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Development, validation and application of a methodology based on solid-phase micro extraction followed by gas chromatography coupled to mass spectrometry (SPME/GC–MS) for the determination of pesticide residues in mangoes

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ABSTRACT

A method was developed for the simultaneous analysis of 14 pesticide residues (clofentezine, carbofuran, diazinon, methyl parathion, malathion, fenthion, thiabendazole, imazalil, bifenthrin, permethrin, prochloraz, pyraclostrobin, difenoconazole and azoxystrobin) in mango fruit, based on solid-phase micro extraction (SPME) coupled to gas chromatography–mass spectrometry (GC–MS). Different parameters of the method were evaluated, such as fiber type, extraction mode (direct immersion and headspace), temperature, extraction and desorption times, stirring velocities and ionic strength. The best results were obtained using polyacrylate fiber and direct immersion mode at 50 °C for 30 min, along with stirring at 250 rpm and desorption for 5 min at 280 °C. The method was validated using mango samples spiked with pesticides at concentration levels ranging from 33.3 to 333.3 μg kg^{-1}. The average recoveries (n = 3) for the lowest concentration level ranged from 71.6 to 117.5%, with relative standard deviations between 3.1 and 12.3%, respectively. Detection and quantification limits ranged from 1.0 to 3.3 μg kg^{-1} and from 3.33 to 33.33 μg kg^{-1}, respectively. The optimized method was then applied to 16 locally purchased mango samples, all of them containing the pesticides bifenthrin and azoxystrobin in concentrations of 18.3–57.4 and 12.7–55.8 μg kg^{-1}, respectively, although these values were below the MRL established by Brazilian legislation. The method proved to be selective, sensitive, and with good precision and recovery rates, presenting LOQ below the MRL admitted by Brazilian legislation.

1. Introduction

The mango is a fruit whose attractive flavor, aroma, color and exotic appearance have gained wide acceptance and led to its high demand in both domestic and export markets, making it a product of increasing economic importance. Its popularity is enhanced by the fact that it is rich in carotenoids, mineral salts, carbohydrates, ascorbic acid and B vitamins [1]. Ripe mangoes contain a considerable amount of vitamin C, which may reach up to 110 mg/100 g of fruit, depending on the variety. Mango production in Brazil is destined primarily for the export markets of Europe and North America [2]. To increase its productivity and obtain good quality fruits, phytosanitary treatments are applied for pest and fungal control during the cultivation and post-harvest stages. The fruit is subject to several diseases during these stages, leading to significant losses, the most important of which is anthracnosis caused by *Colletotrichum gloeosporioides*. The treatment involves the use of contact and systemic fungicides [3] and, after harvesting, immersion of the fruit in water containing fungicides such as thiabendazole [4]. Albeit effective in controlling pests and fungi, pesticides may penetrate the vegetable tissues, remaining in the fruit as residues and posing a potential risk to human health due to their toxicity [5]. To control the levels of residual pesticides in foods, several countries have established maximum residue limits (MRLs) of each active principle as a form of protecting the health of the population.

Environmental and food samples are usually not analyzed without a preliminary preparation, since contaminants are present in low concentrations and the matrices are complex [6]. Preparation of the sample is the most critical step in the determination of pesticide residues in foods, since biological samples present complex chemical compositions, requiring extraction techniques that allow for greater selectivity and concentration of the analytes, allowing for the determination of pesticide residues at increasingly low levels.

Conventional methods for the determination of pesticide residues in foods are laborious and time-consuming, requiring considerable amounts of organic solvents and extracting undesirable

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interferants from the matrix [7]. The most commonly used methods are based on liquid–liquid extraction (LLE), solid-phase extraction (SPE) [8–11], supercritical fluid extraction (SCE), and matrix solid-phase dispersion (MSPD) [12–14]. Solid-phase microextraction (SPME) emerged as a versatile alternative method of analyte extraction and preconcentration, which requires little or no organic solvents, is easily automated, and can also improve the limits of detection [15]. SPME encompasses sampling, extraction, preconcentration and introduction of the sample into the system of analyses in a single uninterrupted process [16], thus avoiding contamination of the matrix.

SPME has been used routinely in combination with gas chromatography, using different types of detectors, especially mass spectrometers and, more recently, with liquid chromatography–diode array detection-mass spectrometry (LC/DAD-MS) [17]. SPME is of increasing interest in the analysis of pesticide residues, and is applied in the determination of various classes of pesticides in aqueous media or in other samples [18]. For example, in a recent study, Cortés-Aguado et al. [19] used SPME in the screening of juice samples to determine pesticide residues by an official methodology, avoiding the analysis of samples that showed no traces of pesticides.

Multi-residue analytical methods have been proposed for both screening and quantifying pesticides of different chemical groups in fruits and vegetables, such as organochlorine and organophosphorous pesticides in fruits and vegetables by HS-SPME/GC/ECD [26], strobilurin, imidazole and oxazole fungicides in grapes and wines by SPME/LC/DAD [27], pyrethroids in vegetables by SPME/LC/PIF/FD [7], and strobilurin fungicides in baby foods by SPME/GC/MS [28]. However, these methods are generally laborious to develop, since the targeted compounds present different degrees of polarity, solubility and volatility, as well as different values of pK_a, making their extraction and analysis difficult [8].

The aim of the present work was to develop a new analytical method for simultaneous determination of 14 pesticides, belonging to different chemical classes, in mango fruits, using SPME/GC/MS. To the best of our knowledge, in the literature to date contains no previous works that report this approach. Seven different classes of common pesticides were investigated: (a) organophosphorous such as diazinon, methyl parathion, malathion and fenthion (insecticides); (b) pyrethroids such as bifenthrin and permethrin (acaricides and insecticides); (c) strobilurins such as azoxystrobin and pyraclostrobin (fungicides); (d) imidazoles such as imazalil and prochloraz (fungicides); (e) triazoles such as thiabendazole and difenoconazole (fungicides); (f) methylcarbamates such as carbofuran (acaricide and nematicides) and (g) tetrazines such as clofentezine (acaricide). These compounds were selected due to their extensive application in the irrigation project "Platô de Neópolis," located in the city of Neópolis, state of Sergipe, Brazil (10°19′12″S, 36°34′46″W), and also based on their authorized use by the National Health Surveillance Agency (ANVISA). The method was then applied to 16 fruit samples purchased from different retailers in the city of Salvador, state of Bahia, Brazil. The results are discussed herein.

2. Materials and methods

2.1. Standards, reagents and solvents

Certified standards of clofentezine, carbofuran, fenthion, thiabendazole, bifenthrin, imazalil, difenoconazole, permethrin, prochloraz, pyraclostrobin and azoxystrobin were acquired from AccuStandard (New Haven, CT, USA); and methyl parathion (1000 μg ml^{-1}), malathion (1000 μg ml^{-1}) and diazinon (1000 μg ml^{-1}) from Absolute Standards (Hamden, CT, USA). All the standards had purities exceeding 97.0%.

HPLC grade methanol and acetonitrile were acquired from J.T. Baker (Phillipsburg, NJ, USA); isopropanol from Merck (Dalmstadt, Germany); and sodium chloride (99.0%) from Nuclear (São Paulo, Brazil).

2.2. Equipment

Extraction and analysis of pesticides were performed in an autosampler (CTC Combi-PAL, Zwinger, Sweden) coupled to a GC–MS (Shimadzu QP2010 Plus, Kyoto, Japan) equipped with a split/splitless injector operating in the splitless mode at 280 °C during the chromatographic run. Pesticides were separated in a capillary column (Restek Rtx®-1 MS Crossbond® 100% polydimethylsiloxane; 30 m × 0.25 mm ID × 0.25 μm, Bellefonte, PA, USA) using helium 99.99% as carrier gas at a 1.0 mL min^{-1} flow rate. The oven temperature was as follows: 60 °C (1 min); followed by 170 °C at 25 °C min^{-1}; and 290 °C at 6 °C min^{-1}, where it was held for 1 min.

The mass detector conditions were: transfer line temperature 250 °C; ion source temperature 230 °C; and ionization mode electron impact at 70 eV. The analyses were done in SIM (Selected Ion Monitoring) mode and for all selected pesticides one target and two qualifier ions were monitored. For each pesticide that follows, the first ion is the target ion and the other two the qualifier ions: 137, 102 and 109 (clofentezine); 164, 149 and 131 (carbofuran); 137, 179 and 152 (diazinon); 125, 109 and 263 (methyl parathion); 127, 93 and 173 (malathion); 278, 125 and 109 (fenthion); 201, 174 and 129 (thiabendazole); 41, 215 and 173 (imazalil); 181, 165 and 166 (bifenthrin); 183, 163 and 165 (permethrin); 70, 43 and 215 (prochloraz); 132, 164 and 325 (pyraclostrobin); 265, 323 and 267 (difenoconazole), and 344, 388 and 329 (azoxystrobin). The quantification and confirmation of the selected pesticides were done in single runs, by monitoring target and qualifier ions together.

The SPME was performed in a holder designed for autosampler use (Supelco, Model 23GA, Bellefonte, PA, USA). The fibers evaluated were the polyacrylate (85 μm), polydimethylsiloxane (100 μm), polydimethylsiloxane–divinylbenzene (65 μm), divinylbenzene–carboxen–polydimethylsiloxane (50 μm) and carboxen–polydimethylsiloxane (85 μm), all of them from Supelco (Bellefonte, PA, USA). Since the selected pesticides belong to different chemical groups, with significant variations in their polarity, these fiber phases were chosen for testing in order to ensure the best extraction efficiency.

2.3. Preparation of standards and spiking of samples

Individual stock solutions of each standard were prepared in methanol in a concentration of 1000 μg mL^{-1} and stored at −18 °C. The exception was clofentezine, which was prepared in acetonitrile. The work standard containing the 14 pesticides was prepared by diluting the stock solution in methanol to a concentration of 10 μg mL^{-1}. This standard was used both to spike the matrix in order to optimize the extraction conditions (50 ng mL^{-1}) and for the validation study at different concentrations (1–500 ng mL^{-1}). Calibration standards with concentrations of 1, 2, 5, 10, 30, 50, 100, 250 and 500 ng mL^{-1} were prepared by diluting the work standard directly in the matrix extract.

2.4. Extraction procedure

The mango samples used in the development of the method were of the pesticide-free-organic-culture-type and acquired directly from producers located in the state of Sergipe (10°37′21″S and 37°28′56″W) in northeastern Brazil. A representative amount

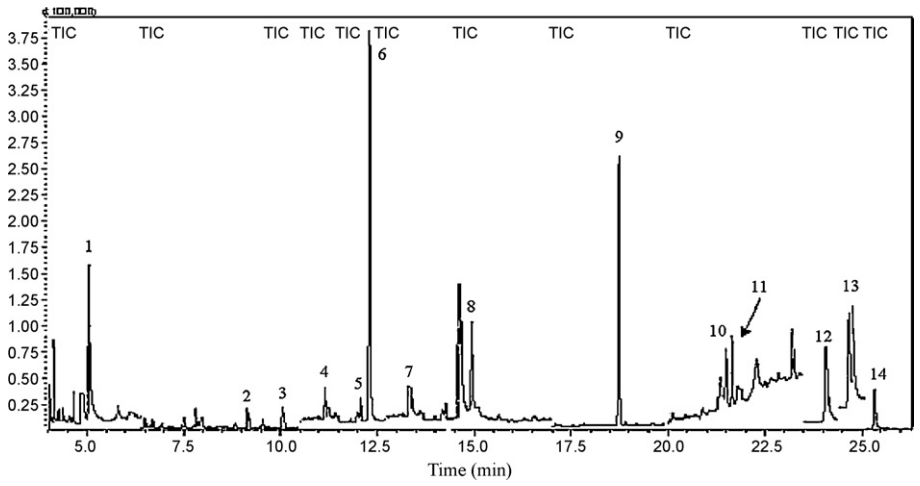

Fig. 1. Chromatogram (SIM mode) of a 50 μg kg^{-1} pesticide standard extracted by DI-SPME from the matrix extract. Peak identification: 1, clofentezine; 2, carbofuran; 3, diazinon; 4, methyl parathion; 5, malathion; 6, fenthion; 7, thiabendazole; 8, imazalil; 9, bifenthrin; 10, permethrin; 11, proclhoraz; 12, pyraclostrobin; 13, difenoconazole; 14, azoxystrobin.

of the fruits (~500 g) was ground and homogenized in a food processor and then transferred to amber glass flasks, which were stored at −18 °C until they were used.

For the extraction, a sample aliquot (3 g) was weighed in a 20 mL vial, fortified with 50 μL of the work standard and allowed to rest for 10 min, followed by the addition of 10 mL of a 20:80 (v/v) isopropyl alcohol:water mixture with 5% NaCl and pH 3, and adjusted by the addition of HCl. This mixture was stirred at 1000 rpm for 10 min, centrifuged at 5000 rpm for 15 min, the upper layer transferred to a 10 mL volumetric flask, and the volume completed with the alcohol:water mixture. The resulting solution was then transferred to a sealed 10 mL headspace vial for the SPME procedure.

The SPME fiber used here (85 μm polyacrylate) was first prepared as recommended. Extraction of the pesticides was done in the direct immersion mode (DI-SPME), at 50 °C for 30 min, while stirring at 250 rpm was applied in alternate cycles (30 s of stirring, followed by 10 s without stirring). Following extraction, the fiber was placed in the GC injector for desorption for 5 min at 280 °C. After each extraction, the fiber was cleaned at 280 °C for 1 min in a helium atmosphere.

3. Results and discussion

The optimization steps for the extraction and desorption conditions were performed with pesticide solutions in the real matrix. Considering the 10 variables that could affect the SPME method were evaluated, a univariate analysis was made to gain a better understanding of the chemical behavior of each variable.

3.1. GC–MS conditions

The optimization of the retention times and chromatographic resolution were done in the SCAN mode using a 1 μg mL^{-1} standard. To quantify the pesticides in the samples, SIM was then chosen and specific ions were selected for each analyte. Fig. 1 shows the chromatogram of a standard extracted directly from the matrix and obtained by GC–MS in the SIM mode. The resolution was considered satisfactory. The pesticides difenoconazole and permethrin, both of which presented stereoisomerism, showed two peaks each, corresponding to the cis(Z) and trans(E)isomers.

3.2. Selection of fiber and extraction mode

The selected pesticides belong to different chemical groups whose octanol–water partition coefficients vary significantly ($\log k_{ow}$) (Table 1), which is a measure of their hydrophilic or lipophilic characteristics and also their tendency toward polar or non-polar media. Due to these differences, five fiber phases were tested, namely: a polar phase (polyacrylate – PA); a non-polar phase (polydimethylsiloxane – PDMS) two bipolar phases (polydimethylsiloxane/divinylbenzene – PDMS/DVB; PDMS/carboxen), and a tripolar phase (DVB/carboxen/PDMS). To ensure the deter-

Table 1
Chemical class, formula and physical properties of the pesticides studied.

Pesticide	Chemical class	Formula	MM (g mol^{-1})	$\log K_{ow}$ (20 °C)	H_2O solubilities (mg L^{-1} at 20 °C)	PV (mPa at 25 °C)
Clofentezine	Tetrazine	$C_{14}H_8Cl_2N_4$	303.1	3.10	0.002	1.4×10^{-3}
Carbofuran	Metylcarbamate	$C_{12}H_{15}NO_3$	221.3	1.70	319	0.031
Diazinon	Organophosphorus	$C_{12}H_{21}N_2O_3PS$	304.3	3.69	60.0	11.97
Methyl parathion	Organophosphorus	$C_8H_{10}NO_5PS$	263.2	3.00	55.0	0.2
Malathion	Organophosphorus	$C_{10}H_{19}O_6PS_2$	330.3	2.75	145.0	0.45
Fenthion	Organophosphorus	$C_{10}H_{15}O_3PS_2$	278.3	4.84	4.2	0.37
Thiabendazole	Benzimidazole	$C_{10}H_7N_3S$	201.2	2.39	30.0	5.3×10^{-4}
Imazalil	Imidazole	$C_{14}H_{14}Cl_2N_2O$	297.1	3.82	22.4	1.6×10^{-6}
Bifenthrin	Pyrethroid	$C_{23}H_{22}ClF_3O_2$	422.8	6.00	0.0025	0.024
Permethrin	Pyrethroid	$C_{21}H_{20}Cl_2O_3$	391.3	6.10	0.2	0.002
Prochloraz	Imidazole	$C_{15}H_{16}Cl_3N_3O_2$	376.7	3.53	34.4	0.15
Pyraclostrobin	Strobilurin	$C_{19}H_{18}ClN_3O_4$	387.8	3.99	1.9	2.6×10^{-5}
Difenoconazole	Triazole	$C_{19}H_{17}Cl_2N_3O_3$	406.2	4.20	15.0	3.3×10^{-5}
Azoxystrobin	Strobilurin	$C_{22}H_{17}N_3O_5$	403.4	2.50	6.7	1.1×10^{-7}

Source: IUPAC [21].

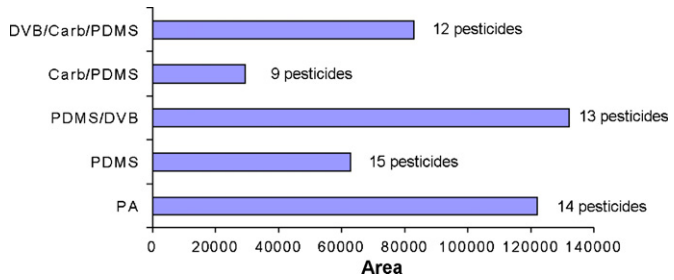

Fig. 2. Efficiency of each fiber on the pesticides extraction by DI-SPME.

mination of as many compounds as possible, extractions were performed in the direct immersion mode. Fig. 2 shows the number of pesticides extracted as a function of the fiber type, and also compares the average peak areas obtained for each fiber. The carboxen-containing fibers (DVB/carb/PDMS and carb/PDMS) were the least efficient, while the PDMS fiber was able to extract the greatest number of compounds. Although the PA and PDMS/DVB fibers extracted fewer compounds than the PDMS, they were able to concentrate larger amounts of each extracted pesticide. Since the PA fiber was capable to extract one more pesticide than the PDMS/DVB fiber, the former was selected for the development of the method, although in principle the three types of fiber could be used successfully in pesticide analysis.

Conventionally, DI-SPME is more sensitive than HS-SPME and is thus the method of choice for the analysis of clean aqueous samples. For complex or dirty samples, e.g., food and soil samples, HS-SPME is frequently chosen. In this study, the two SPME modes were compared using the PA fiber (Fig. 3). In agreement with Cai et al. [20], headspace extractions were more efficient in extracting the more volatile compounds, such as clofentezine, imazalil, diazinon, methyl parathion, malathion, fenthion, permethrin and bifenthrin. Nevertheless, they were not successful in extracting the less volatile pesticides, such as thiabendazole, prochloraz, pyraclostrobine, difenoconazole and azoxystrobine. Since the DI-SPME mode successfully extracted all the 14 pesticides, albeit with less sensitivity than HS-SPME in the case of the more volatile ones, this mode was elected as the best choice for the development of the method.

3.3. Optimization of desorption conditions

The complete desorption of analytes from the fiber enhances the detector response and eliminates memory effects. The injector temperature was varied from 250 to 300 °C and it was observed that responses had improved up to 280 °C, with no significant changes between 280 and 300 °C. Desorption times of 3, 4, 5 and 6 min were also evaluated and the desorption of all the pesticides was completed in 5 min. The conditions chosen were therefore 280 °C and 5 min.

3.4. Extraction solution

Due to the sample's characteristics (processed mango fruits), in order to reduce matrix interferences it was necessary to use an extraction liquid that would facilitate the transfer of analytes from the matrix to the fiber [19]. Compared with pure water, the addition of small portions of an organic solvent has proved to yield better results, probably due to a reduction in the solvent's polarity. Binary mixtures (80:20) of water:ethanol, water:isopropylalcohol and water:acetonitrile were tested and the second mixture yielded the best results in a first approach. Fig. 4 compares different water:isopropylalcohol mixtures against pure water and shows that, on average, the 80:20 mixture was able to efficiently extract nine pesticides, while three were extracted more efficiently in pure water. Also, the detector responses to the analytes were evaluated by comparing the signals resulting from standard added to pure water against the signals resulting from standard added to the mixture containing the sample matrix and the water:alcohol extraction solution. It was observed that peak areas were larger in the second case, so this extraction mode was selected.

3.5. Effect of the extraction temperature

The influence of temperature was evaluated starting from values near ambient temperature, since the more volatile compounds can already be extracted at such temperatures, while certain pesticides can undergo thermal decomposition at higher temperatures. The extractions were performed at 30, 40, 50 and 60 °C. The majority of pesticides showed a signal enhancement at temperatures up to 50 °C, indicating that increasing the temperature favored the mass transfer of the analytes from the matrix to

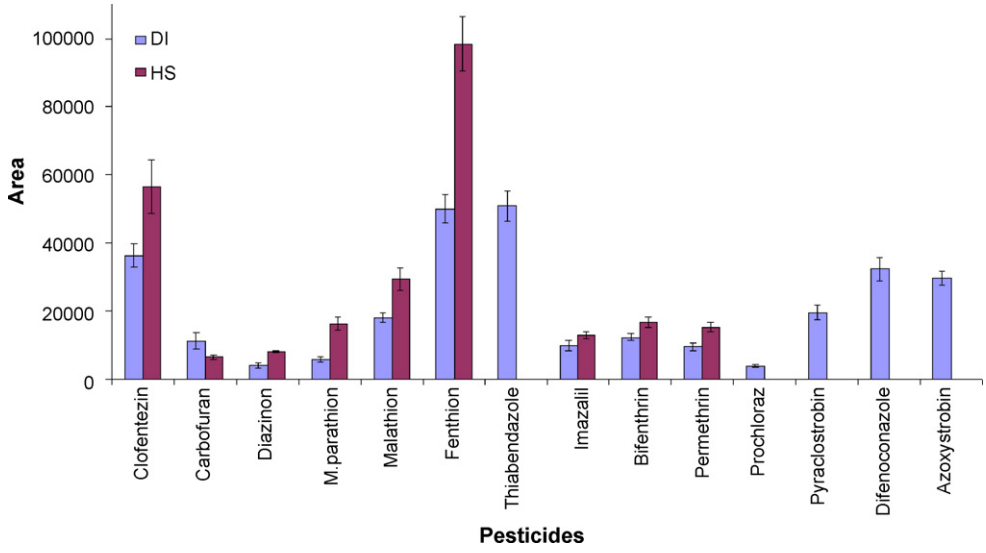

Fig. 3. Comparison between the SPME extraction efficiencies in the direct fiber immersion mode and in the headspace mode.

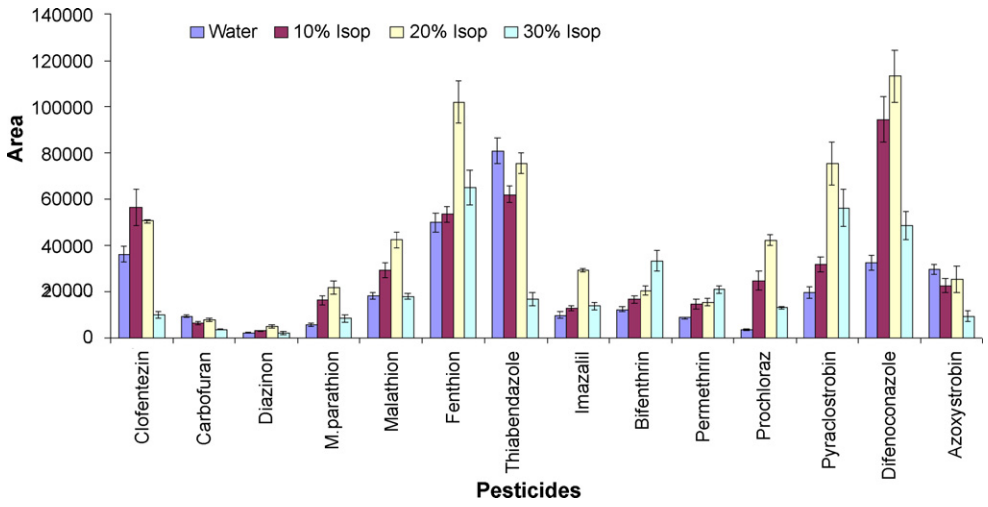

Fig. 4. Comparison amongst the SPME extraction efficiencies using different water:isopropylalcohol compositions as extraction solutions.

the solution and from there to the fiber. Temperatures above 50 °C favored the volatilization of the organophosphorus compounds, thereby removing them from the liquid phase. It was also observed that carbofuran underwent thermal decomposition above 40 °C.

Clofentezine and the piretroids bifenthrin ($\log k_{ow} = 6.0$) and permethrin ($\log k_{ow} = 6.1$) have high affinities for the mango's lipid-rich matrix. For these compounds, an increase in temperature up to 60 °C favored a break in the interactions between the analyte and the matrix, transferring the compounds to the liquid phase and increasing their diffusion in the fiber. Nevertheless, higher temperatures may also reduce the sorption of the analytes, since this is generally an exothermic process [22]. Fig. 5 shows the peak area for each pesticide as a function of the extraction temperature. A temperature of 50 °C was chosen, since it was found to yield the best analytical signal for most of the compounds.

3.6. Evaluation of the extraction time

The time required to reach equilibrium in the fiber stationary phase, the sample solution and the pesticides was evaluated through direct immersion extractions at 50 °C for 10, 20, 30 and 40 min (Fig. 6). It was observed that the analytical signal for most of the compounds was enhanced for times up to 30 min. This time was therefore selected, since on average it represents the best condition for the set under study.

3.7. Stirring velocities

The stirring velocity applied to the extraction system affects the mass transfer of the analytes from solution to the fiber. The extraction was carried out at 250, 400 and 600 rpm. With the exception of clofentezine, the signal for all the pesticides decreased as the stirring velocity increased. The velocity of 250 rpm was thus chosen.

3.8. Ionic strength

An increase in the ionic strength weakens the interaction between the analytes and the sample matrix, thus facilitating their extraction by fiber. The influence of ionic strength was evaluated by adding different amounts of NaCl to the extraction solution and also by modifying the pH with diluted HCl (pH 3) and diluted NaOH (pH 8). The salting out effect was evaluated through additions of 0, 5 and 12% (w/v) of NaCl. The 12% concentration yielded the best results for methyl parathion, malathion, thiabendazole, imazalil and prochloraz. However, this concentration impaired the repeatability of the results and also the stability of the solution due to precipitation of NaCl while the sample was at rest. On the other hand, 5% of NaCl yielded the best results for clofentezine, carbofuran, diazinon, fenthion, permethrin, difenoconazole and azoxystrobin (by more than 12%) and did not affect the stability of the solutions, and was therefore chosen.

The effect of pH was evaluated at pH 3, pH 8 and without the addition of a pH modifier solution. The pH 3 yielded the best

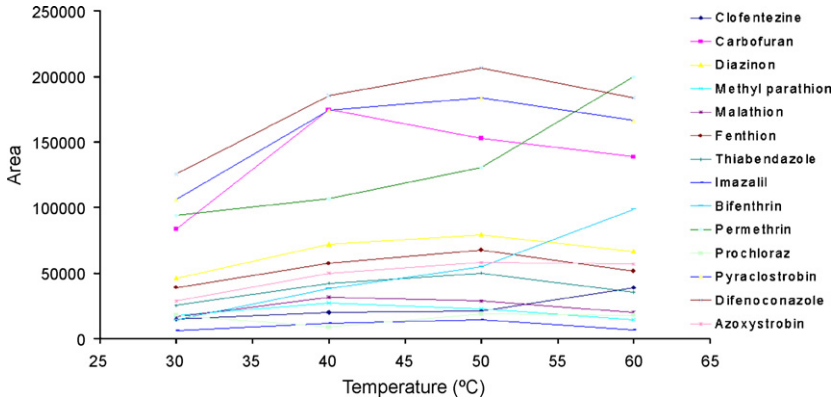

Fig. 5. Influence of temperature on the extraction efficiency.

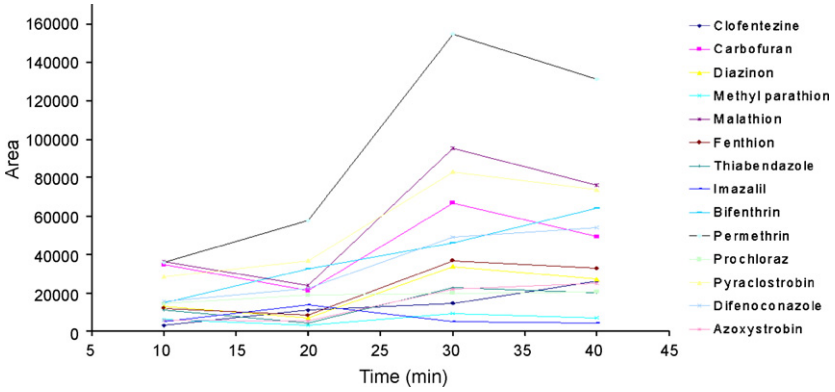

Fig. 6. Influence of the extraction time on the extraction efficiency.

Table 2
Analytical figures of merit obtained and the Brazilian Maximum Residue Levels of the pesticides studied.

Pesticide	Regression equation	R^2	Linear range ($\mu g\,kg^{-1}$)	LOD ($\mu g\,kg^{-1}$)	LOQ ($\mu g\,kg^{-1}$)	MRL[a] ($\mu g\,kg^{-1}$)
Clofentezine	$2061.50x + 60577$	0.9967	3.33–1665.00	1.00	3.33	–
Carbofuran	$42.54x - 1295.4$	0.9906	33.33–1665.00	10.00	33.33	–
Diazinon	$39.58x - 5.217$	0.9987	16.65–1665.00	5.00	16.65	–
Methyl parathion	$453.69x - 12124$	0.9960	16.65–1665.00	5.00	16.65	–
Malathion	$395.65x - 22928$	0.9907	6.66–1665.00	2.00	6.66	–
Fenthion	$3917.3x + 165795$	0.9966	3.33–1665.00	1.00	3.33	50
Thiabendazole	$267.40x + 17590$	0.9948	33.33–1665.00	10.00	33.33	2000
Imazalil	$114.65x - 3382.2$	0.9980	33.33–1665.00	10.00	33.33	1000
Bifenthrin	$6792.7x - 286761$	0.9973	6.66–1665.00	2.00	6.66	100
Permethrin	$1537.10x - 106775$	0.9903	16.65–1665.00	5.00	16.65	–
Prochloraz	$335.83x - 852.13$	0.9960	6.66–1665.00	2.00	6.66	200
Pyraclostrobin	$1689.80x + 4841.3$	0.9991	16.65–1665.00	5.00	16.65	100
Difenoconazole	$4357.3x + 29790$	0.9986	3.33–1665.00	1.00	3.33	200
Azoxystrobin	$1857x - 19938$	0.9929	6.66–1665.00	1.00	6.66	500

[a] *Source*: ANVISA [25].

results for most of the pesticides, although the amount of extracted clofentezine was reduced. As expected, pH 8 reduced the amounts of the extracted organophosphorus compounds diazinon, methyl parathion, malathion and fenthion, which undergo hydrolysis in alkaline solutions [23]. Imazalil, piretroids bifenthrin and permethrin were better extracted in alkaline solutions, bifenthrin and permethrin being stable between pH 5 and 9.

3.9. Method validation

The method was validated under the optimized conditions by determining the limits of detection (LOD) and quantification (LOQ), the inter- and intra-day precisions (RSD), the linearity, the absolute recovery and the relative recovery at different levels of fortification. The results are presented in Tables 2–5. The external standard calibration curve was constructed with nine concentrations, each of which was analyzed in triplicate. The standard deviations indicated that the dispersion of analysis was independent of the sample's concentration. The developed method presents a wide linear range of applications, and is in line with the maximum residue limits (MRLs) established by Brazil's National Health Surveillance Agency (ANVISA), which range from 3.33 to 1665.00 $\mu g\,kg^{-1}$ except for thiabendazole, whose MRL is 2000 $\mu g\,kg^{-1}$. The LOD and LOQ ranged, respectively, from 1.00 to 3.33 $\mu g\,kg^{-1}$ and 3.33 to 33.33 $\mu g\,kg^{-1}$. Among the pesticides under study, only fenthion, thiabendazole, imazalil, bifenthrin, prochloraz, pyraclostrobin, difenoconazole and azoxystrobin are authorized by Brazilian legislation for use in mango culture. The LOQ determined for these pesticides were below their MRL. For fenthion, which presents the lowest MRL among all the pesticides studied here, the LOQ was 15-fold lower than that value, demonstrating that the sensitivity of the method developed here is adequate for use in the proposed application.

The precision of the method was evaluated based on its repeatability, which was ascertained by performing seven sample extractions on the same day plus three extractions per day for 5 days. In the first case, the variation coefficients (VC) were below 15% except for thiabendazole (17.05%), probably due to its high affinity for the PA fiber, which caused a peak tailing effect during the gradual desorption. In the second case, the variation coefficients were 7.04 and 22.03%, respectively, for fenthion and imazalil.

Tables 3 and 4 show the VC and the relative recoveries at different concentrations, respectively. The recoveries ranged from 71.6 to 117.5% at the lowest concentration (33.3 $\mu g\,kg^{-1}$) and from 52.2 to

Table 3
Intra- and Inter-day repeatability of the DI-SPME method developed.

Pesticide	RSD (%) spiked amount (50 $\mu g\,kg^{-1}$)	
	Intra-day ($n = 7$)	Inter-day ($n = 15$)
Clofentezine	7.42	9.13
Carbofuran	5.82	12.11
Diazinon	4.04	13.69
Methyl parathion	6.71	11.52
Malathion	10.88	12.96
Fenthion	8.33	7.04
Thiabendazole	17.05	19.90
Imazalil	12.50	22.03
Bifenthrin	5.84	13.29
Permethrin	10.08	13.66
Prochloraz	8.42	10.28
Pyraclostrobin	7.55	12.72
Difenoconazole	7.58	9.29
Azoxystrobin	6.61	12.00

Table 4
Mean relative recoveries and RSD, at three concentration levels, of the DI-SPME method developed.

Pesticide	Concentration level ($\mu g\,kg^{-1}$)	Mean recovery (%)	RSD (%)
Clofentezine	33.33	89.63	7.78
	166.50	71.94	3.83
	333.33	52.85	3.15
Carbofuran	33.33	89.32	12.37
	166.50	83.96	4.51
	333.33	77.33	6.66
Diazinon	33.33	117.52	12.33
	166.50	89.48	4.56
	333.33	77.35	6.68
Methyl parathion	33.33	91.85	7.60
	166.50	82.03	6.75
	333.33	68.93	3.73
Malathion	33.33	83.87	4.85
	166.50	75.34	5.72
	333.33	72.05	6.04
Fenthion	33.33	75.81	5.93
	166.50	72.39	4.11
	333.33	52.25	3.93
Thiabendazole	33.33	90.08	10.82
	166.50	95.06	15.62
	333.33	78.54	5.84
Imazalil	33.33	102.25	11.56
	166.50	94.64	15.93
	333.33	72.54	5.84
Bifenthrin	33.33	71.64	5.61
	166.50	67.56	7.83
	333.33	59.59	3.84
Permethrin	33.33	80.85	3.14
	166.50	82.24	8.22
	333.33	86.04	6.74
Prochloraz	33.33	108.76	5.93
	166.50	80.72	4.46
	333.33	82.65	10.62
Pyraclostrobin	33.33	115.95	10.04
	166.50	74.24	3.47
	333.33	77.07	2.88
Difenoconazole	33.33	110.96	8.62
	166.50	79.33	4.56
	333.33	81.26	4.84
Azoxystrobin	33.33	84.75	7.32
	166.50	82.68	5.06
	333.33	80.02	4.75

86.0% at the highest (333.3 $\mu g\,kg^{-1}$). The best recoveries at the lowest concentration have already been reported in the literature [24], which states that in more diluted samples the interaction of pesticides with the constituents of the matrix is attenuated, increasing their availability to the fiber's solid-phase. Another possibility is that fiber saturation may occur when samples are more concentrated.

Table 5
Absolute recoveries and RSD of the DI-SPME method developed.

Pesticide	Absolute recovery (%) and RSD (%) ($n = 3$)
Clofentezin	4.07 ± 025
Carbofuran	5.26 ± 0.48
Diazinon	0.83 ± 0.05
Methyl parathion	2.40 ± 0.15
Malathion	3.54 ± 0.13
Fenthion	3.84 ± 0.24
Thiabendazole	9.72 ± 1.32
Imazalil	1.09 ± 0.05
Bifenthrin	1.67 ± 0.09
Permethrin	1.62 ± 0.15
Prochloraz	1.55 ± 0.10
Pyraclostrobin	12.36 ± 1.17
Difenoconazole	17.83 ± 0.90
Azoxystrobin	16.08 ± 1.36

The absolute recoveries were calculated by comparing the average ($n = 3$) detector signals produced when directly injecting a volume of standard mixture containing 20 ng of each pesticide into the GC–MS against the average ($n = 3$) detector signals produced by the DI-SPME analysis of samples to which the same amount (20 ng) of each pesticide was spiked into the sample matrix. Because SPME is a non-exhaustive extraction technique, recoveries are usually low [16]. Table 5 shows absolute recoveries obtained by this method. The values ranged from 0.83 ± 0.05% for diazinon to 17.83 ± 0.90% for difenoconazole. Extractions done by direct immersion of the fiber yielded better results for the less volatile compounds such as difenoconazole and azoxystrobin. On the other hand, the organophosphorus compounds showed recoveries of 0.83–3.84%, probably due to their volatilization during the extraction performed at 50 °C. The piretroids bifenthrin ($\log k_{ow} = 6.0$) and permethrin ($\log k_{ow} = 6.1$) both showed recoveries of about 1.6%, probably due to their high affinity for the lipid-rich matrix.

Table 6
Pesticides levels determined for sixteen mango samples locally purchased.

Pesticides	Pesticide level (μg kg^{-1}) (RSD (%) $n = 3$)															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Clofentezine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Carbofuran	93.36 (4.32)	74.33 (3.86)	64.26 (3.43)	n.d.	62.34 (3.63)	n.d.	n.d.	68.54 (4.73)	n.d.	54.04 (3.48)	n.d.	75.39 (4.68)	n.d.	n.d.	n.d.	n.d.
Diazinon	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Metyl parathion	43.14 (3.74)	40.81 (4.78)	n.d.	n.d.	37.43 (4.21)	n.d.	n.d.	31.95 (2.68)	n.d.	41.80 (3.42)	n.d.	57.88 (4.18)	n.d.	n.d.	n.d.	n.d.
Malathion	47.90 (2.16)	n.d.	43.65 (3.04)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	48.58 (4.07)	n.d.	n.d.	n.d.	n.d.
Fenthion	4.46 (0.86)	3.80 (0.61)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	6.98 (1.04)	n.d.	n.d.	n.d.
Thiabendazole	n.d.	42.94 (5.37)	47.72 (4.23)	n.d.	42.61 (3.78)	n.d.	n.d.	43.01 (6.04)	n.d.	46.62 (4.05)	n.d.	43.18 (5.14)	n.d.	n.d.	n.d.	n.d.
Imazalil	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Bifenthrin	42.87 (2.36)	39.94 (3.69)	34.96 (4.65)	54.65 (4.24)	42.61 (6.02)	38.56 (3.06)	57.35 (3.89)	43.01 (4.67)	48.02 (3.57)	42.52 (2.96)	29.74 (2.57)	43.18 (3.62)	26.75 (3.26)	37.45 (4.57)	18.34 (2.85)	37.57 (4.34)
Permethrin	104.96 (8.35)	77.12 (6.34)	81.39 (4.43)	n.d.	76.59 (3.75)	n.d.	n.d.	78.06 (4.04)	n.d.	69.48 (3.75)	n.d.	80.41 (4.67)	n.d.	n.d.	n.d.	n.d.
Prochloraz	38.45 (4.32)	38.07 (2.75)	18.32 (2.32)	21.45 (3.46)	19.38 (1.97)	n.d.	n.d.	28.28 (2.64)	21.32 (2.56)	23.00 (2,,25)	34.78 (3.57)	n.d.	n.d.	n.d.	n.d.	19.69 (3.76)
Pyraclostrobin	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Difenoconazole	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Azoxystrobin	37.85 (2.38)	39.78 (3.67)	45.89 (4.23)	35.64 (3.46)	55.79 (4.34)	16.32 (1.02)	24.58 (1.87)	48.37 (3.74)	23.91 (2.04)	37.73 (3.14)	14.37 (2.36)	52.20 (3.59)	18.67 (2.05)	12.67 (1.97)	19.63 (1.05)	27.89 (2.46)

n.d.: not detected.

3.10. Analysis of commercial samples

To ascertain its applicability, the method was employed to determine pesticide residues in sixteen mango samples which were cultivated according to conventional agricultural procedures. The samples were purchased from different retailers in the city of Salvador, state of Bahia, Brazil and immediately processed following the above described procedure. Analysis were made in triplicate and, between two samples, a fiber blank was carried out in order to check for the absence of carryover effects. Table 6 shows the results obtained. The pesticides bifenthrin and azoxystrobin were detected in all the samples, at concentrations of 18.34–57.35 $\mu g\,kg^{-1}$ and 12.67–55.79 $\mu g\,kg^{-1}$, respectively. Nevertheless, all the concentrations were below the MRL established by Brazilian legislation.

4. Conclusions

The results indicate that the method developed here, based on DI-SPME followed by GC–MS analysis, can be applied to the qualitative and quantitative determination of pesticide residues in mangoes and very likely also to other types of fruits and food matrices. The method is selective and sensitive, and allowed for the determination of 14 pesticides in samples – namely clofentezine, carbofuran, fenthion, thiabendazole, bifenthrin, imazalil, difenoconazole, permethrin, prochloraz, pyraclostrobin, azoxystrobin, parathion-methyl, malathion and diazinon – with good precision and recovery rates and LOQ below the MRL admitted by legislation.

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