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Pesticide residues analysis in passion fruit and its processed products by LC–MS/MS and GC–MS/MS: Method validation, processing factors and dietary risk assessment

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ABSTRACT

A method for the determination of 80 pesticides (including five metabolites) in passion fruit using ethyl acetate extraction and dispersive solid-phase extraction followed by LC–MS/MS and GC–MS/MS was validated at LOQ of 0.005 or 0.010 mg kg^{-1} (70 to 120% recovery; RSD $\leq$ 20%). Fifty-five passion fruit samples were obtained from producers, and 30 samples of frozen pulp and 12 samples of flour purchased. About 27% of the pesticides were detected; at least one in 60% of the peel samples, mainly imidacloprid and carbendazim (max. of 0.274 mg kg^{-1}). Median processing factor was 0.5 for washed peel and 6.5 for dried peel (flour). About 63% of frozen pulp samples were positive, and 4 flour samples contained residues, mainly methamidophos. About 70% of the detected pesticides are not authorized in passion fruit in Brazil. Chronic and acute exposure from the consumption of passion fruit products did not indicate a health concern.

1. Introduction

The genus *Passiflora* is the largest of the *Passifloraceae* family and includes more than 500 species, which are known for their edible fruits (passion fruit), ornamental flowers and pharmaceutical properties (Panelli et al., 2018). Brazil is the world's largest producer and consumer of passion fruit , with at least 140 native species, mainly *Passiflora edulis*, which accounts for 98% of the Brazilian production (Ferreira, 2005). Passion fruit juice is the main industrial product, and the peel, responsible for approximately 60% of the total fruit weight, has been the focus of studies for its good nutritional value, including soluble fiber (pectins and mucilages), vitamin B3, calcium, and phosphorus (Córdova, Gama, Winter, Neto, & Freitas, 2005). Flour made of passion fruit peel has functional properties in metabolic syndromes, reducing cholesterol levels, glycated hemoglobin and triglycerides and increase in HDL cholesterol in type II diabetes mellitus patients (Claro, Rodrigues, & Teixeira, 2018; Janebro et al., 2008).

In Brazil, 33 pesticides from different chemical groups are registered for use in passion fruit, including mancozeb and metiram (dithiocarbamates), imidacloprid (neonicotinoids), deltamethrin (pyrethroid), difeconazole (triazole), and pyraclostrobin (strobilurin) (ANVISA, 2021a). Monitoring the residue levels of these compounds in food is important to verify the use of good agricultural practices in the field, including only the use of pesticides registered in the crop and residues not higher than the maximum residue limit (MRL) (ANVISA, 2021a). Additionally, the results are used to assess human exposure to pesticides and the potential health risks (Jardim, Brito, van Donkersgoed, Boon, & Caldas, 2018; Jardim, Mello et al., 2018).

Different methods have been used for analyte extraction and sample clean-up for pesticide determination. The QuEChERS (quick, easy, cheap, effective, rugged, and safe) method was primarily developed using acetonitrile extraction, salt partitioning and dispersive solid-phase extraction (d-SPE) with PSA (primary-secondary amine) for analysis by liquid chromatography–triple-quadrupole mass spectrometry (LC–MS/MS) (Anastassiades, Lehotay, Stajnbaher, & Schenck, 2003; Kemmerich, Demarco, & Bernardi, 2018; Musarurwaa, Chimukab, Pakadec, & Tavengwa, 2019; Kandaswamy, Anandaram, Presley, & Shabeer, 2021). Ethyl acetate extraction with d-SPE has also been used, which can improve the extraction of less polar compounds to be analyzed by gas chromatography–-triple-quadrupole mass spectrometry (GC–MS/MS)

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(Chen et al., 2011; Jardim, Mello, Goes, Frota Junior, & Caldas, 2014; Grande-Martinez, Arrebola-Liebanas, Martinez-Vidal, Hernandez-Torres, & Garrido-Frenich, 2016). However, very few studies analyzed pesticide residues in passion fruit, and included samples originating from Colombia (Hjorth et al., 2011; Botero-Coy, Marín, Ibáñez, Sancho, & Hernández, 2012) and India (Narenderan, Meyyanathan, Karri, Babu, & Chintamaneni, 2019). The European Union includes passion fruit in its monitoring programs, but the origin of the samples is not stated in the report (EFSA, 2021). In the only study conducted in Brazil with passion fruit, only dithiocarbamate fungicides were analyzed (Mozzaquatro et al., 2019).

The presence of pesticides in food commodities, however, does not alone imply a potential risk to consumers, requiring the conduction of dietary risk assessment studies. In this process, in addition to residue data, the amount of food consumed by a given population and the health-based guideline level (HBGL) of the pesticide are also considered (Caldas & van der Velde-Koerts, 2017, 2017). Furthermore, processing factors are important to determine the levels of residue in the food as consumed (FAO, 2016).

This study aimed to validate a multi-residue method to determine 80 pesticides (including five metabolites) in passion fruit peel and pulp by LC–MS/MS and GC–MS/MS; analyze passion fruit samples obtained from producers in different regions of Brazil and frozen pulp and passion fruit flour commercialized in the country; determine processing factors for flour production (washing and drying the peel) and conduct a dietary risk assessment of the Brazilian population from the consumption of passion fruit juice and flour.

2. Material and methods

2.1. Chemicals and reagents

Ethyl acetate (EtAc), acetonitrile (ACN) and HPLC-grade methanol (MeOH) were purchased from Merck (Darmstadt, Germany), and acetone from Dinâmica (Brazil). HPLC-grade toluene was purchased from Mallinckrodt Baker (Phillipsburg, NJ), acetic acid and sodium acetate anhydrous (H_3COONa, 99.5%) from J.T. Baker (Phillipsburg, NJ), magnesium sulfate anhydrous ($MgSO_4$) from Sigma-Aldrich (≥99.5 %) (St. Louis, MO), PSA from Supelco (Bellefonte, PA), and ammonium formate from Fluka (Buchs, Switzerland).

Analytical standards (94 to 100% purity) of 80 pesticides (including five metabolites) investigated in the study were purchased from Accu Standard (USA). Except for the metabolites aldicarb sulfoxide and omethoate, and the pesticides carbendazim and EPN (*O*-ethyl *O*-(4-nitrophenyl) phenylphosphonothioate), all were certified reference standards (ISO 17034). Individual stock solutions of the analytes (1 mg mL^{-1}) were prepared in 10 mL (calibrated volumetric flasks with "A" certification) of toluene, acetone, MeOH, or EtAc, according to the solubility and stability of each compound. Mixed working solutions (10 ng μL^{-1}) of 50 analytes determined by LC–MS/MS (carbamates, triazoles, organophosphates and other chemical groups; Table S1, Supplementary Material) were prepared in methanol; 30 analytes determined by GC–MS/MS (organophosphates, pyrethroids and other chemical groups; Table S2) were prepared in EtAc. The mixed solutions were diluted to prepare working solutions, and all solutions were stored in amber vials at –15 °C.

2.2. Extraction and clean-up

The method used in this study was based on that developed by Jardim et al. (2014), which uses EtAc as extraction solvent. In summary, 15 g of previously homogenized sample (passion fruit peel or pulp) were weighed in a 50-mL Falcon tube, 15 mL of EtAc (1% acetic acid) added, the tube manually shaken, 6 g of $MgSO_4$ and 1.55 g of H_3COONa added, followed by shaking and centrifugation (3500 rpm for 5 min.). Six milliliters of organic phase were transferred to a 15-mL Falcon tube containing PSA and $MgSO_4$ (300 and 900 mg, respectively), followed by manual vigorous shaking for about 1 min; 1.5 mL of extract were transferred to a vial, evaporated under N_2 at room temperature, the residues were resuspended in 300 μL MeOH:H_2O (50:50) and filtered with a 0.45 μm filter for analysis by LC–MS/MS, or resuspended in EtAc for analysis by GC–MS/MS. The passion fruit peel flour samples (2 g) were previously hydrated with 13 mL of Milli-Q water for 30 min before analysis.

2.3. Instrumentation

LC–MS/MS analyses were performed on the Shimadzu UFLC system (LC-20AD/SIL-20AC; Kyoto, Japan), coupled to a 4000QTRAP triple quadrupole mass spectrometer (SCIEX, Framingham, MA), with Turbo Ion Spray source with ESI ionization (electrospray ionization) in positive mode. Data were acquired by the software Analyst® V 1.5.2 (SCIEX). MS/MS parameters for each analyte were optimized by direct infusion of analytical standard solutions (50–200 ng mL^{-1}; dissolved in MeOH/H_2O, containing ammonium formate, 5 mM), at a flow rate of 10 μL/min. A Synergi 4u Fusion RP 80 A 50 × 2.00 mm, 4 μm column (Phenomenex) with precolumn (Fusion-RP 4x2.0 mm) was used. The oven operated at 40 °C, and the mobile phases were: (A) water: ACN (80:20) + 5 mmol L^{-1} ammonium formate and (B) MeOH + 5 mmol L^{-1} ammonium formate. The flow rate was 0.25 mL/min, with an elution gradient from 0 to 10 min to 90% B, maintained between 10 and 13 min, and in 13–13.1 min it returned to 0% B. The total analysis time was 18.1 min. Data were acquired in Scheduled MRM (multiple reaction monitoring) mode. Ion source optimization conditions were: 20 psi curtain gas; high nitrogen gas collision; ion spray with a voltage of 4500 V; temperature 450 °C; 40 psi ion source gas (GS1 and GS2). For each analyte, two transitions were selected, one of quantification and one of qualification. The parameters of the 50 analytes in the LC–MS/MS system are shown in Table S1 (Supplementary Material).

The GC–MS/MS system used was the Trace GC Ultra (Thermo Scientific) with a programmed vaporization temperature (PTV) injector coupled to a triple quadrupole mass spectrometer (TSQ Quantum XLS; Thermo Scientific). A TR-Pesticide II column (Thermo Scientific) 30 m × 0.25 mm × 0.25 μm ID, preceded by a guard column (5 m × 0.25 mm), was used, with a flow of 1.2 mL/min of helium gas (99.99% purity). The mass spectrometer was operated in positive ionization mode, and data were acquired using selected reaction monitoring, SRM). Argon (1.5 mTorr) was used as collision gas and ion source temperature was 250 °C. The injection volume was 1 μL in splitless mode. Initially, analytical standard solutions at 1000 ng mL^{-1} were injected into the mass spectrometer and data acquired in full scan mode. For each analyte, two product ions were selected, one for quantification and one for qualification, from the same precursor ion or from different precursor ions. Table S2 shows the optimized parameters for determination of 30 analytes in the GC–MS/MS system.

2.4. Method validation

Method validation was performed for passion fruit pulp and peel according to the European Union recommendations (SANTE, 2019). No validation was necessary for flour (dried peel) as the samples were hydrated before analysis. Organically grown passion fruit samples from Bahia and the Federal District were used as controls. Selectivity was evaluated by analyzing the LC–MS/MS and GC–MS/MS chromatograms of fortified and unfortified control samples (peel and pulp), checking the existence of interferents with the same retention time, the transition ions, and their ratios as the pesticides of interest. Linearity was evaluated for each analyte at five concentration levels, three replicates, using external matrix-matched calibration, i.e., the control sample extract was fortified (post extraction) with appropriate working mixed solution volumes containing the analytes (10 ng μL^{-1}). Studies conducted by our and other research groups have shown that matrix effects due to co-

extractives are commonly observed in multiresidue LC–MS/MS (mainly signal suppression) and GC–MS/MS (mainly signal enhancement) methods for pesticides in food (Jardim et al., 2014; Kandaswamy et al., 2021). Hence, matrix-matched calibration curves were used to compensate for any matrix effects that affected instrument response. Linear regression parameters were estimated by the ordinary least squares method, the presence of outliers verified by the Grubbs test, the homogeneity of the variances by the Cochran test and the coefficient of determination (R^2) and the significance of the regression obtained by ANOVA. For heteroscedastic data, different weighting factors were tested and the one that produced the smallest sum of relative errors was chosen for the regression. The concentrations of the calibration curves were 20, 50, 100, 300 and 500 ng mL^{-1} on the LC–MS/MS and 20, 50, 100, 500 and 1100 ng mL^{-1} on the GC–MS/MS.

The analyte recovery tests in each matrix were evaluated at levels of 0.005, 0.010, 0.020 and 0.100 mg kg^{-1} for LC–MS/MS and 0.005, 0.010 and 0.050 mg kg^{-1} for GC–MS/MS analysis, expressed in %. The experiment was performed with five replicates on the same day and by the same analyst, and repeatability expressed as relative standard deviations (%RSDr). Intermediate precision was assessed by repeating the experiment on different days (% RSDp). For each pesticide, the limit of quantification (LOQ) was defined as the lowest level for which the method was satisfactorily validated (recovery between 70 and 120%; RSDr and RSDp $\leq$ 20%).

2.5. Samples

Fifty-five samples of passion fruit (*P. edulis*) cultivated using a conventional system were obtained directly from 51 producers in the Brazilian states of Rio de Janeiro, Goiás and Bahia, and in the Federal District, from February 2016 to February 2018, covering two production seasons per year. Collection detail is described elsewhere (Mozzaquatro et al., 2019). Upon arrival at the laboratory, the sample units were manually separated into peel, pulp and seed with the aid of a knife and a sieve, avoiding contact between the pulp and the outer part of the peel, and the seeds were discarded. The peels were cut in small pieces and frozen before homogenized in a blender. The pulps were also frozen before homogenization and both matrices were kept at –15 °C until the analysis.

Additionally, 30 samples of frozen passion fruit pulp from 11 different brands and 12 samples of peel flour from 11 different brands were purchased in supermarkets and food stores. The frozen pulp samples were kept at –15 °C and the flour samples at room temperature (23–25 °C) until the analysis.

To assess the performance of the validated method in routine analysis, quality control samples (QC) containing all the analytes, with two authentic replicates at two fortification levels (0.005 and 0.010 mg kg^{-1}) for each matrix, were included in the analysis batches. The pesticide concentration in the total fruit for each sample was calculated from the pesticide concentrations detected in the peel and pulp samples and the weight of each portion. To calculate the concentration in the fruit, residues detected at levels below the LOQ of the method were considered as ½ of the LOQ. Residues were reported at trace levels when detected at < LOQ. Samples of commercial passion fruit pulp and flour were analyzed only by LC–MS/MS.

2.6. Processing factors

Unwashed fruits that showed peel with quantified results ($\geq$ LOQ) were used to produce passion fruit flour. Fruits were left in a hypochlorite solution (2%) for 5 min, the peel manually separated and analyzed to determine the processing factor (PF) for washing (unwashed peel → washed peel). A portion of the washed peel was dried in an industrial dryer with hot air circulation at an average temperature of 60 °C for about 10 h (average moisture content of 10%), according to the patented procedure by Srur (2003). The dried peels were blended and sieved to obtain the flour, which was analyzed to determine the PF of washed peel → flour. PF is defined as the ratio between pesticide concentrations in the sample after and before food processing (FAO, 2016).

2.7. Dietary risk assessment

Chronic and acute intakes of pesticides by the consumption of passion fruit juice (prepared from commercial frozen pulp) and of commercial flour were estimated by the deterministic method for pesticides detected at levels $\geq$ LOQ in at least one sample. Intake is equal to pesticide concentration × consumption per body weight (FAO, 2016; Caldas & van der Velde-Koerts, 2017). Passion fruit juice consumption and body weight (bw) were obtained from the 2017/2018 Household Budget Survey (HBS) conducted by the IBGE (Brazilian Institute for Geography and Statistics) with 46,164 individuals from 10 years of age, considering the sample expansion factor to represent the entire Brazilian population (IBGE, 2020).

For chronic exposure assessment, the average consumption of passion fruit juice (9.4 g/person), the average body weight of the entire population of HBS 2017/2018 (69.0 kg; n = 46,164) and the average concentration of pesticides found in the samples were used to estimate the intake. For acute exposure assessment (within 24 h), the 97.5 percentile of consumption (97.5P; 720 g/person), the average body weight of participants who reported passion fruit juice consumption (63.5 kg; n = 3,090), and the maximum concentration found in the samples were used. The estimated pesticide intake from juice consumption considered a pulp dilution factor of 70%, which was applied to the residue levels quantified in the commercial frozen pulp samples.

The estimated chronic and acute pesticide intakes from commercial passion fruit flour considered the consumption recommendation on the product labels, which ranged from 1 to 2 tablespoons (15 to 30 g) 1–3 times a day (15 to 90 g/day), with an average of 48 g/person and a 97.5P of 87 g/person, and average body weight estimated by HBS 2017/2018 (69.0 kg).

Risk of exposure was assessed by comparing the estimated intake with the HBGL for chronic (acceptable daily intake, ADI) or acute (acute reference dose, ARfD) exposure established by the Food and Agriculture Organization/World Health Organization Joint Meeting on Pesticide Residues (FAO/WHO JMPR, 2021). Risk may exist when intake is greater than the ADI or the ARfD.

3. Results

3.1. Validation of the multi-residue method by LC–MS/MS

The chromatograms of the peel and pulp matrix controls did not show any interference peak eluting at the same retention time of the analytes under evaluation (Table S1), indicating satisfactory selectivity of the method. The matrix-matched analytical curves obtained by the least squares method showed homoscedasticity behavior ($C_{calc} < C_{tab}$; 5;3) for 39 analytes. For those that had heteroscedastic behavior ($C_{calc} > C_{tab}$; 5.3), different weighted linear regressions were tested (Miller & Ambrus, 2000), and the best weighting factors were selected: $1/x$ for carbendazim, carbofuran and pyrymicarb, $1/x^2$ for aldicarb, azoxystrobin, boscalide, dimethoate and paraoxom-methyl and $1/y^2$ for aldicarb sulfoxide, methiocarb, and buprofezin. Coefficients of determination (R^2) were greater than 0.99 for all analytes, regressions were significant (p less than 0.05), and there was no lack of adjustment for the regressions used in calibration procedures. Figure S1 shows the LC–MS/MS ion chromatogram of a passion fruit pulp matched matrix analytical curve point at 100 ng mL^{-1}.

Fig. 1 summarizes the results of recovery, repeatability (RSDr) and intermediate precision (RSDp) obtained during validation at different concentrations (0.005 to 0.100 mg kg^{-1}) for the 50 analytes in passion fruit peel analyzed by LC–MS/MS. The results for each analyte are shown in Table S3 (Supplementary Material). Most analytes had

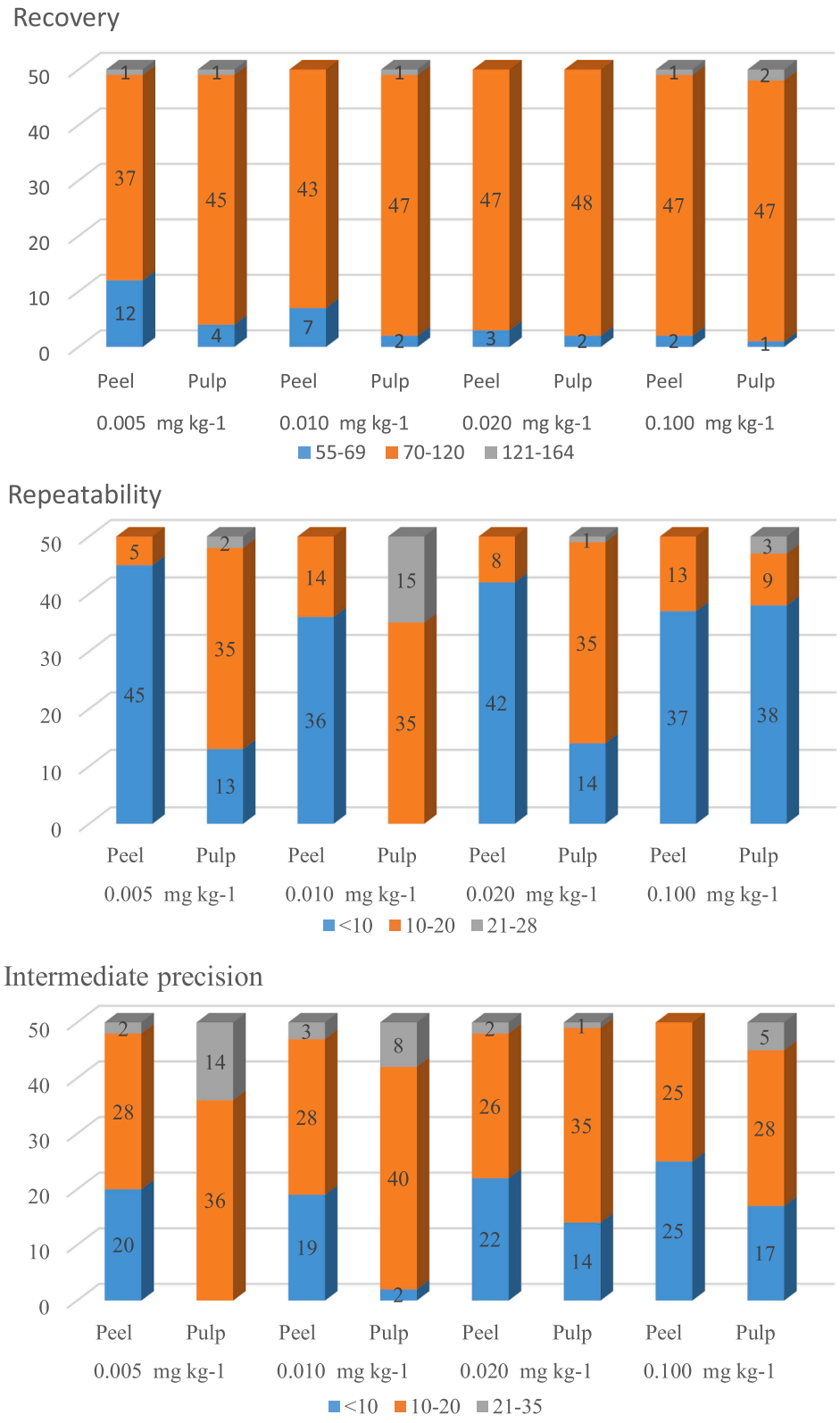

Fig. 1. Summary of the validation data for the determination of 50 pesticides in passion fruit peel and pulp by LC–MS/MS, showing the number of compounds within each range.

recoveries between 70 and 120% and precision (RSDr and RSDp) of less than 20%. For 17 analytes, the mean recovery was less than 70% in at least one level, with the lowest percentage of recovery (55%) found for chlorpyriphos and diazinon at the lowest fortification level (Table S3). Recoveries were greater than 120% for methidathion at the lowest and higher levels (164 and 133%). Repeatability was less than 20% for all analytes at all levels, and intermediate precision was greater than 20% (21–28%) at least at one level for six analytes (aldicarb-sulfoxide, boscalide, carbofuran-3-OH, kresoxim methyl, methidathion and diazinon (Table S3). The LOQ for the peel matrix was defined as 0.005 mg kg^{-1} for all analytes, except diazinon, chlorpyriphos, and methidathion, which was defined as 0.010 mg kg^{-1}.

Fig. 1 also summarizes the results of the passion fruit pulp validation data and the individual values are shown in Table S4. Average recoveries less than 70% in at least one fortification level were found for four analytes, with methamidophos showing the lowest recoveries (63 to 65%), and the average recovery for trichlorfon was 143% in the highest level. Average repeatability between 22 and 26% was obtained for pirimiphos, tebuconazole and trichlorfon at one fortification level, and average intermediate precision above 20% for twenty analytes, with kresoxim methyl and methiocarb showing the highest values at the lowest fortification level (29 and 30%), and omethoate and trichlorfon at the highest level (30 and 35%). The deviations from the validation parameters were considered acceptable and the LOQ was established as 0.005 mg kg^{-1} for all analytes in the passion fruit pulp matrix.

3.2. Validation of the multi-residue method by GC–MS/MS

Control samples of passion fruit peel and pulp did not show the presence of interfering peaks at the same retention time as the 30 analytes analyzed by GC–MS/MS (Table S2), indicating good selectivity of the method. The matrix-matched analytical curves showed homoscedastic behavior ($C_{calc} < C_{tab}$; 5;3) for all analytes, coefficients of determination (R^2) were greater than 0.99, regressions were significant (p less than 0.05), and there was no lack of adjustment for the regressions used in the calibration procedures.

A summary of validation data for passion fruit peel and pulp matrices by GC–MS/MS are shown in Fig. 2, and the results for each analyte are shown in Tables S5 and S6. Average recoveries below 70% (values between 61 and 69%) in peel were obtained for 4 analytes and none had average recovery above 120%. Intermediate precision was satisfactory for most analytes, except fenthion, λ-cyalothrin and parathion methyl (between 22 and 24% in at least one of the fortification levels).

For passion fruit pulp matrix, average recovery of less than 70% was obtained only for azinphos methyl (69%) (Table S6). Repeatability was less than 20% for all analytes, except of azinphos methyl (31% at the highest fortification level) and cyfluthrin (33 and 21% at the lowest and highest fortification level, respectively). RSDp values were less than 20% with the exception of 4 analytes (up to 32% for chlortiophos at the 0.010 mg level kg^{-1}). A LOQ value of 0.005 mg kg^{-1} was defined for the 30 analytes analyzed in passion fruit peel and pulp matrices by GC–MS/MS..

3.3. Passion fruit samples

Of the 80 analytes evaluated by the multi-residue method validated in this study, 19 were detected, of which 12 were by LC–MS/MS (24% of the 50 investigated analytes) and seven by GC–MS/MS (23.3% of the 30 investigated analytes). The results of the QC samples (0.005 and 0.010 mg kg^{-1}) included in each extraction batch were within the acceptable range for recoveries and repeatability, confirming the method performance during the routine analysis on the two systems.

Table 1 summarizes the results of positive samples (at least trace levels) in peel and pulp of passion fruit collected in different regions of the country and Table S7 (Supplementary Material) shows the levels of residues present in each positive sample. About 60% of the 55 peel passion fruit samples analyzed were positive for at least one pesticide (Table S7). Imidacloprid was the analyte with the highest detection frequency, present in 40.6% of positive peel samples ($n = 13$), with a maximum concentration of 0.071 mg kg^{-1}. The second highest detection frequency was found for carbendazim (37.5%; maximum 0.148 mg kg^{-1}), followed by methamidophos (25%; 0.274 mg kg^{-1}). Acephate and cypermethrin were present at the highest level (~0.5 mg kg^{-1}).

About 25% of the pulp samples ($n = 14$) showed residues of at least one of the pesticides detected in the peel (Table S7). Concentrations varied between traces (<LOQ) and 0.090 mg kg^{-1} (acephate). For all samples analyzed, the levels detected in the pulp were lower than those of the peel, representing on average 13.8% of the concentration in the five quantified samples. One of the passion fruit peel samples analyzed contained seven analytes, being two pyrethroids (cypermethrin and λ-cyalothrin), two triazoles (difenoconazole and tebuconazole), two organophosphates (dimethoate and omethoate) and one neonicotinoid (imidacloprid) (Table S7). When the pulp of this fruit was analyzed, only cypermethrin (0.010 mg kg^{-1}) and traces of imidacloprid (<LOQ) were detected. Nine peel samples had 4 or 5 analytes (Table S7).

The maximum concentrations estimated in the fruit from the concentrations in the peel and in the pulp ranged from traces (pyraclostrobin and tebuconazole) to 0.366 mg kg^{-1} (cypermethrin) (Table 1).

3.4. Processing factor

A total of 24 samples of unwashed fruits containing residues in the peel at ≥ LOQ levels were subjected to wash and drying to estimate PFs for washed peel and for flour. The results are shown in Table 2. During fruit washing, the PF (unwashed peel → washed peel) of the five organophosphate insecticides evaluated ranged between 0.1 and 0.7, with a median for the group ($n = 17$) of 0.5 (residue reduction of 50%). PF for the dehydration process to produce flour (washed peel → flour) for organophosphates ranged between 1.9 and 10, indicating the concentration of residues, with a median PF of 4 for the group. Residue reduction in the peel after washing and concentration after dehydration were also estimated for carbendazim (median PF of 0.4 and 6.6, respectively) and imidacloprid (median 0.6 and 7.6, respectively). When considering all pesticides, the median PF was 0.5 for washing and 6.5 for drying ($n = 32$).

3.5. Commercial product samples and risk assessment

Table 3 shows the eleven analytes detected (at least traces) in 19 of the 30 frozen passion fruit pulp samples analyzed by LC–MS/MS (63%) and Table S8 details the results. Carbendazim was the most frequent pesticide, detected in 68.4% of positive samples, followed by imidacloprid and acephate (63.2 and 52.6% of positive samples, respectively). The carbendazim concentration reached 0.098 mg kg^{-1}, being the maximum concentration found among the analyzed samples. Most samples had more than one analyte detected, reaching six analytes in one of them (carbendazim, imidacloprid, tebuconazole, malathion, pyraclostrobin, and trifloxystrobin; Table S8). Fig. 3A shows a chromatogram of a frozen pulp sample containing four pesticides.

Of the 12 commercial passion fruit flour samples analyzed by LC–MS/MS, four contained pesticide residues, with methamidophos present in all positive samples at a maximum concentration of 0.050 mg kg^{-1} (Table 3; Table S8). Two samples also contained imidacloprid or pirimiphos methyl. Fig. 3B shows a chromatogram of a flour sample containing two pesticides.

The chronic and acute risk assessments through passion fruit juice consumption were estimated for the compounds shown in Table 3, except azoxystrobin (traces) and pirimiphos-methyl (not detected). The results are shown in detail in Table S9 (Supplementary Material). Chronic intake ranged from 9×10^{-9} mg kg^{-1} bw (trifloxystrobin) to 5×10^{-7} mg kg^{-1} bw (carbendazim), with no significant contribution to the ADI of each compound (0.00% ADI); the acute intake ranged from

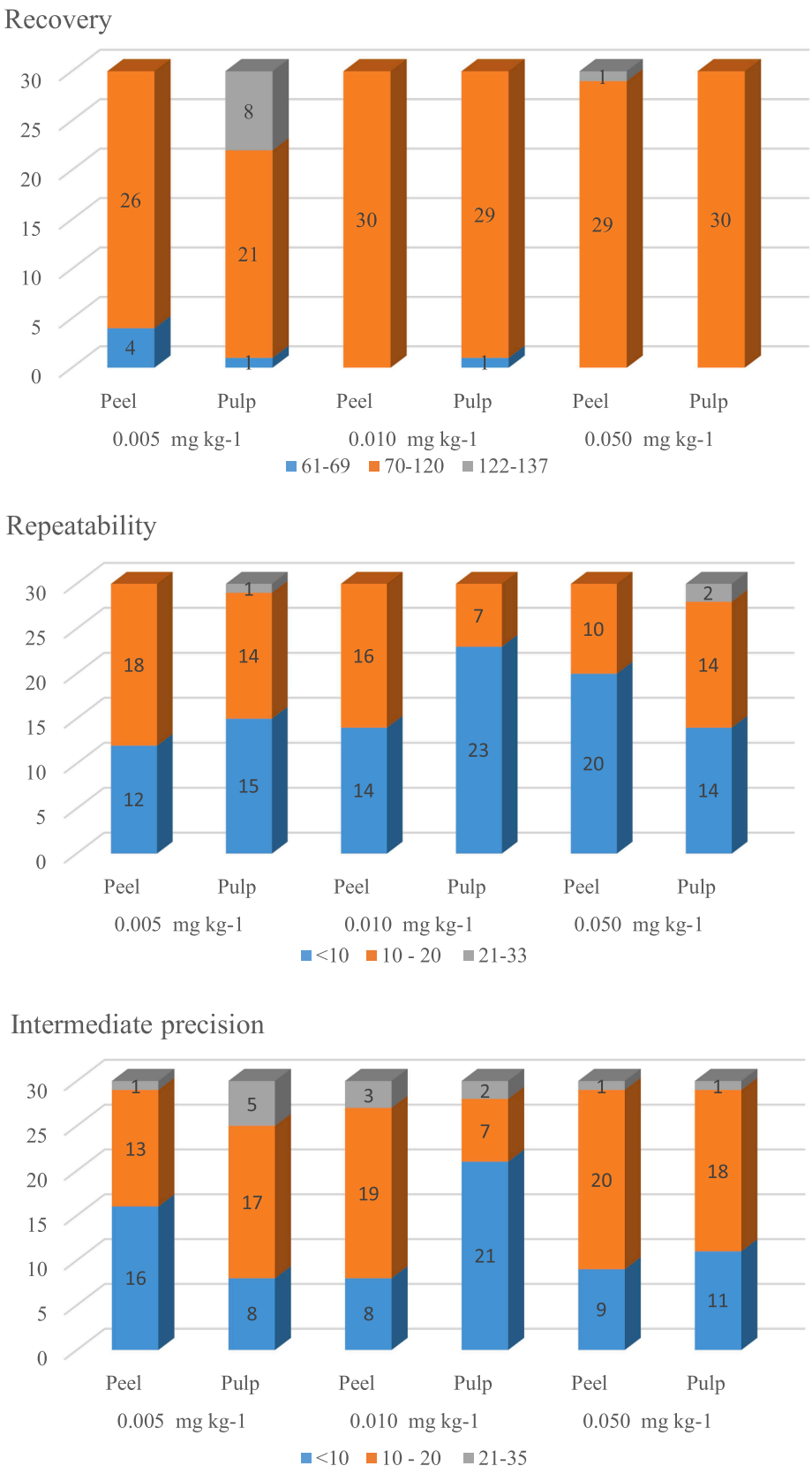

Fig. 2. Summary of the validation data for the determination of 30 pesticides in passion fruit peel and pulp by GC–MS/MS, showing the number of compounds within each range.

Table 1
Pesticides detected in 32 of the 55 passion fruit samples analyzed by LC–MS/MS or GC–MS/MS.

		Peel			Pulp			Fruit[b] Max., mg kg^{-1}
Pesticida		≥ LOQ, n	Traces,n[a]	Max., mg kg^{-1}	≥ LOQ, n	Traces,n[a]	Max., mg kg^{-1}	
Imidacloprid	LC	11	2	0.071	7	1	0.008	0.056
Carbendazim	LC	10	2	0.148	nd	1	nd	0.069
Methamidophos	LC	6	2	0.274	1		0.03	0.163
λ-Cyalothrin	GC	6	1	0.037	nd		nd	0.027
Acephate	LC	6		0.522	5		0.090	0.248
Chlorothalonil	GC	5		0.053	1		0.005	0.027
Chlorfenapyr	GC	5		0.046	nd		nd	0.023
Deltamethrin	GC	3		0.017	nd		nd	0.013
Cypermethrin	GC	3		0.528	3		0.098	0.366
Omethoate	LC	3		0.031	nd		nd	0.023
Dimethoate	LC	2		0.171	nd		nd	0.125
Tebuconazole	LC	2		0.019	nd		nd	0.013
Methomyl	LC	1	2	0.013	nd		nd	0.006
Difenoconazole	LC	1	1	0.005	nd		nd	traces
Profenophos	LC	1		0.014	nd		nd	0.009
Malathion	LC	1		0.022	nd		nd	0.012
Esfenvalerate	GC	1		0.023	nd		nd	0.005
Fenvalerate	GC	1		0.043	nd		nd	0.010
Pyraclostrobin	LC		1	Traces	nd		nd	traces

n = number of positive samples; nd = not detected; [a] < LOQ; [b] trace levels were considered at ½ LOQ and samples with non-detected residues were considered at 0 to calculate the concentration in the fruit. Sample results are shown in Table S8 (Supplementary Material).

Table 2
Summary of the residues found in passion fruit peel at levels ≥ LOQ and estimated processing factors (PF).

	Peel	Washed peel		Passion fruit flour	
Pesticide	Range, mg kg^{-1}	Range, mg kg^{-1}	PF[a], range (median)	Range, mg kg^{-1}	FP[c], range (median)
Acephate (n = 5)	0.010 – 0.522	0.006 – 0.321	0.2 – 0.6 (0.6)	0.018 – 1.09	2.0 – 5.7 (3.2)
Chlorpyrifos (n = 1)	0.127	0.085	0.7	0.161	1.9
Dimethoate (n = 2)	0.037 – 0.155	0.005 – 0.113	0.1 – 0.7 (0.4)	0.057 – 0.410	3.6 – 10 (6.8)
Omethoate (n = 3)	0.006 – 0.031	traces[b] – 0.014	0.3 – 0.5 (0.4)	0.001	1.3 – 7.5 (2.8)
Malathion (n = 1)	0.021	0.010	0.5	0.074	7.3
Methamidophos (n = 5)	0.010 – 0.274	0.005 – 0.118	0.4 – 0.5 (0.4)	0.019 – 0.768	4.0 – 6.8 (6.2)
Organophosphates (n = 17)	0.006 – 0.522	0.005 – 0.321	0.1 – 0.7 (0.5)	0.019 – 0.768	1.9 – 10 (4.0)
Carbendazim (n = 5)	0.015 – 0.148	0.006 – 0.049	0.2 – 0.5 (0.4)	0.040 – 0.328	5.7 – 12 (6.6)
Imidacloprid (n = 10)	0.007 – 0.070	0.006 – 0.072	0.1 – 1.0 (0.6)	0.041 – 0.535	4.5 – 15 (7.6)
All (32)			0.1 – 1.0 (0.5)		1.3 – 15 (6.5)

[a] In relation to unwashed peel; [b] considered at ½ LOQ; [c] in relation to the washed peel.

Table 3
Pesticides detected in frozen passion fruit frozen pulp and passion fruit flour by LC–MS/MS.

Pesticide	Frozen pulp (N = 30) (n) mean[a]/max., mg kg^{-1}	Flour (N = 12) (n) mean[a]/max., mg kg^{-1}
Carbendazim	(13) 0.012/0.098	nd
Imidacloprid	(12) 0.002/0.008	(1) 0.006/0.066
Acephate	(10) 0.004/0.030	nd
Difenoconazole	(4) 0.001/0.006	nd
Tebuconazole	(3) 0.001/0.008	nd
Pyraclostrobin	(3) 0.0004/0.008	nd
Malathion	(2) 0.0003/0.007	nd
Methamidophos	(1) 0.0003/0.008	(4) 0.014/0.050
Trifloxystrobin	(1) 0.0002/0.005	nd
Azoxystrobin	(1) traces	nd
Pirimiphos methyl	nd	(1) 0.003/0.036

n: samples with detected residues; nd: not detected; [a]trace levels were considered at ½ LOQ and samples with non-detected residues were considered at 0. Sample results are shown in Table S8 (Supplementary Material).

8.3 × 10^{-6} (difeconazole) to 1.4 × 10^{-4} mg kg^{-1} bw (carbendazim), accounting for up to 0.14% of the ARfD for carbendazim. For passion fruit flour, the chronic intake reached 0.26% of the ADI for methamidophos, which also had the highest contribution to the ARfD (1.3%).

4. Discussion

The LOQ values established in this work for passion fruit matrices (0.005 or 0.010 mg kg^{-1}) are similar to other multiresidue methods that used GC–MS/MS or LC–MS/MS for fruit matrices (Ferreira et al., 2016; Machado, Gérez, Pistón, Heinzen, & Cesio, 2017). Kemmerich et al. (2019) obtained LOQ values of 0.0025 mg kg^{-1} for 90% of the 170 analytes monitored in the multi-residue method validated in the pear matrix using UHPLC–MS/MS, while values greater than 0.010 mg kg^{-1} had been reported, including in passion fruit (Hjorth et al., 2011; Botero-Coy et al., 2012; Paz et al., 2015; Volpatto et al., 2016; Concha-Meyer et al., 2019). The LOQs of the analytical method are below the MRLs established by the Brazilian legislation for passion fruit (ANVISA, 2021a). It is important to point out that the responses of the analytes in the LC–MS/MS and GC–MS/MS vary, as can be seen in Fig. 3 and Fig. S1. In this work, the lowest fortification level was established from the response of the compounds for which the method showed less sensitivity, which was conservative for some compounds, such as carbofuran, for which the method was about 10 times more sensitive than for paraoxon methyl (Fig. S1).

The recoveries of 34% of pesticides analyzed by LC–MS/MS were less than 70% at least in one assessed level (minimum of 55%). Among them is diazinon, which was not detected in any sample analyzed in this work, and chlorpyriphos, which was detected in only one sample at a level higher than the LOQ established for this analyte (0.010 mg kg^{-1}). Methamidophos showed the lowest recoveries in the passion fruit pulp matrix (down to 63%), but the RSDr was ≤ 20%. For high polarity compounds, such as methamidophos, it is possible that full partitioning does not occur during extraction with EtAc (Jardim et al., 2014). Similar results were reported by other authors in other matrices (Mol et al., 2007; Jardim et al., 2014), while other studies report good recoveries for methamidophos using EtAc as extracting solvent (Ferrer, Martinez-

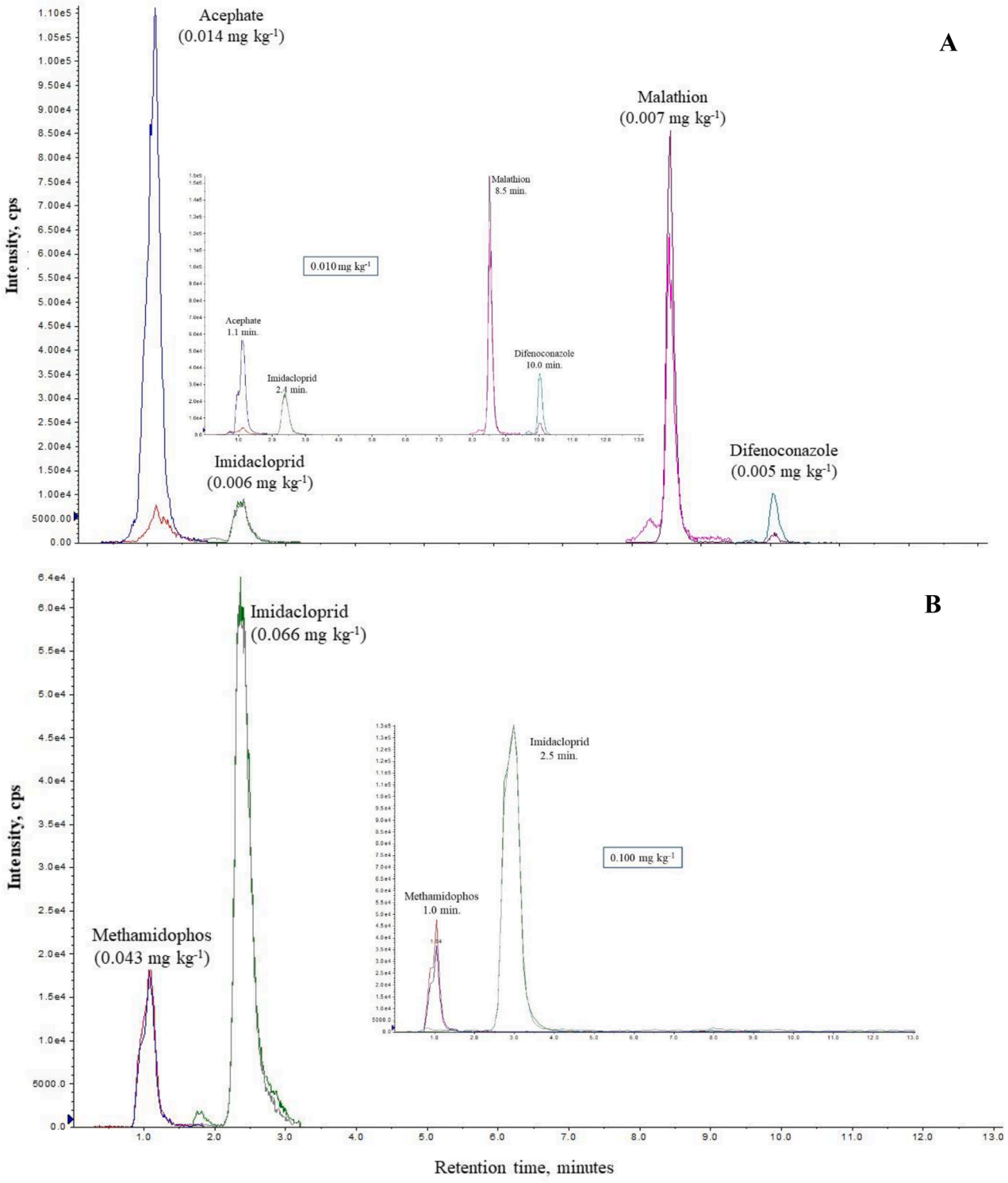

Fig. 3. LC–MS/MS ion chromatograms of A: commercial frozen pulp of passion fruit (sample 49/20) and B: commercial passion fruit flour (sample 16/20). Inserted in each case is a point of the in-matrix analytical curve analyzed in the same day (corresponding to 0.010 mg kg^{-1} for pulp and 0.100 mg kg^{-1} for flour).

Bueno, & Fernandez-Alba, 2011; Kemmerich et al., 2019).

Imidacloprid and carbendazim were the most detected pesticides in passion fruit samples, present in 23.6 and 21.8% of the analyzed peel samples, respectively. Of the 19 analytes detected, only six are allowed to be used on the crop (chlorfenapir, difenoconazole, λ-cyalothrin, imidacloprid, pyraclostrobin, and tebuconazole) and the pesticide levels present in passion fruit samples were below the MRL (between 0.05 and 1 mg kg^{-1}; ANVISA, 2021a).

Omethoate and methamidophos had their registrations canceled in Brazil in 2002 and 2011, respectively (ANVISA, 2021b). In this study, two samples of passion fruit peel contained dimethoate and its metabolite omethoate, similar to what was reported by Jardim et al. (2014) in other fruits in Brazil. Similarly, acephate and its metabolite methamidophos were detected together in 5 peel samples, results also reported by Jardim et al. (2014). In both cases (omethoate/dimethoate and methamidophos/acephate), all samples have illegal residues, as the

use of dimethoate or acephate is not allowed in passion fruit. On the other hand, thiophanate methyl, not included in this study, is allowed to be used in passion fruit (MRL = 0.5 mg kg^{-1}), and its metabolite carbendazim (not registered for the crop) was the second most detected in peel samples (up to 0.069 mg kg^{-1} in the fruit). In this case, it cannot be said that carbendazim residues are illegal. Most of the pesticides detected in the samples are also not registered in Europe or do not have permitted use in passion fruit (EC, 2021), which could pose a potential problem to Brazilian exporters. Some exceptions include tebuconazole, difeconazole and pyraclostrobin. In the 2019 EU report on pesticide residues, 22.5% of the passion fruit samples had residues above the regional MRL, and about 16 % of the samples had at least 5 different pesticide residues, a multiple residue situation also found in the present work (EFSA, 2021).

The use of unauthorized pesticides for crops is common in Brazil, being reported by Brazilian monitoring programs, the Program on Pesticide Residue Analysis in Food (PARA), coordinated by the Brazilian National Regulatory Health Agency (ANVISA), and the National Residue and Contaminant Control Program (PNCRC), coordinated by the Ministry of Agriculture, Livestock and Food Supplies (MAPA). Data from these two programs for the period 2001 to 2010 indicated that 72% of the irregularities found referred to the use of unauthorized pesticides for the crop (Jardim & Caldas, 2012). Similar results were found in the PARA 2017/2018, which analyzed 4616 samples of 14 foods, including 5 fruits (orange, pineapple, mango, guava and grapes) (ANVISA, 2019). During this period, imidacloprid, tebuconazole and carbendazim were the most detected pesticides. None of the Brazilian monitoring programs included passion fruit, which makes the present study very relevant.

While 32 peel samples contained residues of at least one analyte (~60%), 14 pulp samples (25%) contained residues. This percentage is much higher than that found for dithiocarbamate fungicides in the same samples by Mozzaquatro et al. (2019), when only one pulp sample contained residues (0.09 mg kg^{-1} CS_2), which is expected, since dithiocarbamates are non-systemic pesticides. In the present study, the fruit pulp was manually separated without coming into contact with the outside of the peel, which directly receives the foliar application of the pesticide. This indicates that the residue found in the pulp in this study is due to the pesticide's systemic action, and, as expected, detected in much lower concentrations than those in the peel. Results of field trials conducted with passion fruit in Colombia, showed the presence of all investigated pesticides in the pulp (including difenconazole, mancozeb, tebuconazole, deltamethrin, and imidacloprid), but also at much lower concentration compared to the peel (Juraske, Fantke, Ramírez, & González, 2012).

The high percentage of commercial passion fruit pulp samples that contained pesticide residues (63%) indicates cross-contamination during the industrial process. Residues still present in the peel after washing the fruits maybe be transferred to the pulp, as washing may not remove 100% of residues present, as observed in this study. Jardim et al. (2014) also detected pesticide residues in most samples of commercial pulps of cashew apple, peach and guava, fruits with very thin peel, which increases the transfer of pesticides to the pulp.

In theory, two major parameters may play a role in the washing PF for a commodity: water solubility and systemic properties of the pesticides (Chung et al., 2018). However, the passion fruit washing PF did not show a significant impact of either parameter, as the range of PF and the medians were similar (0.4–0.6) among the pesticides, which have different water solubility (e.g., 8 mg L^{-1} for carbendazim, 610 mg L^{-1} for imidacloprid; Chung et al., 2018) and systemic properties. Furthermore, median peel washing PF for dithiocarbamates (non-systemic, very low water solubility) in the same passion fruit samples was also 0.5 (Mozzaquatro et al., 2019). Previous studies have also reported that the washing PF was unrelated to the pesticide solubility, being more related to the fruit characteristics, mainly fruit surface and waxy layer (Chung et al., 2018; Yigit & Velioglu, 2020).

A large range of PFs for dried passion fruit peel (flour) was also found among the pesticides (1.3 to 15, median of 6.5). This large PF variability has also been reported by the JMPR (JMPR, 2021). For example, buprofezin PFs ranging from 1.1 to 16 (n = 14) for orange dried pulp and from 0.2 to 3.4 (n = 10) for dried grapes (raisin) were reported by the 2019 JMPR, in addition to dimethoate PFs ranging from 0.22 to 3.2 (n = 4) for wholemeal flour. Furthermore, PFs for pyriofenone in dried grapes ranging from 1.5 to 5 (n = 8) were reported by the 2018 JMPR. During the drying process in the oven, two parameters affect the residue concentration of the final product: loss of water, which concentrates the residues, and degradation of the pesticides, which reduces the pesticides. Raisins are normally dried in the field, so in addition to degradation by the heat, pesticide photo-degradation may also occur (Yigit & Velioglu, 2020; Thekkumpurath, Girame, & Hingmire, 2020).

The commercial flour samples analyzed in this study contained three of the 50 analytes investigated by LC–MS/MS: methamidophos and imidacloprid, also detected in peel samples, and pirimiphos methyl, only detected in this matrix throughout the study. In addition to the small number of compounds detected in the flour, the levels were lower than those expected from the levels found in the peel taking the PFs into account. For instance, considering the maximum methamidophos level in the peel (0.274 mg kg^{-1}) and the washing and drying processes (PF_{med} = 0.4 and 6.2), the expected level of methamidophos in the flour would be 0.679 mg kg^{-1}, much higher than the higher value found in the commercial flour sample (0.050 mg kg^{-1}). Although this conclusion is based on the analysis of a limited number of flour samples (12), it is likely that fruits destined for flour production come from agricultural production without intensive use of pesticides. The flour product label does not indicate the origin of the passion fruit used in its production, and a higher number of flour samples should be analyzed to confirm this hypothesis.

Considering consumption (juice) and body weight data from HBS 2017/2018, label consumption recommended for flour, and concentrations obtained in this study, it was possible to estimate chronic and acute exposure to pesticides from passion fruit juice and flour consumption for compounds that were quantified in at least one analyzed sample. The results showed that the chronic intake from pulp consumption did not impact the respective ADIs, and the acute intake reached 0.14% of ARfD for carbendazim. As the levels found in the passion fruit pulp manually processed are much lower than the commercial samples, the risks from the consumption of pulp processed in the household, are expected to be much lower. For flour, methamidophos intake contributed the most to the toxicological parameter, reaching 1.3 % of the ARfD.

This is the first study conducted in Brazil that considered the consumption of passion fruit or its products in dietary risk assessment. Jardim et al. (2014) conducted a cumulative acute risk assessment study for organophosphate and pyrethroid insecticides by consumption of cashew apple, guava, persimmon, and peach. Guava consumption showed the highest percentages of ARfD for exposure to organophosphates (45%) and pyrethroids (12%). In the latest PARA results (2017/2018), carbendazim intake from pineapple consumption represented up to 100% of ARfD, and methamidophos intake represented at most 40% of ARfD (ANVISA, 2019). Chronic (dithiocarbamates and triazoles) and acute (organophosphorus, carbamates, pyrethroids and triazoles) risk assessment studies using the probabilistic method (consumption data from the HBS 2008/2009) indicated that the total intake in the 99th percentile of the intake distribution represented a maximum of 59% for organophosphates (Jardim et al., 2018a,b). The results of the present study indicate that the contribution of the consumption of passion fruit products (juice and flour) does not have a significant impact on the chronic dietary intake of the evaluated pesticides by Brazilian consumers.

5. Conclusion

In this study, a multi-residue method for the determination of 80 pesticides, including five metabolites, in passion fruit peel and pulp by

LC–MS/MS and GC–MS/MS was satisfactorily validated (recovery ranging from 70 to 120% and precision $\leq$ 20%) at LOQ of 0.005 mg kg^{-1}, except for diazinon, chlorpyriphos, and methidathion in passion fruit peel by LC–MS/MS, for which a LOQ of 0.010 mg kg^{-1} was established. At least one analyte was detected in 60% of the 55 samples of passion fruit collected from Brazilian producers. Peel dehydration process concentrates the residues, information that must be considered when the fruit is used for flour production. Most of the frozen pulp samples acquired in the commerce were positive for at least one analyte, indicating a possible transference of pesticides present in the peel during the industrial pulp production. The estimated risks of exposure to pesticides assessed by the consumption of commercial products did not indicate a health concern for Brazilian consumers.

The high incidence of pesticides not authorized by Brazilian legislation present in passion fruit indicates that farmers may not be receiving adequate assistance in the field to guide them in the application of good agricultural practices. The monitoring of this crop by Brazilian programs is important, since the country is the world's largest producer and consumer of passion fruit.

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CRediT authorship contribution statement

Joseane de Oliveira Mozzaquatro: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft. **Izabelle Araujo César:** Investigation. **Anna Eduarda Barbosa Pinheiro:** Investigation. **Eloisa Dutra Caldas:** Conceptualization, Resources, Data curation, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2021.131643.

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