temperature ($\sim 22^\circ\text{C}$) for about one hour, manually cut into small pieces, and homogenised.

2.3. Multiresidue method and instrumentation

A 100-g portion of the homogenised sample was blended, and 15 g (± 0.01 g) weighed in a 50-mL screw-cap centrifuge tube. Fig. 1 illustrates the extraction and clean-up procedure. Organophosphorus compounds (OP) were quantified by GC-FPD, and pyrethroids, chlorothalonil, pendimethalin and trifluralin by GC- μ ECD; permethrins, cyfluthrins and cypermethrins were quantified as the sum of their isomers. The identity of the detected compounds was confirmed by GC-MS/MS. *N*-methyl carbamate pesticides were quantified by LC-MS/MS.

2.3.1. GC-FPD- μ ECD

An Agilent Model 7890A gas chromatograph equipped with a 7683B Series autosampler and ChemStation B.03.02 was used. The injector temperature was set at 250°C . N₂ (99.999% purity) was used as carrier gas at a constant flow rate 1.0 mL min^{-1} . FPD temperature was 250°C , and the N₂ make-up, H₂, and synthetic air flows were 60, 85 and 100 mL min^{-1} , respectively. μ ECD temperature was 300°C , and the N₂ makeup flow 60 mL min^{-1} . The column coupled to the FPD was an OV-17 (50% diphenyl/50% dimethyl-polysiloxane), and to the μ ECD was an OV-5 (5% diphenyl/95% dimethyl-polysiloxane), both with $30\text{ m} \times 0.25\text{ mm ID}$ and $0.25\text{ }\mu\text{m}$ film thickness. The oven temperature for both columns was programmed as follows: initial temperature of 50°C was held for 1 min, ramped to 150°C at $30^\circ\text{C min}^{-1}$, followed by 5°C min^{-1} until 280°C and held for 5 min, resulting in a total run time of 35.33 min. The injection volume was $1\text{ }\mu\text{L}$, in splitless mode.

2.3.2. GC-MS/MS

A Trace GC Ultra (Thermo Scientific) equipped with a programmed temperature vaporiser (PTV) injector and a Quantum XLS Triple Quadrupole was used. A TR-Pesticide II column (Thermo Scientific) of $30\text{ m} \times 0.25\text{ mm ID} \times 0.25\text{ }\mu\text{m}$ was used for chromatographic separation, with helium (99.999% purity) as carrier gas (1.2 mL min^{-1}). The mass spectrometer was operated in positive ionisation mode, and data were acquired using selected reaction monitoring (SRM). Argon (1.5 mTorr) was used as collision gas and the source temperature was set at 280°C . The injection volume was $1\text{ }\mu\text{L}$, in splitless mode.

2.3.3. LC-MS/MS

A Shimadzu (Kyoto, Japan) LC system coupled to a triple-quadrupole mass spectrometer (4000QTRAP, Applied Biosystem/MDS Sciex, Foster City, CA). Chromatographic separation was carried out at 40°C using a Synergi $4\text{ }\mu\text{m}$ Fusion RP 80A, $50 \times 2.00\text{ mm}$ column (Phenomenex, Torrance, CA) with a pre-column (Fusion-RP $4 \times 2.0\text{ mm}$). Mobile phases were methanol + 5 mM ammonium

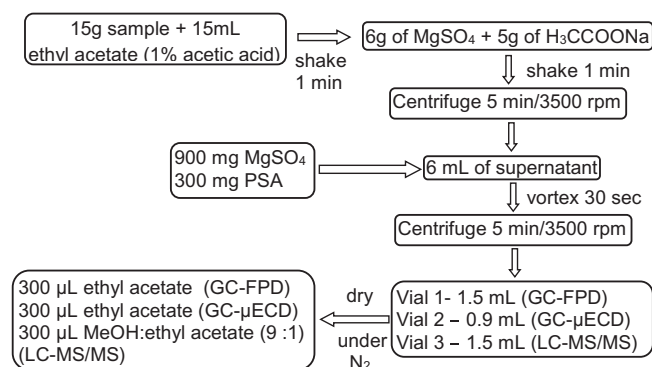


Fig. 1. Flowchart for the multiresidue method.

formate (B) and water:acetonitrile (80:20) with 5 mM ammonium formate (A). Gradient elution at 0.25 mL min⁻¹ flow: 0–7 min from 0% to 90% B, 7–12 min at 90% B, 12–13 min to 0% B and 5 min equilibration time. Injection volume was 5 µL. ESI (electrospray ionisation) was operated in positive mode and data were acquired in multiple reaction monitoring mode (MRM). The iMethod™ Test of Cliiquid® Software for pesticides was used, and recommends the following instrument settings: entrance potential of 10 V, curtain gas of 30 psi; nitrogen collision gas as medium; ion spray voltage of 5000 V; temperature of 500 °C; ion source gas of 40 psi (GS1) and 60 psi (GS2). The precursor and product ions of the *N*-methyl carbamates analysed are listed in Table 1.

2.4. Multiresidue method validation

Selectivity was checked by analysing the GC–FPD, GC–µECD and LC–MS/MS chromatogram profiles of a blank and a fortified sample, verifying for interferences at the same retention time of the pesticides and the ions of interest. The trueness (recovery) and precision (repeatability and intermediate precision, expressed as relative standard deviation, RSD, %) were determined by analysing replicate samples fortified at four levels ($n = 5$ at each level). For each commodity, the fortifications were performed in two sets: (i) the organophosphorus group 1 plus pyrethroids, chlorothalonil, pendimethalin and trifluralin, and (ii) organophosphorus group 2 plus the *N*-methyl carbamates. Intermediate precision was evaluated for all commodities through the analysis of 5 fortified samples at 20 µg/kg for GC compounds and 10 µg/kg for LC–MS/MS compounds (*N*-methyl carbamates), performed by the same analyst on two different days. Linearity was accessed by injecting 3 different external matrix-matched calibration standards curves, each with 6 points. The calibration standard curves ranged from 8 to 1100 pg/µL for GC–FPD compounds, 10 to 650 pg/µL for GC–µECD compounds, and 4 to 550 pg/µL for *N*-methyl carbamates. The linear regression was evaluated by checking the residues' variance (Cochran's test), the correlation coefficient (r), and the significance of the regression (ANOVA) (INMETRO, 2011). The method limit of quantification (LOQ) was defined as the lowest concentration that could be quantified with acceptable recovery (70–120%) and precision (RSD ≤ 20%) (SANCO, 2012). The method limit of detection (LOD) was set at 1/3 LOQ.

Two procedures were included as quality controls during sample analysis: (a) addition of 100 µL of the surrogate standard solution (chlorpyrifos methyl; 3 ng/µL in ethyl acetate) to the samples before extraction; samples with surrogate recoveries <70% were re-analysed; (b) extraction of 5 samples fortified with all pesticides at 20 µg/kg; these results were also used to evaluate the intermediate precision.

2.5. Dithiocarbamate analysis

Dithiocarbamates were determined according to Caldas, Conceição, Miranda, Souza, and Lima (2001). Briefly, 150 g of homogenised sample were transferred to a two-neck round-bottom flask, and stannous chloride acid solution was added. The flask was placed in a heating mantle, connected to a N₂ inlet and to the CS₂ vertical reaction system containing sodium hydroxide (trap 1) and the complexant solution of copper (II) acetate monohydrate/diethanolamine (trap 2). After 45 min heating under reflux, the complexant solution was transferred to a 25-mL volumetric flask (certified "A" class), the volume completed with ethanol and the absorbance measured at 435 nm against a CS₂ standard curve (Shimadzu UV 1650 PC spectrophotometer). The eight calibration points ranged from 0.21 to 8.4 µg of CS₂/mL, corresponding to 0.04 to 1.4 mg of CS₂/kg in the food samples. The calibration curves were homoscedastic (the standard deviations of the residues did not change with concentration), and the least squares method was used to quantify the residues. Method validation was performed with samples fortified with thiram at levels of 0.05, 0.1, 0.5 and 1 mg/kg CS₂ ($n = 3$ or 4 at each level). Recoveries ranged from 70% to 120%, and RSD were lower than 20% at all tested levels, with a LOQ of 0.05 mg/kg CS₂ for all matrices. The Laboratory of Toxicology of the University of Brasília is ISO 17025 certified by INMETRO, the Brazilian accreditation body, to perform this method.

2.6. Cumulative acute dietary risk assessment

The cumulative acute exposures to organophosphorus and pyrethroid residues through the consumption of kaki, guava, cashew apple or peach fruits were estimated based on the FAO/WHO Joint Meeting on Pesticide Residues (JMPR) approach for the calculation of the International Estimated Short-Term Intake (IESTI) for raw commodities (FAO, 2003).

$$IESTI = \frac{U * HR * v + (LP - U) * HR}{bw}$$

where U = unit weight of the crop; HR = highest residue found in the crop; v = variability factor of 3; LP = large portion (97.5th percentile of consumers) and bw = body weight.

Fruit unit weights were 90 g for cashew apple, 110 g for kaki, 170 g for guava and 110 g for peach.

In this study, HR is the total residue (HR_t) present in a sample after normalisation for the reference compound for each group with the same mode of action (organophosphorus or pyrethroids), using the relative potency factors (RPF) estimated by Caldas, Boon, and Tressou (2006) and EPA (2006, 2011). Acephate (acute

Table 1
LC–MS/MS conditions for *N*-methyl carbamates determination.

Pesticide	RT (min)	Precursor ion	<i>m/z</i>	Product ions (<i>m/z</i>) ^a	DP	CE	CXP	IR (RSD, %) $n = 72$
Aldicarb	4.60	[M+NH ₄] ⁺	208	<u>116</u> :89	31	11;20	3;3	1.4 (2.0)
Aldicarb sulfone	1.49	[M+H] ⁺	223	<u>86</u> :148	50	20;12	3;3	1.3 (10.3)
Aldicarb sulfoxide	1.16	[M+H] ⁺	207	<u>132</u> :89	41	9;19	3;3	1.2 (2.6)
Carbaryl	6.06	[M+H] ⁺	202	<u>145</u> :127	51	16;39	3;3	3.4 (4.1)
Carbofuran	5.75	[M+NH ₄] ⁺	222	<u>165</u> :123	50	17;29	2;2	1.2 (2.6)
Carbofuran 3-oh	2.91	[M+NH ₄] ⁺	238	<u>163</u> :181	62	21;15	4;2	1.4 (2.8)
Carbosulfan	9.88	[M+H] ⁺	381	<u>118</u> :160	52	27;20	3;3	1.1 (1.6)
Methomyl	1.71	[M+H] ⁺	163	<u>88</u> :106	35	13;13	3;3	1.9 (1.7)

RT: retention time; DP: declustering potential (V); CE: collision energy (V); CXP: collision cell exit potential (V); IR: mean ion ratio (quantifier/qualifier); RSD: relative standard deviation.

^a Quantifier ion underlined.

reference dose (*ARfD*) of 0.1 mg/kg bw; JMPR, 2005) and methamidophos (*ARfD* of 0.01 mg/kg bw; JMPR, 2002) were used as reference compounds for organophosphorus, and deltamethrin was used as the reference compound for pyrethroids (*ARfD* of 0.01 mg/kg bw; EPA, 2011). To characterise the cumulative risk for each group, the intake was expressed as % *ARfD* of the respective reference compound. Risk may exist when % *ARfD* is higher than 100.

Large portion and body weight data were obtained from the 2008/2009 POF survey (*Pesquisa de Orçamento Familiar*; IBGE, 2012) in which 34003 individuals 10 years or older from all 26 Brazilian states and the Federal District completed a two non-consecutive-days dietary report. Mean body weight for fruit consumers was 65.5 kg.

3. Results and discussion

3.1. Multiresidue method validation

GC–FPD and GC– μ ECD chromatograms showed no interfering peaks present in the blank samples of each crop at the same retention time for all pesticides, indicating that the extraction, clean-up and instrument conditions were satisfactory and the method was selective. No interfering responses were seen at the same retention time for the *N*-methyl carbamates for all transition ions in the LC–MS/MS system. Figs. 2 and 3 show the chromatograms of fortified control fruit samples analysed by GC–FPD (organophosphorus compounds), GC– μ ECD (pyrethroids, chlorothalonil, pendimethalin and trifluralin) and LC–MS/MS (*N*-methyl carbamates).

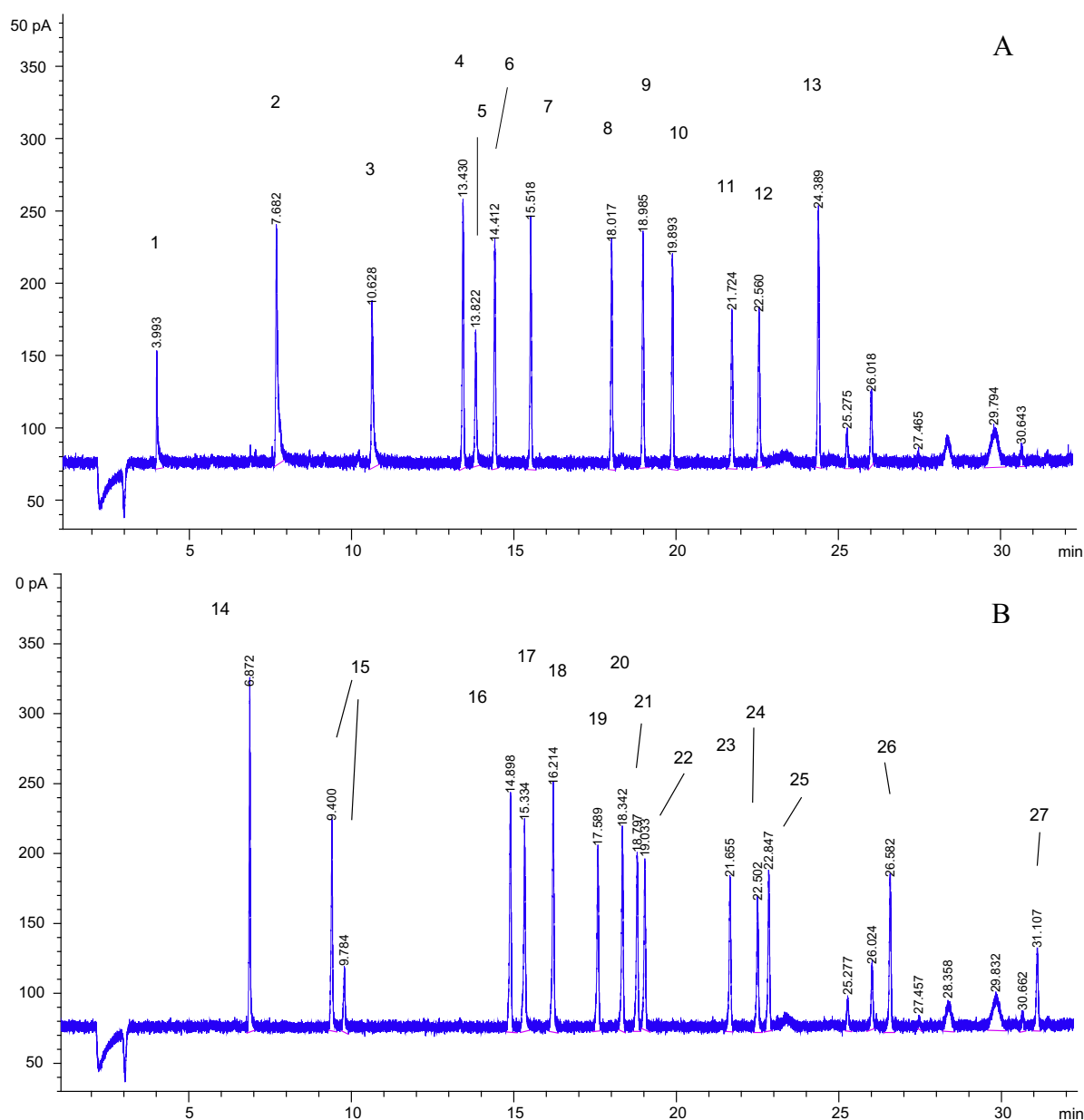


Fig. 2. GC–FPD chromatograms (OV-17 column: 50% diphenyl/50% dimethyl-polysiloxane) of a kaki matrix fortified with 50 pg/ μ L of organophosphorus compounds. (A) Group 1: 1. trichlorfon; 2. methamidophos; 3. acephate; 4. phorate; 5. omethoate; 6. terbufos; 7. disulfoton; 8. parathion methyl; 9. fenitrothion; 10. fenthion; 11. prothiophos; 12. fenamiphos; 13. ethion. (B) Group 2: 14. dichlorvos; 15. mevinphos (*E* and *Z*); 16. diazinon; 17. monocrotophos; 18. dimethoate; 19. chlorpyrifos-methyl; 20. pirimiphos; 21. chlorpyrifos; 22. malathion; 23. phentoate; 24. profenofos; 25. methidathion; 26. triazophos; 27. azinphos-methyl.

In most published methods, anhydrous sodium sulfate (Na_2SO_4) is the salt used to force the partition between the aqueous and the ethyl acetate organic phase (Banerjee et al., 2007; Chen et al., 2011) and sodium hydrogen carbonate (NaHCO_3) (Aysal et al., 2007; Pihlström, Blomkvist, Friman, Pagard, & Österdahl, 2007) or phosphate buffer solution to adjust the extract pH (Mol et al., 2007). In this study, we used MgSO_4 and CH_3COONa , the same as the modified QuEChERS method (Lehotay, Tóvská, & Lightfield, 2005). MgSO_4 was chosen due to its higher ability to remove water compared to Na_2SO_4 , in addition to the fact that the dissolution of MgSO_4 is an exothermic process, and can facilitate the extraction of non-polar compounds.

The linearity study of the matrix-match calibration curve showed that for most compounds, in all matrices, the standard deviations of the calibration curve residues increased with pesticide concentrations, an indication of heteroscedasticity ($C_{\text{calculated}} < C_{\text{critical};6;3}$). Hence, the weighted linear regression was chosen to calculate recoveries and to quantify the residues

(Miller & Ambrus, 2000). The correlation coefficients were 0.99 or higher for almost all compounds, and even when it was lower (0.96), the ANOVA showed that the weighted regressions were significant ($p < 0.05$).

Fig. 4 summarises the results of the mean recoveries found during the method validation. Overall, about 80% of the crop-compound combinations had recoveries between 70% and 120% at all tested levels. Supplementary Material (Tables S1–S3) shows the recoveries for all matrices and levels, in addition to repeatability data (% RSD).

Over 10% of the crop-OP combinations had recoveries lower than 50% at the lowest level, including cases where nothing was detected ($< \text{LOD}$ for acephate, azinphos-methyl, methamidophos, omethoate, phorate and trichlorfon). When detected, recoveries of acephate and methamidophos were between 50% and 70% in most cases. These compounds have higher polarity ($\log K_{\text{ow}} \sim -0.9$), which probably prevented a total partitioning into the ethyl acetate phase. Similar results were found by other authors

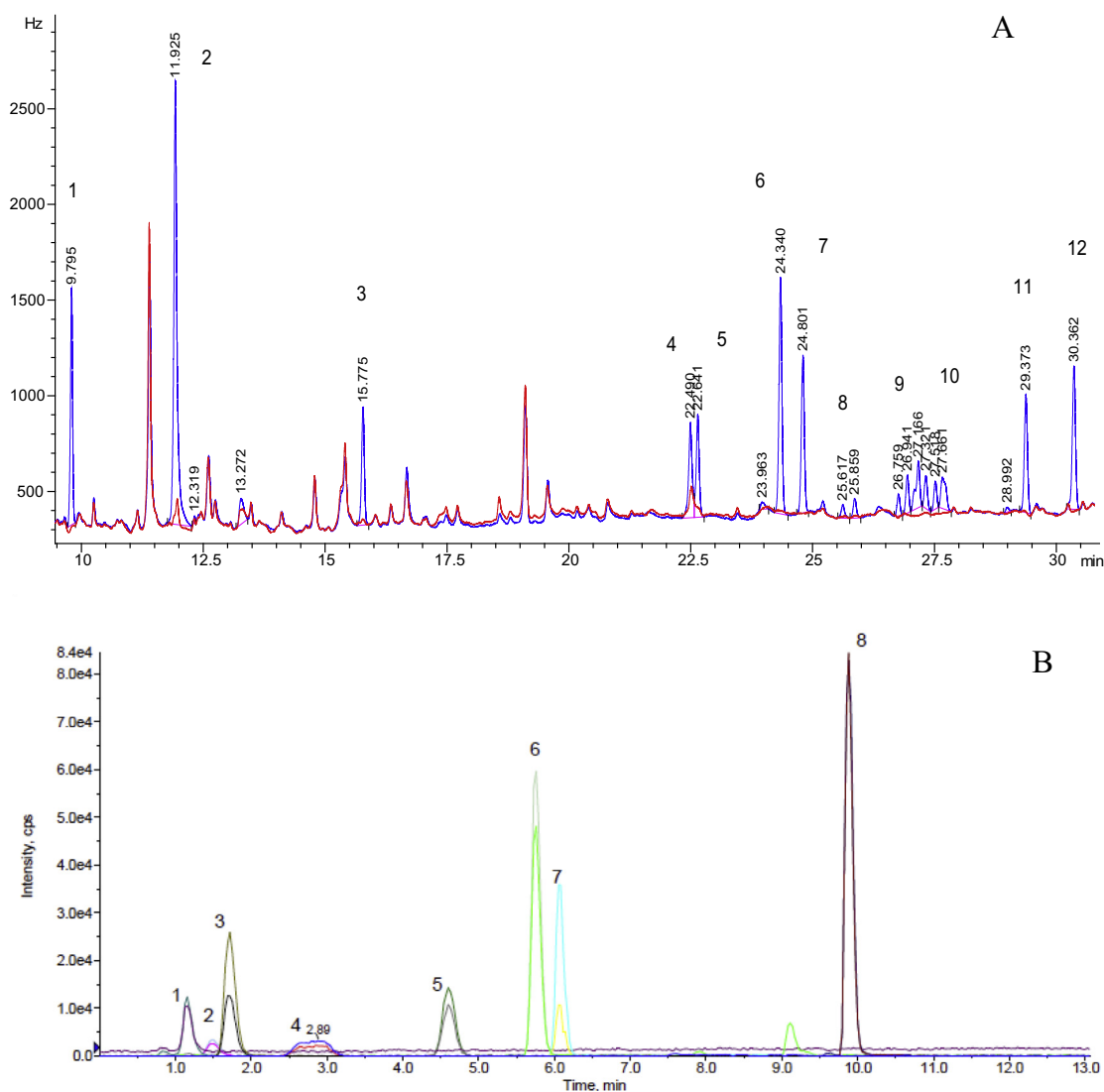


Fig. 3. (A) GC- μ ECD chromatograms (OV-5 column: 5% diphenyl/95% dimethyl-polysiloxane) of a cashew apple matrix blank (red) and fortified at 50 pg/ μL (blue). 1. Trifluralin; 2. chlorothalonil; 3. pendimethalin; 4. bifenthrin; 5. fenpropathrin; 6. acrinathrin; 7. λ -cyhalothrin; 8. permethrins (2 isomers); 9. cyfluthrins (4 isomers); 10. cypermethrins (4 isomers); 11. esfenvalerate; 12. deltamethrins; (B) LC-MS/MS chromatogram (Synergi 4 μm Fusion RP 80A column) for a guava matrix fortified at 20 pg/ μL , showing the two product ions for each pesticides: 1. aldicarb sulfone; 2. aldicarb sulfoxide; 3. methomyl; 4. carbosulfan 3-OH; 5. aldicarb; 6. carbofuran; 7. carbaril; 8. carbosulfan. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

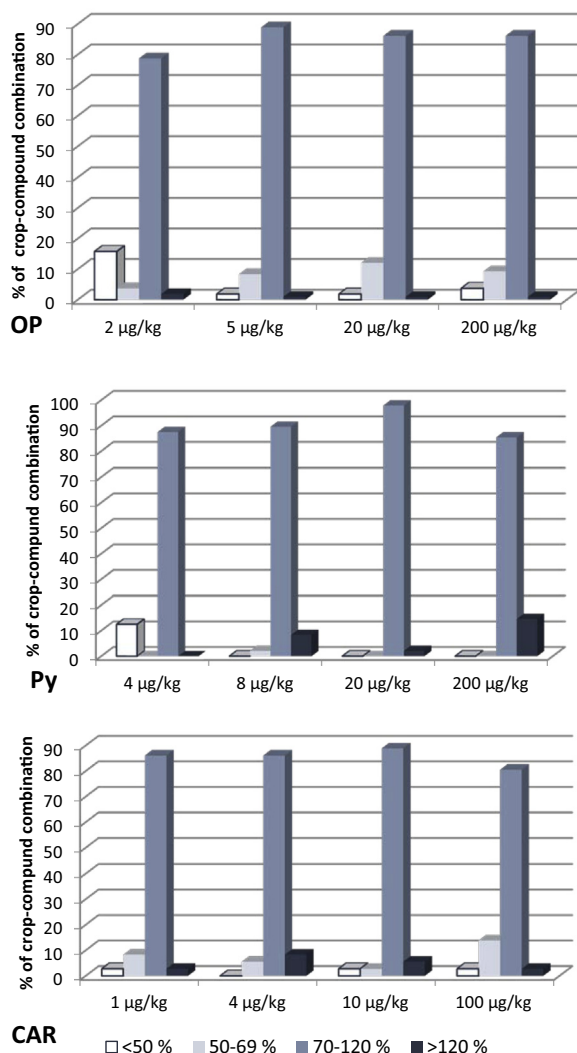


Fig. 4. Percentage of crop-compound combinations (in fruits and pulps) grouped by % recovery range at different fortification levels (mean of 4–5 replicates for each combination). OP = organophosphorus (GC–FPD); Py = pyrethroids and others compounds (GC– μ ECD); CAR = *N*-methyl carbamate (LC–MS/MS).

(Berrada et al., 2010; Lehotay et al., 2010; Mol et al., 2007). Repeatability was within the acceptable range ($RSD \leq 20\%$) in most cases, with the exception of dichlorvos at all fortification levels in certain crops (up to 46% in peach). Dichlorvos was not detected in kaki at the lower fortification levels, and recovery from peach was over 250% at all levels.

About 10% of the pyrethroids had recoveries below 50% or above 120% (Fig. 4). Bifenthrin, cypermethrins and permethrins were not detected in fortified peach samples at 4 μ g/kg level (see Supplementary Material). Higher recoveries ($>120\%$) were found for cyfluthrins, cypermethrins, deltamethrin and esfenvalerate in cashew apple (at 200 μ g/kg) and guava (at 8 μ g/kg), probably due to an enhancement of the μ ECD response (Hunter et al., 2010). Type II pyrethroids (which contain a cyano group at the α -carbon of the 3-phenoxybenzyl alcohol moiety), such as λ -cyhalothrin and deltamethrin, can suffer isomerisation in the GC liner, a process that increases with the number of injections (Mastovska & Lehotay, 2004). Reduction of this phenomenon was achieved by replacing the silanised liner and cutting 15 cm of the column after each batch of 100–120 injections, and by using an acidified ethyl acetate matrix extract. Repeatability of pyrethroid analysis was satisfactory, with RSD mostly below 20% (see Supplementary Material).

Fewer than 3% of the crop-*N*-methyl carbamate combinations had recoveries $<50\%$ (Fig. 4), mainly aldicarb sulfoxide for kaki (down to 15%). Some recoveries obtained for carbosulfan in cashew apple were extremely high (125–210%), as were the RSD s at the highest levels.

Intermediate precision was below 30% in most cases (Table S4; Supplementary Material). Exceptions were aldicarb sulfoxide in kaki, aldicarb in peach, carbosulfan in kaki and cashew apple, and dichlorvos in all matrices.

LOQ (recovery 70–120%, $RSD < 20\%$) was 2 μ g/kg for most organophosphorus compounds, with the exception of trichlorfon and azinphos-methyl in all matrices, disulfoton, methamidophos and omethoate in kaki and acephate in kaki, guava and peach (5 μ g/kg). Dichlorvos was not satisfactorily validated in this study due to low or higher recoveries and high RSD . LOQ for the pyrethroids was 4 μ g/kg, except for permethrins in cashew apple, guava and peach, and for bifenthrin and cypermethrins in peach (8 μ g/kg). LOQ for *N*-methyl carbamates was 1 μ g/kg, with the exception of aldicarb sulfoxide in cashew apple and carbosulfan in guava (4 μ g/kg). The LOQs established in this study were below the set value of 10 μ g/kg required for most regulatory agencies for compliance with maximum residue level (MRL) (European Commission, 2014), but are desirable when the data are to be used in exposure assessment. In most published studies, reported LOQs for multiresidue methods using either selective or mass spectrometry detectors are in the range of 10–50 μ g/kg (Aysal et al., 2007; Berrada et al., 2010; Hunter et al., 2010; Mol et al., 2007; Wang et al., 2013).

3.2. Residues in fruit samples

The on-going performance verification during sample extraction (See Table S4 in Supplementary Material) showed recoveries ranging from 60% to 140% in most cases, within the acceptable range for routine analyses (SANCO, 2012). The surrogate chlorpyrifos-methyl had recoveries below 70% in 6 samples, which required reanalysis. The identity of the pesticides found in the samples analysed by GC–FPD or GC– μ ECD was confirmed by the GC–MS/MS in all cases, confirming the selectivity of these detectors for the compounds investigated.

Almost 70% of the 238 samples analysed had at least one pesticide residue. Guava was the crop with the highest percent of positive samples (77.6%), and cashew apple with the lowest (27.9%). Almost all guava pulp samples (13), all peach pulp samples and 2 of the 11 cashew apple pulp samples were positive. Dithiocarbamates were the pesticides most present (33.2% of all samples, 46.5% of positive samples). Of the 45 pesticides and metabolites analysed by the validated multiresidue method, 23 were detected at least once. The 22 compounds not found in any sample were acrinathrin, aldicarb, aldicarb sulfone, aldicarb sulfoxide, azinphos methyl, bifenthrin, carbaryl, carbofuran, carbosulfan, disulfoton, ethion, fenamiphos, mevinphos, monocrotophos, pendimethalin, permethrins, phorate, pirimiphos-methyl, prothiophos, terbufos, trichlorfon and trifluralin. Dichlorvos was also not detected in any sample, but the method did not perform well for this compound.

Fig. 5 shows the pesticides most found in the fruit samples (including pulps). Dithiocarbamates were the compounds most found in kaki, cashew apple and peach, and λ -cyhalothrin the most found in guava. Dithiocarbamates were also the main pesticides found in the Brazilian monitoring programs (20% of all samples, Jardim & Caldas, 2012), which did not include the fruits analysed in this study. Residues of at least one organophosphorus or pyrethroid were detected in all positive samples. Only six samples contained *N*-methyl carbamates (methomyl or carbofuran-3OH). Among the pesticides analysed by the multiresidue method, dimethoate was present at the highest levels (up to 0.229 mg/kg

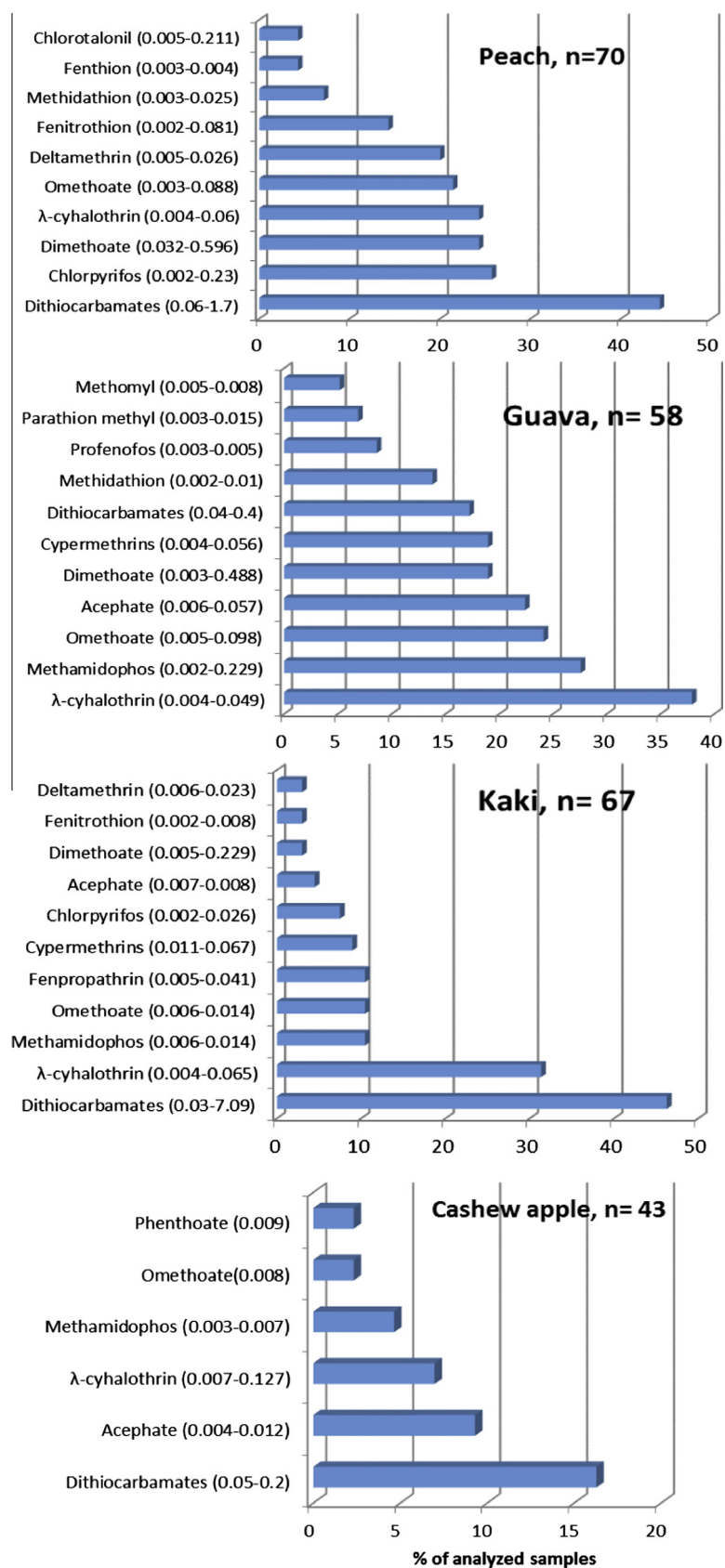


Fig. 5. Main pesticides (in % of analysed samples) found in the fruits and pulps (concentration range, mg/kg). All detected pesticides are shown for cashew apple.

in kaki). The highest dithiocarbamate concentration was found in a kaki sample (7.09 mg/kg CS₂). Fig. 6 shows the GC–FPD (Fig. 6A)

and GC– μ ECD (Fig. 6B) chromatograms of a cashew apple sample containing methamidophos, acephate and λ -cyhalothrin.

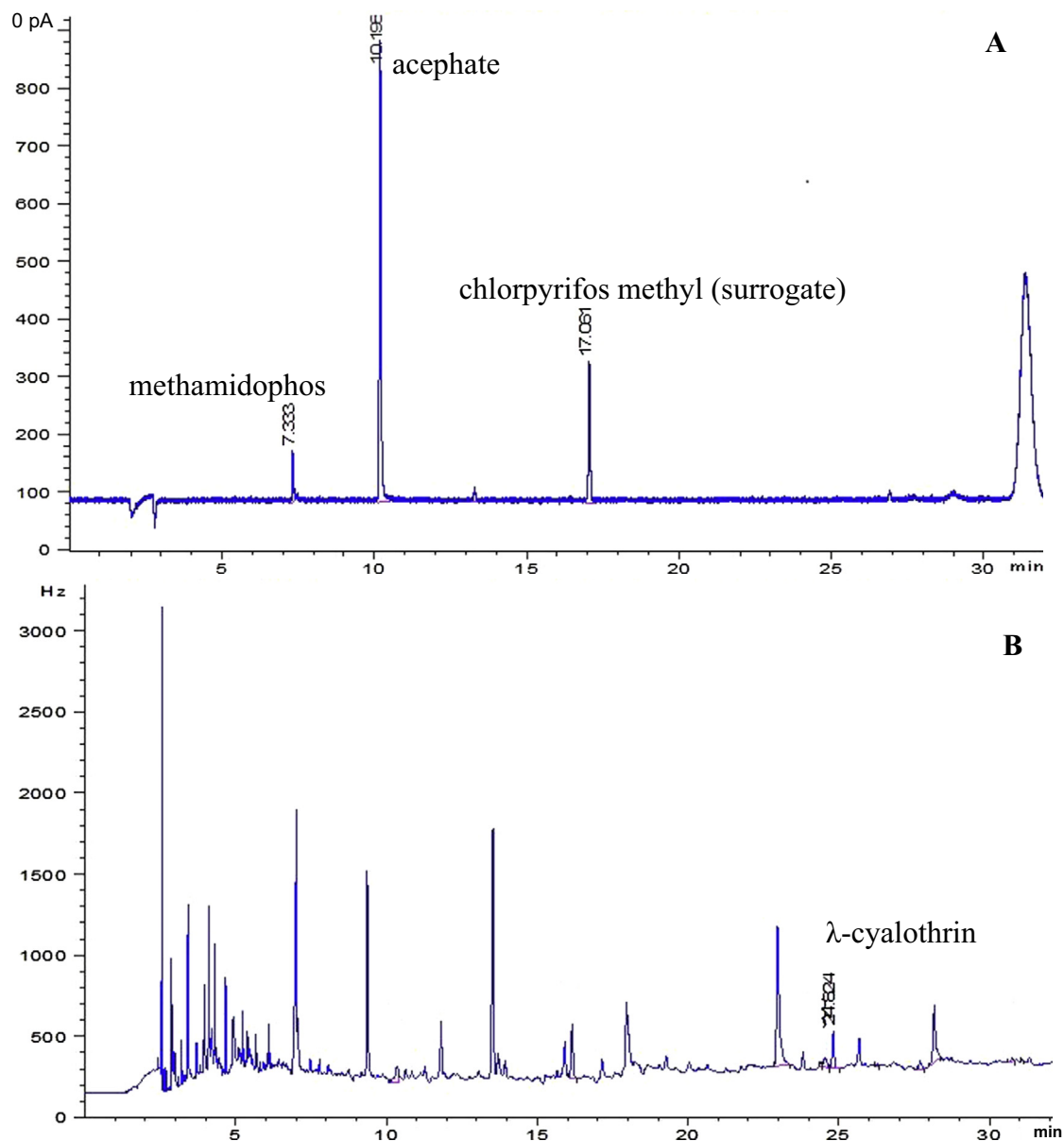


Fig. 6. Chromatogram of a cashew apple sample with incurred residues. (A) GC–FPD (OV-17 column: 50% diphenyl/50% dimethyl-polysiloxane), showing methamidophos at 7.33 min (3 µg/kg) and acephate at 10.198 min (41 µg/kg); (B) GC–μECD chromatogram (OV-5 column: 5% diphenyl/95% dimethyl-polysiloxane), showing the presence of λ-cyhalothrin at 24.82 min (4 µg/kg). The identities of the compounds were confirmed by GC–MS/MS.

In general, the pesticides and their levels found in the fruit and fruit pulp were similar. In Brazil, edible peel fruit pulp production includes washing in chlorinated water, seed removal, grinding, sieving, packing (generally 100 g in a plastic bag) and freezing. Washing the fruit should remove a significant amount of non-systemic pesticides, such as dithiocarbamates. Although the levels of dithiocarbamates found in guava fruit (0.16 ± 0.14 mg/kg CS₂; $n = 6$) were higher than that in guava pulp fruit (0.06 ± 0.10 mg/kg CS₂; $n = 4$), the difference was not significant ($p > 0.05$). Frozen fruit pulp is largely commercialised in Brazil, and used mostly for homemade juice.

The pesticides found in kaki, cashew apple and guava are not authorised for use on these crops in Brazil. Only four pesticides found in peach are registered (deltamethrin, fenthion, malathion and the dithiocarbamate mancozeb), and were present at levels below the Brazilian MRLs (ANVISA, 2014). The use of non-authorised pesticides is a major issue in the country, accounting for 72% of all irregularities found in Brazilian monitoring programs

(Jardim & Caldas, 2012). Of the 67 peach fruit samples collected, 34 were imported from USA or Chile, of which 47% had no residues detected. On the other hand, all the peach fruit and frozen pulp samples from national producers were positive for at least one pesticide, of which only five samples had authorised pesticide residues. These results are expected. Imported commodities generally contain fewer irregularities as exporters are more aware of trade barriers.

About 65% of the positive samples contained multiple residues of the various classes investigated (all whole fruit samples). Guava and peach had the highest percent of fruit samples with multiple residues among the positive samples (72–73%), followed by kaki (58.8%) and cashew apple (25%). Four peach samples had seven or eight different residues.

Table 2 summarises the information for the fruit samples containing multiple residues belonging to the organophosphorus and pyrethroid classes. In total, 59 samples (34.6% of positive samples) contained multiple residues of organophosphorus, mainly

Table 2

Fruit samples with multiple residues of organophosphorus and pyrethroid classes.

Food	Organophosphorus	Pyrethroids
Guava ^a	27 (17/2r, 6/3r, 4/4r)	5 (2r)
Peach ^a	19 (7/2r, 7/3r, 2/4r, 3/6r)	9 (2r)
Kaki ^a	10 (9/2r, 1/6r)	5 (2r)
Cashew apple ^a	3 (2/2r, 1/3r)	0
Main residues ^b	dim + ome (28) ^c	λ-cyhal + cyp (9)
	ace + meth (11) ^c	λ-cyhal + delt (6)

^a Number of samples/number of residues r.^b Number of samples in parenthesis.^c Some samples include other OP residues; dim = dimethoate; ome = omethoate; ace = acephate; meth = methamidophos; λ-cyhal = λ-cyhalothrin; cyp = cypermethrins; delt = deltamethrin.

guava samples. Almost half of these samples contained residues of dimethoate plus its metabolite omethoate. Methamidophos and acephate were present together in 11 samples. Methamidophos is a plant metabolite of acephate and a pesticide on its own. Registration for methamidophos was recently cancelled in Brazil, with the phase-out period ending in July 2012 (RDC 1/2011; ANVISA), after the samples for this study were collected. Hence, the methamidophos found in the samples could be either from the use of acephate or methamidophos. Nineteen samples of guava, peach and kaki had two pyrethroids, mainly λ-cyhalothrin combined with cypermethrins or deltamethrin (Table 3).

Pesticide residue data for kaki and guava are very scarce in the literature. In Spain, out of the 32 pesticides investigated in 24 kaki samples, only dithiocarbamates were detected (33.3% of the samples), at levels up to 0.12 mg/kg CS₂ (Berrada et al., 2010), much lower than what was found in this study. In India, mean levels of organophosphorus (including malathion) and pyrethroids (including cypermethrins) in guava were 0.05 mg/kg (Kumari, Madan, & Kathpal, 2006). To the best of our knowledge, this is the first time that monitoring residue data on pesticides are reported for cashew apple.

3.3. Cumulative acute dietary risk assessment

Organophosphorus and pyrethroids are neurotoxic pesticides, and compounds from each class have a common mechanism of action: organophosphorus compounds are acetylcholinesterase inhibitors (EPA, 2006) and pyrethroids interact with voltage-gated sodium channels (EPA, 2011). The acute intakes of these pesticide groups from the consumption of each of the fruits investigated in this study were calculated using the *IESTI* approach. Acephate and methamidophos were used as reference compounds for organophosphorus, and deltamethrin for pyrethroids. Relative potency factors (*RPF*) were applied to the residues of each class found in the samples to normalise to the reference compounds and estimate the

highest total residue (*HR_t*). As the *HR_t* were always from samples containing multiple residues in whole fruit, this assessment does not apply to fruit pulps.

A summary of the data and the risk assessment results are shown in Table 3. The highest percentages of *ARfD* for organophosphorus and pyrethroid exposures were found for guava, 45% (acephate as the reference compound) and 12%, respectively. The results indicate that the cumulative acute exposure to organophosphorus or pyrethroids from the consumption of guava, kaki, peach or cashew apple is unlikely to represent a health concern. It is important to emphasise that this assessment does not apply to individuals younger than 10 years old, as consumption data for this population are not available in the country.

The *IESTI* concept was developed to estimate the acute exposure to a single pesticide residue using data from supervised trials, and its application to cumulative exposure using monitoring data has some limitations. Most of all, the variability factor used in the *IESTI* equation reflects the unit-unit variability in a single lot (FAO, 2003), and samples collected in the market do not necessarily come from the same lot. Furthermore, the multiple residues found in the samples (a composite of about 10 units of each crop per sample) analysed in this study may have come either from the application of multiple pesticides to a single field (lot) or from the application of different pesticides to different fields (various lots).

4. Conclusion

A multiresidue method for the analysis of 45 pesticides and metabolites using GC-FPD, GC-μECD and LC-MS/MS in kaki, cashew apple, guava and peach was successfully validated. Residue data obtained in this study indicated that good agricultural practices are not being followed by Brazilian farmers, since most of the pesticides found in the samples are not authorised for use on the crops. Guava, peach, kaki and cashew apple are seasonal fruits highly consumed in the country during certain periods, when their availability is high and the price more affordable. High consumers (of 10 years or older) of these fruits treated with organophosphorus or pyrethroid pesticides considered in this study are unlikely to be at risk. The data showed the need to include these fruits in the scope of the Brazilian pesticide monitoring programs for *MRL* enforcement and for acute and chronic dietary exposure assessments. Individual consumption data for younger children are necessary in the country to allow the dietary intake estimation of pesticides for this population group.

Funding

This work was financially supported by the Funding Authority for Studies and Projects (FINEP 01.08.0288.00) and the National Council of Scientific and Technological Development (CNPq).

Table 3Cumulative acute risk assessment of organophosphorus (OP) and pyrethroid (Py) pesticides in fruits. Large portion (*LP*) is given in kg/person.

	Guava <i>LP</i> = 0.87	Peach <i>LP</i> = 0.34	Kaki <i>LP</i> = 0.42	Cashew apple <i>LP</i> = 1.6
<i>OPs detected^a</i>	<i>dim, ome</i>	<i>dim, ome, met</i>	<i>ace, meth, dim, ome, chlor,</i>	<i>ace, meth, phen</i>
<i>HR_t</i> , mg/kg	2.31	2.68	1.03	0.22
% <i>ARfD_{ace}</i>	45	21	10	7
<i>HR_t</i> , mg/kg	0.187	0.216	0.09	0.11
% <i>ARfD_{meth}</i>	36	17	8.6	32
<i>Py detected^a</i>	<i>cyp, λ-cyhal</i>	<i>fen, λ-cyhal</i>	<i>cyf, fen</i>	<i>λ-cyhal</i>
<i>HR_t</i> , mg/kg	0.060	0.070	0.077	0.012
% <i>ARfD_{delt}</i>	12	6	7	3

^a In the sample with the highest total residue (*HR_t*); dim = dimethoate; ome = omethoate; met = methidathion; ace = acephate, meth = methamidophos, chlor = chlorpyrifos; phen = phenothate; fen = fenprothrin; cyp = cypermethrins; cyf = cyfluthrin; λ-cyhal = λ-cyhalothrin; delt = deltamethrin; *ARfD_{ace}* = 0.1 mg/kg bw; *ARfD_{meth}* = 0.01 mg/kg bw; *RPF_{ace}* = 3.96 for dim, 11 for om, 12.4 for meth, 0.67 for chlor and 0.58 for phen; *RPF_{meth}* = 0.32 for dim, 0.89 for om, 0.08 for ace, 0.05 for chlor and 0.07 for phen; *RPF_{delt}* = 0.19 for cyp, 1.15 for cyf, 1.63 for λ-cyhal and 0.5 for fen; *ARfD_{delt}* = 0.01 mg/kg bw.

401754/2010-3). A.N.O. Jardim was supported with a PhD scholarship from CNPq. F.C.S. Goes and D.C. Mello were supported by master scholarships from the Coordination for the Improvement of Higher Education Personnel (CAPES). E.F. Frota Jr. received a training grant from the University of Brasília.

Notes

The authors declare no competing financial interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.foodchem.2014.05.030>.

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