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Research Article

Development of a Methodology for the Determination of Pesticide Residues in Cajá-Manga Pulp (*Spondias dulcis* L.) Using Solid-Liquid Extraction with Low-Temperature Partitioning

Escarlet T. D. Santana,[1] Dayane F. Soares,[2] and Anizio M. Faria[1]

[1]*Instituto de Ciências Exatas e Naturais do Pontal, Universidade Federal de Uberlândia, Ituiutaba, MG 38304-402, Brazil*
[2]*Instituto Federal de Educação, Ciência e Tecnologia do Triângulo Mineiro, Ituiutaba, MG 38305-200, Brazil*

Correspondence should be addressed to Anizio M. Faria; anizio@ufu.br

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Emergent fruits with functional properties and sui generis flavours are increasingly gaining international market, mainly due to the pleasant taste and its nutritional properties. However, methodologies for determination of pesticide residues in these fruits are still incipient or nonexistent. In this work, a simple, rapid, and accessible methodology to small laboratories was developed, optimized, and validated for the determination of four pesticide residues in the cajá-manga pulp: chlorpyrifos, thiabendazole, carboxin, and difenoconazole. The extraction of pesticides from the cajá-manga pulp was performed by the liquid-solid extraction with low-temperature partitioning, achieving efficiency in the range of 75.3–129.3% with limits of quantification between 170 and 430 $ng \cdot g^{-1}$ for all compounds. The methodology was validated according to the SANTE/11813/2017 document, demonstrating good detectability, selectivity, precision, and accuracy with limits of quantification within the range of the maximum residual limits preconized for the compounds. The quantification was performed by gas chromatography with flame ionization detection which, although it does not present the best detectability for the compounds, is a lower cost instrumentation and is available in several analysis laboratories in Brazil, making the method more accessible to evaluate the pesticide residues in fruit pulp. Thus, in this work, a methodology for the determination of pesticides in the cajá-manga pulp is available for the monitoring of pesticide residues in fruit pulp, in an efficient and accessible way.

1. Introduction

The healthy foods consumption has been growing every year at rates higher than those of the world economy. Among these foods, fruits stand out as the preferred and most consumed by the population [1–3]. Sources of vitamins, fibers, minerals, and various other substances of nutritional and/or therapeutic value, fruits, have pleasant sensorial characteristics that intensify their consumption. A fruit that has had a growing commercialization in several countries around the world, whether in natural or processed form as pulps, juices, popsicles, and ice creams, is the cajá-manga [4, 5]. The cajá-manga (*Spondias dulcis*) belongs to the Anacardiaceae family, is native of the Society Islands in Polynesia, has a *sui generis* flavour and a high nutritional value, and is rich in vitamins A and B, fiber, calcium, phosphorus, and iron, essential nutrients for a healthy lifetime [5–7].

The exploitation of the cajá-manga is mainly carried out in an extractivistic way in Brazil [8], and the cajazeira is frequently affected by diseases and plagues, such as anthracnose (*Colletotrichum gloeosporioides*), verrugose (*Sphaceloma fawcetti*), fruit flies, and sauva ants, which affect the production and the quality of fruits [9–11]. To supply an increasingly growing market for cajá-manga consumption, the use of pesticides is a faster and more effective solution to guarantee productivity and meet the growing demand. However, when applied incorrectly, pesticides leave residues in foods that cause harmful effect to human health, being one of the main problems of food intoxication today [12–17].

In Brazil, the monitoring of pesticide residues in food is carried out by the National Agency for Sanitary Vigilance (ANVISA) through the Programa de Análise de Resíduos de Agrotóxicos em Alimentos (PARA) [18]. Annually, the PARA publishes reports with the indexes of pesticide residues in the main foods of vegetal origin. Nevertheless, emerging fruits such as cajá-manga do not present methodologies for the determination of pesticide residues and, therefore, are not included in the reports of the PARA, despite the increasing popularization of its consumption.

In this work, a simple, fast, and easy-to-implement method was developed for the determination of four pesticide residues in the cajá-manga pulp by solid-liquid extraction with low-temperature partitioning (SLE-LTP) and quantification by gas chromatography with flame ionization detection (GC-FID). SLE-LTP is a simple extraction method involving a sequence of steps: addition of a small volume of water to the solid sample, addition of a water-miscible extractor solvent, vortex homogenization, and freezing of the system for phase separation [19–23]. The organic extract, which will remain liquid after the freezing step, is removed by using a syringe and introduced into a scientific instrumentation suitable for quantification of the analyte(s). SLE-LTP has already been used for the extraction of pesticides from different matrices, such as yacare eggs [19], soil [20], fresh food [21, 23, 24], and processed foods [22]. Due to the simplicity of use and efficiency in the extraction process, the optimal conditions for extraction of pesticide residues from the cajá-manga pulp by SLE-LTP will be determined in this work. The gas chromatography with flame ionization detection will be the analytical technique used to quantify the pesticide residues due to the possibility of applying the method in various laboratories, from those with a simpler structure to the larger ones. So, one of the objectives of this work is to provide a simple, efficient, and, mainly, accessible method to laboratories that do not have sophisticated instrumentation for pesticide residue analysis. The pesticides studied were difenoconazole, carboxin, chlorpyrifos, and thiabendazole, as they have an efficient action against the most common plagues and diseases that affect the cajá tree and are recommended by the Ministry of Agriculture in Brazil. The methodology was optimized, validated, and applied to cajá-manga samples acquired from local greengrocers in Triângulo Mineiro, Brazil.

2. Materials and Methods

2.1. Reagents and Solvents. HPLC-grade methanol, acetonitrile, hexane, and pesticide-grade ethyl acetate were purchased from Tedia (Rio de Janeiro, Brazil), while HPLC-grade dichloromethane was from Merck (Darmstadt, Germany). Ultrapure water (Millipore, São Paulo, Brazil) was used throughout. The solvents used in this work were filtered using a 0.22 μm Teflon membrane from Millipore. The pesticide standards chlorpyrifos (99%), carboxin (99%), difenoconazole (98%), and thiabendazole (98%) were obtained from Pestanal (Riedel-de-Häen, PA, USA). Standard stock solutions (1000 mg·L^{-1}) of these pesticides were prepared in methanol. Cajá-manga samples for application testing were collected from the greengrocers in Ituiutaba, MG, Brazil.

2.2. Solid-Liquid Extraction with Low-Temperature Partitioning. Cajá-manga fruits were washed, peeled, and pitted, and their pulps were cut into little pieces. Cajá-manga pulp pieces were processed in a conventional multiprocessor (Mallory, Oggy Black model; Madrid, Spain). 2.000 g of the cajá-manga processed pulp was spiked with stock solutions of the pesticide mixture. 0.500 g of the fortified pulp was submitted to the solid-phase extraction with low-temperature partition (SLE-LTP). Spiked samples were disposed off in Falcon tubes adding 4 mL of distilled water and different volumes (4, 6, 8, and 10 mL) of the solvent (methanol, acetonitrile, acetone, or ethyl acetate). The mixture was stirred in a vortex mixer for 1 min and allowed to stand by at room temperature for 5 min. After that, Falcon tubes were cooled at −4°C for different periods (0.5, 1.5, 3.0, and 6.0 h). All measures were performed in triplicates.

After the congelation time, the aqueous phase containing the cajá-manga pulp was frozen and the organic counterparts were maintained in the liquid form. 1 mL of the organic phase was collected and filtered in a paper filtration containing a clean-up sorbent (Si(PDAS), C_{18}, PSA, or silica). Si (PDAS) is a sorbent obtained by thermal immobilization of poly(alkylmethylsiloxane-co-dimethylsiloxane), a copolymer with C_{16}/C_{18} groups intercalated by C_1 groups, onto silica particles [25, 26]. The filtered organic extract was injected into a gas chromatographic system.

2.3. Method Validation. The methodology for the determination of the pesticide residues: chlorpyrifos, carboxin, thiabendazole, and difenoconazole in the cajá-manga pulp, using solid-liquid extraction with low-temperature partitioning, was validated according to the Guidance Document on Analytical Quality Control and Method Validation Procedures for Pesticides Residues Analysis in Food and Feed (SANTE/11813/2017 document) [27]. The analytical parameters of the methodology evaluated in this validation study were the selectivity, linearity, range, limits of detection and quantification, matrix effect, precision (repeatability and within-laboratory reproducibility), and accuracy.

2.4. Chromatographic Analyses. The extraction of the samples by the SLE-LTP method was performed in a gas chromatography (GC) model Focus GC, equipped with a flame ionization detector (FID) (Thermo, Austin, TX, USA) using a dimethylpolysiloxane OV-5-fused silica capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness) using the following conditions: N^2 was used as the carrier gas at a flow rate of 1.5 mL·min^{-1}, column inlet split ratio of 1 : 5, and temperature program at 100°C for 1 min and then heated at a rate of 25°C min^{-1} to 200°C and heated again at a rate of 40°C min^{-1} to 300°C and held isothermal for 4.5 min, injector temperature of 280°C, and a detector with a temperature of 300°C.

The presence of each pesticide residue in the sample was determined by comparing its retention time with that obtained for the pesticide reference standard, under the same GC analysis conditions formerly described. The GC retention times for chlorpyrifos, thiabendazole, carboxin, and difenoconazole were 4.87, 5.37, 6.40, and 8.87 min, respectively.

3. Results and Discussion

The pesticides evaluated in this study, carboxin, chlorpyrifos, difenoconazole, and thiabendazole, are those recommended for the diseases mitigation that usually affects the cajá-manga fruit. The main physicochemical properties of compounds are presented in Table 1, as well as their chemical structures in Figure 1.

According to the physicochemical properties of compounds in Table 1, the four pesticides have strong retention in the GC system with the Kováts retention index above 1900, which require a higher column temperature for elutions of them. The order of the compound elutions in the 5% phenylmethylsiloxane-95% dimethylsiloxane capillary column is observed by the values of the Kováts retention index (RI): chlorpyrifos, thiabendazole, carboxin, and difenoconazole, requiring temperature programming to separate substances with retention indexes close to each other and accelerate the elution of difenoconazole, which presents higher RI, justifying the GC temperature programming performed in this work. It is important to mention that these substances are normally analyzed by both HPLC and GC systems [29–33], and therefore, gas chromatography was chosen because it is a cleaner, less-expensive, and more accessible technique for the pesticide quantifications compared to HPLC. Typically, GC detection is performed by mass spectrometry, but flame ionization detection is also used for detection and quantification of these species and was chosen in this work due to the greater availability of this system in smaller laboratories, making the methodology more accessible.

3.1. Optimization of Solid-Liquid Extraction with Low-Temperature Partitioning. The solid-liquid extraction with low-temperature partitioning (SLE-LTP) was used in this study because it is an efficient method in the extraction of pesticide residues from different samples [19–23, 34–37] and, mainly, because it is an easy application method involving few steps and low consumption of solvents and sorbents compared to other extraction methods. The SLE-LTP was optimized for the extraction of pesticide residues from the cajá-manga pulp to obtain maximum recovery of the analytes. The studied parameters were the extractor solvent, the extractor solvent volume, the freezing time of the sample, and the addition of the clean-up sorbent.

SLE-LTP is a solvent extraction method in which the extractor solvents are usually miscible in water and their choice is essential to obtain an efficient extraction because the selectivity of the extraction method is associated with the polarity characteristics of them. In addition to the efficiency, two other characteristics are required in the solvent extractor: the first one is that its melting temperature should be below the melting temperature of the water so that in the freezing step, the extract can be separated from the sample matrix and remain in the liquid state during the freezing step, and the second one is that its density should be lower than that of the water, as this fact facilitates the removal of the extract in the extraction process. The ethyl acetate, acetone, acetonitrile, and methanol solvents were evaluated in this step, and the results are shown in Figure 2.

Acetonitrile was the solvent which resulted in the higher recoveries of pesticide residues from the cajá-manga pulp. Acetonitrile was also the solvent that extracted the least amount of lipophilic interferences from the matrices when compared with acetone and ethyl acetate, besides being completely miscible in water, being its density lower than that of water, and remaining liquid at temperatures lower than 0°C. The polarity indices of evaluated solvents are ethyl acetate 4.4, methanol 5.1, acetone 5.1, acetonitrile 5.8, and water 10.2. As can be observed, the polarity index of acetonitrile is the closest to that of water (more polar among the studied solvents), whereas methanol and acetone have similar polarity indices. These three solvents solubilize better in water than ethyl acetate, which may have led to similar behaviors in the extraction of pesticide residues compared to this latter solvent (Figure 2). Thus, considering all these characteristics, acetonitrile was chosen as the extractor solvent of the SLE-LTP method of pesticide residues from the cajá-manga pulp.

In the SLE-LTP, it is necessary to add water so that the sample matrix remains frozen in the partitioning step. For the sample used in the SLE-LTP process, a volume of 4.0 mL was required to ensure freezing of the sample and facilitate the process of compound partitioning into the organic phase. According to the pKa values of pesticides shown in Table 1, all compounds are in their nonionized molecular forms at pH 5-6 (pH from the aqueous matrix of cajá-manga), and therefore, the extraction does not require pH adjustment for a higher partition into the organic phase. In the literature, the ratio of water volume and extractor solvent volume suggested for the maximum extraction efficiency should be 1 : 2 [19, 34]. Thus, the volume of acetonitrile used in the SLE-LTP for a higher recovery of pesticide residues from the cajá-manga pulp was evaluated. The results obtained are shown in Figure 3.

Extraction using a smaller volume of acetonitrile did not allow adequate separation of the liquid and frozen phases in the freezing step. Despite the addition of 4 mL of acetonitrile, the nonfrozen liquid phase had a lower volume than 1 mL, and an amount of mass of the sample remained dispersed in this small acetonitrile volume, making it difficult to obtain a clean extract (free of fractions of the cajá-manga pulp). Thus, matrix components were concentrated in this small volume, causing strong interference in the elution of the compounds and leading to high extraction percentages, as seen in Figure 3. On the other hand, using volumes greater than 6 mL of acetonitrile, recovery of the residues becomes practically constant, with a satisfactory efficiency between 70 and 110%, for the four evaluated compounds. As the

Table 1: Physicochemical properties of the studied pesticides.

Compounds	log P	Molar mass (g·mol^{-1})	Water solubility (mg·L^{-1})*	Density (g·cm^{-3})*	Kováts retention index	pKa
Chlorpyrifos	4.96	350.58	2	1.4	1947	—
Thiabendazole	2.47	201.25	50	1.1	2010	4.08
Carboxin	2.14	235.30	147	1.7	2138	13.24
Difenoconazole	4.67	406.26	15	1.5	3136	1.95

*Measured at 25°C. All data are extracted from Reference [28].

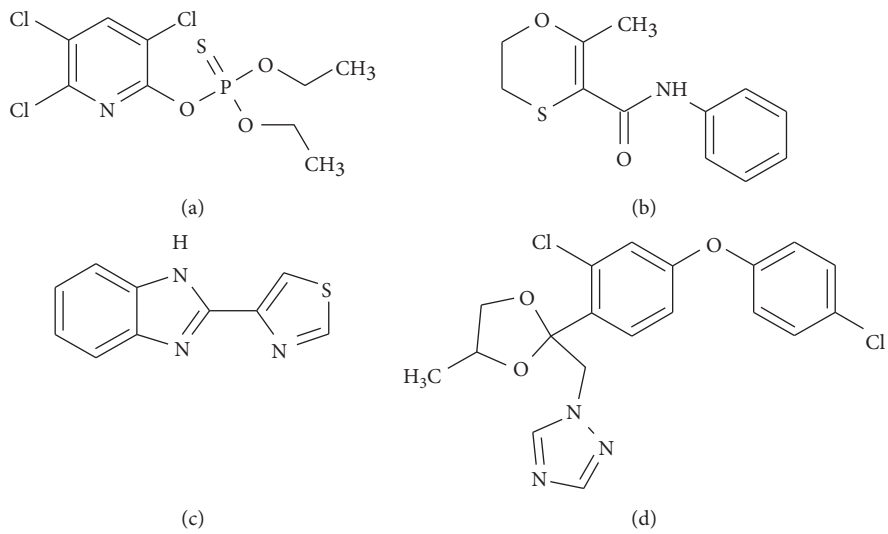

Figure 1: Chemical structures of pesticides: (a) chlorpyrifos, (b) carboxin, (c) thiabendazole, and (d) difenoconazole.

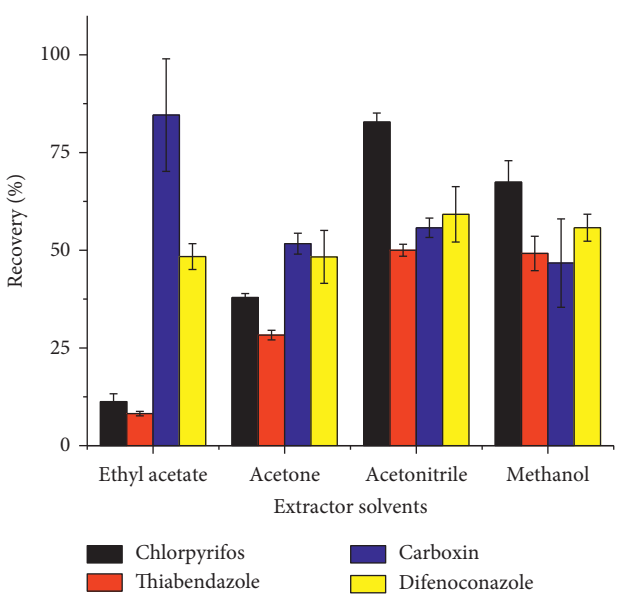

Figure 2: Pesticide residue recoveries in cajá-manga by the SLE-LTP method using different extractor solvents.

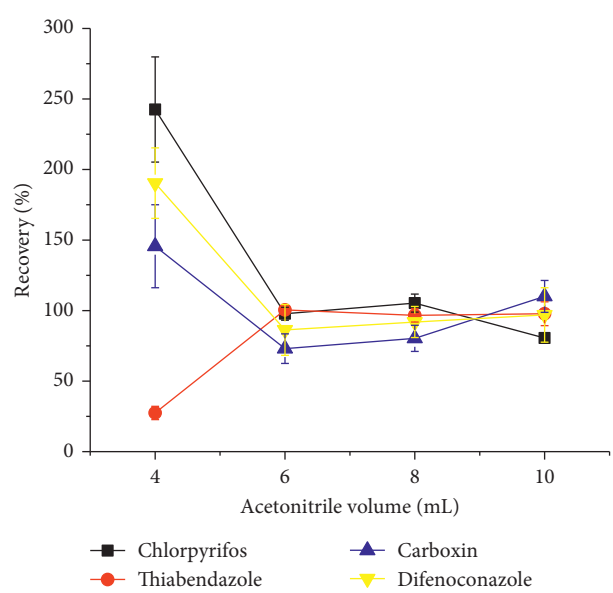

Figure 3: Pesticide residue recoveries in cajá-manga by the SLE-LTP method using different volumes of acetonitrile.

pesticide recoveries did not improve significantly with increasing acetonitrile volume from 6 mL, the lowest volume was defined for the SLE-LTP method. Therefore, the relation between the volumes of water and extractor solvent, 1 : 1.5, obtained for the extraction of pesticide residues from the cajá-manga pulp is lower than that suggested for some determinations in the literature using this method, minimizing costs and organic waste generation [19–22, 34]. It is also important to mention that according to the pesticide solubilities (Table 1), the four compounds are fully

solubilized in the aqueous fruit matrix at the concentrations evaluated in this study. However, their partitioning for less-polar solvents is favored since they have partition coefficients (log P) with a tendency to distribute to nonpolar phases. For this reason, the recovery rates obtained by the ESL-PBT method for the four pesticides were close to 100%.

The freezing in the solid-liquid extraction for partitioning the compounds at low temperature is a very important step for an efficient and concomitant-free extraction from the sample matrix. In this step, the separation of the phases occurs due to lowering of the temperature, condition in which there is the decrease of the solubility of acetonitrile in an aqueous phase. For the phase separation to take place efficiently, the freezing time of the sample should be long enough for the cajá-manga pulp solidification to occur preventing their transfer to the organic phase, and the distribution of the pesticides to the organic phase be as complete as possible. Thus, the minimum freezing time required for maximum recovery of pesticide residues from the cajá-manga pulp was evaluated, and the results are found in Figure 4.

The freezing time of 30 min was not enough to separate the phases by lowering the temperature because the aqueous phase and the pulp of the sample remained liquefied at this time. From 1 h and 30 min, the whole aqueous phase was frozen, during which time the highest recoveries of pesticide residues were recorded. From 3 h, there is a continuous drop in the pesticide residue recoveries. After 3 h of freezing time, the compounds probably decant within the organic liquid phase, since pesticides have higher densities than acetonitrile (Table 1). This decantation appears to increase gradually with increasing freezing time, leading to the pesticide adsorptions on the frozen phase surface and reducing their recoveries. In the literature, freezing times greater than 3 h are recorded for efficient extraction of pesticides from other matrices [19, 22, 23, 35]. As the composition of the cajá-manga pulp is approximately 70% water and low content of liposoluble components, the extraction of residues of the compounds studied occurred more quickly [5]. This lower freezing time leads to a reduction of the total sample preparation time, increasing the analytical frequency for this determination.

The cajá-manga pulp has an intense yellow coloration and constituents potentially interfering with the quantification of pesticides in the chromatographic system. Removal of these interferents in the sample preparation step is important to reduce the matrix effect on the response of compounds. Therefore, after the partition of pesticide residues into the organic phase of acetonitrile, different sorbents were evaluated for the removal of the constituents from the sample through a filtration step of the organic extract. The pesticides were recovered in the filtrate, and the results are presented in Figure 5.

According to Figure 5, the use of clean-up sorbents significantly reduces the amount extracted from the four compounds analyzed, independently of the polarity of evaluated sorbents. This result, however, does not represent a negative factor in the analyses, since the extracts obtained in this work presented clear and colorless aspects and, therefore, do not require a previous purification step for GC analysis. The cajá-manga has a pulp rich in carbohydrates, sugars, proteins, fibers, volatiles (terpenes), and minerals, besides an intense yellow coloration. Thus, a major advantage of SLE-LTP, in the case of a complex matrix such as cajá-manga pulp, is that the freezing of the sample presents itself as a clean-up step for the extracts.

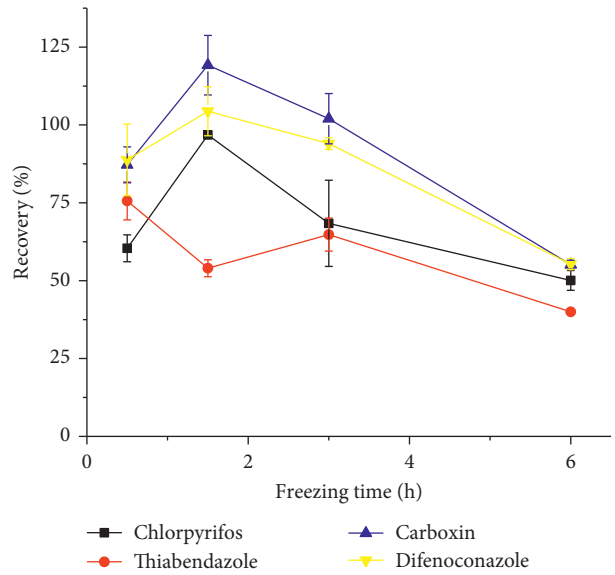

Figure 4: Pesticide residue recoveries in cajá-manga by the SLE-LTP method after different freezing times of samples.

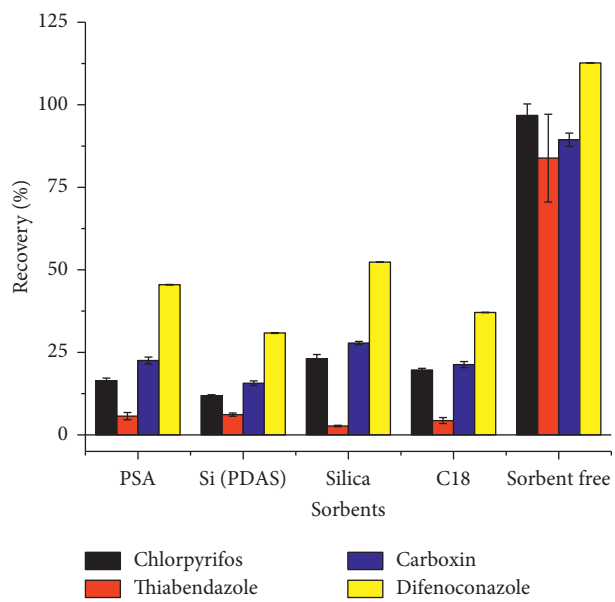

Figure 5: Pesticide residue recoveries in cajá-manga by the SLE-LTP method after the clean-up step of the extract, using different sorbents.

3.2. SLE-LTP Method Validation. After the optimization of SLE-LTP of pesticide residues from the cajá-manga pulp, the analytical parameters of the method were validated

according to the Guidance Document on Analytical Quality Control and Method Validation Procedures for Pesticides Residues Analysis in Food and Feed (SANTE/11813/2017 document) in relation to selectivity, linearity, range, limits of detection and quantification, accuracy, and precision [27].

The selectivity of the method was evaluated by the separation of the analytes by gas chromatography with a flame ionization detector. The chromatogram of the separation of the four pesticides extracted from the cajá-manga pulp is presented in Figure 6, in which the discrimination of the peaks for each pesticide from other eluted substances can be observed. So, the method can be considered selective for the pesticides studied.

Some analytical parameters of the method were determined from the calibration curve plots for each compound by the standard addition method, in which portions of the cajá-manga pulp were spiked with pesticide standards in different concentrations, 0.17 to 11.75 $\mu g \cdot g^{-1}$, submitted to the optimized SLE-LTP, and the extracts were analyzed by GC-FID. The analytical parameters obtained for the method are presented in Table 2. The limit of detection (LOD) and limit of quantification (LOQ) for each pesticide were also estimated from the analytical curves for each pesticide. The LOD was calculated by using the following equation: LOD = $3.3 \times s_y/b$, where b is the slope of the analytical curve and s is the residual standard deviation of the analytical curve. The LOQ was calculated as LOQ = 3 × LOD.

The LOQ for the four pesticide residues was in the range of 0.17 to 0.43 $\mu g \cdot g^{-1}$ in the cajá-manga pulp using the SLE-LTP method with analysis by GC-FID. As there is no reference for determination of residues of pesticides in cajá-manga, the values obtained were compared with the maximum residue limits (MRLs) of these pesticides in different cultures preconized by Brazilian legislation. For different food types, the MRLs are in the range of 0.05–0.20 $mg \cdot kg^{-1}$ for carboxin, 0.01–2.00 $mg \cdot kg^{-1}$ for chlorpyrifos, 0.01–1.00 $mg \cdot kg^{-1}$ for difenoconazole, and 0.01–10 $mg \cdot kg^{-1}$ for thiabendazole [38]. Therefore, it is observed that the LOQ values found for the pesticides in the proposed method are within the range of MRLs for different types of food monitored by ANVISA, although GC-FID does not have a high detectability for the compounds studied. The analytical curves presented excellent adjustments, with determination coefficients higher than 0.996. The adjustments were linear for the chlorpyrifos, carboxin, and difenoconazole compounds, whereas for thiabendazole, the curve showed a better fit in the quadratic model. Figure 7 shows the analytical curves obtained by the standard addition method and by the external standard method for each pesticide to evaluate a possible matrix effect from the cajá-manga pulp.

According to the slopes of the analytical curves (Figure 7), comparing each compound by the two calibration methods constructed under the same concentrations, all compounds showed a positive matrix effect [36]; that is, the sensitivity of the proposed method increases when the analytical curve is prepared in the cajá-manga pulp itself and submitted to the SLE-LTP process. The positive matrix effect indicates that components of the sample matrix have

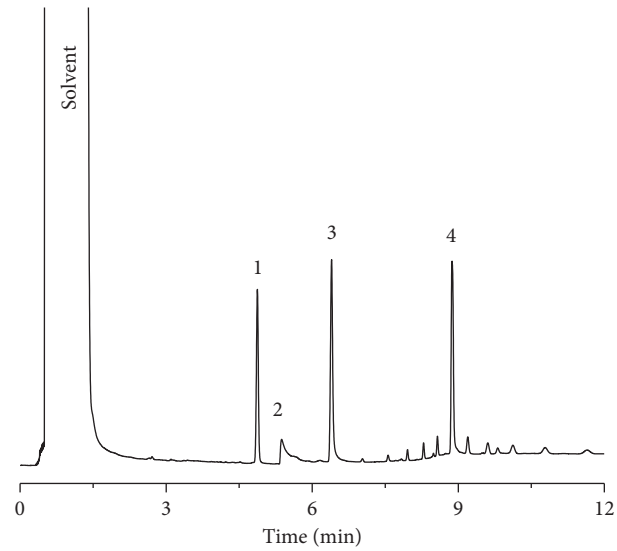

Figure 6: Separation of pesticide residues extracted from the cajá-manga pulp by the SLE-LTP method, using gas chromatography with flame ionization detection. Conditions: capillary column 30 m × 0.32 mm i.d. × 0.25 μm film thickness, 5% phenylmethylsiloxane-95% dimethylsiloxane, split injection 1 : 5, injected volume 1 μL, injector temperature 280°C, FID temperature 300°C, N_2 flow rate 1.5 $mL \cdot min^{-1}$, and oven temperature 100°C (1 min), 25°C min^{-1}, 200°C, 40°C min^{-1}, 300°C (4.5 min). (1) Chlorpyrifos; (2) thiabendazole; (3) carboxin; (4) difenoconazole.

a synergistic effect on the compound response in the sample, enhancing the analytical signal. This matrix effect is suppressed by the calibration using the standard addition method.

The precision and accuracy of the method for the determination of pesticide residues in the cajá-manga pulp were evaluated according to the recommendations of the SANTE guidelines [27]. The precision of the method was evaluated based on its repeatability and within-laboratory reproducibility, which were obtained by performing nine sample extractions on the same day in three different levels and by nine sample extractions in the same level on three different days, respectively. The relative standard deviation for repeatability (RSD_r) and relative standard deviation for within-laboratory reproducibility (RSD_{wR}) are presented in Table 3. The accuracy of the method was investigated by the recovery of pesticide residues from the cajá-manga pulp fortified at three different levels. Accuracy was obtained by percent recovery of each pesticide, and the results are presented in Table 3.

The SLE-LTP method presented adequate precision for the extraction of residues from the four pesticides selected for the study. As shown in Table 3, the method repeatability (RSD_r) was generally less than 20% at the different concentration levels evaluated, except for carboxin and chlorpyrifos at the lowest concentration level where the dispersion of the results was higher than 20%. The laboratory reproducibility (RSD_{wR}) evaluated at the 4 × LOQ level showed the low dispersion of the results with RSD values lower than 8% for all pesticides, indicating that the

Table 2: Analytical parameters of the SLE-LTP method of pesticide residues from the cajá-manga pulp.

Pesticides	Linearity (R^2)	Range ($\mu g \cdot g^{-1}$)	LOD ($\mu g \cdot g^{-1}$)	LOQ ($\mu g \cdot g^{-1}$)
Chlorpyrifos	0.996	0.18/9.05	0.06	0.18
Thiabendazole	0.999	0.43/10.80	0.13	0.43
Carboxin	0.996	0.17/8.35	0.06	0.17
Difenoconazole	0.998	0.24/11.75	0.14	0.24

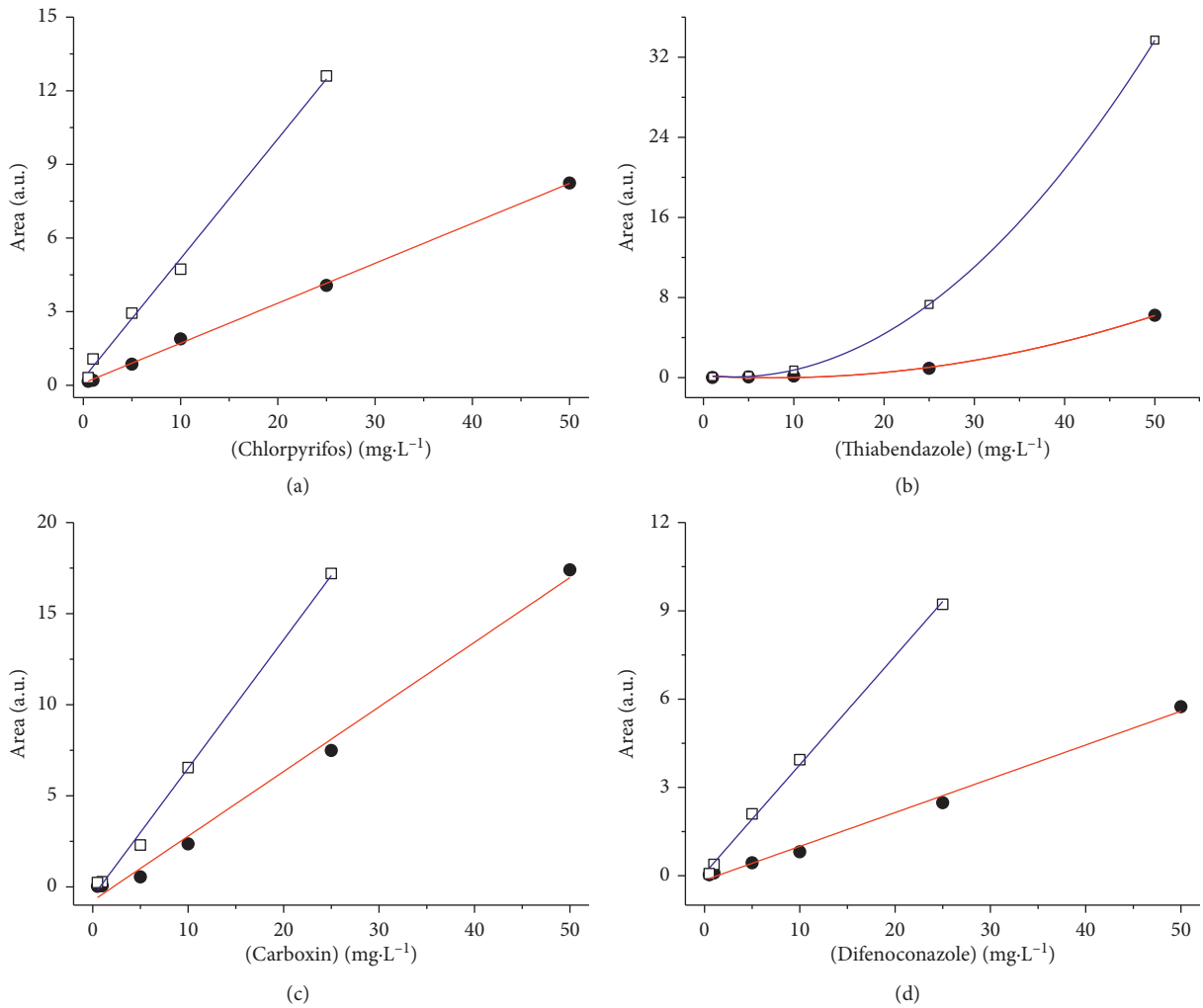

Figure 7: Analytical curves prepared by the external standard method and by the standard addition method for the compounds: (a) chlorpyrifos, (b) thiabendazole, (c) carboxin, and (d) difenoconazole.

Table 3: Accuracy (% recoveries) and precision (repeatability and within-laboratory reproducibility) of the SLE-LTP method developed ($n = 5$).

Pesticides	Repeatability						Within-lab reproducibility	
	2 × LOQ		3 × LOQ		4 × LOQ		4 × LOQ	
	Recovery (%)	RSD_r (%)	Recovery (%)	RSD_r (%)	Recovery (%)	RSD_r (%)	Recovery (%)	RSD_{wR} (%)
Chlorpyrifos	96	22	95	13	89	6	88	2
Thiabendazole	188*	14	129	7	117	6	111	5
Carboxin	69	26	78	8	82	11	76	7
Difenoconazole	80	13	90	4	109	9	102	6

* = Outlier value. A value above the acceptable range of recoveries for compounds in complex matrices.

application of the method on different days does not affect the recovery of the residues. The method also showed good accuracy, except for thiabendazole that showed recoveries always above 100%, probably due to the long tailing peak for its peak in the chromatogram, which makes more difficult the peak integration due to the possibility of coelution with constituents from the matrix or even due to the baseline instability. The lower the concentration of thiabendazole in the cajá-manga pulp, the more pronounced the interference becomes in the thiabenzadole quantification, reaching approximately 190% of recovery (Table 3). For the other compounds, the residue recoveries were in the range of 69 to 109%, being in accordance with the criteria recommended by the SANTE guidelines for this parameter, 70–120% [27].

3.3. Analysis of Real Samples. Two samples of different cajá-manga varieties, obtained from the greengrocer in Triângulo Mineiro, Brazil, were submitted to SLE-LTP with analysis by GC-FID to evaluate the accuracy and precision of the method. Both samples were spiked with a concentration equivalent to 4 × LOQ for each pesticide, and the recovery results are shown in Table 4.

The pesticide residue recoveries from the cajá-manga pulp of two commercially available varieties presented acceptable recovery ranges of 75.3 to 129.3%, considering the complexity of the matrices. Only, thiabendazole was not recovered in any of the three replicates of sample A. In this way, the method has a good accuracy of the measurements performed for the recovery of pesticides from the cajá-manga pulp, in which all the relative standard deviation (RSD) values were below 20%, as recommended by the SANTE document [27].

Table 4: Pesticide residue recoveries from two samples of the cajá-manga pulp fortified with pesticides, purchased from greengrocers in Triângulo Mineiro, Brazil ($n = 3$).

Pesticides	Sample A		Sample B	
	Recovery (%)	RSD	Recovery (%)	RSD
Chlorpyrifos	106.8	11.1	129.3	4.2
Thiabendazole	—	—	75.3	3.8
Carboxin	76.7	7.2	104.3	8.0
Difenoconazole	86.8	12.7	104.4	1.8

4. Conclusions

In this work, an SLE-LTP method was proposed to extract carboxin, chlorpyrifos, difenoconazole, and thiabendazole from the cajá-manga pulp. SLE-LTP has been successfully used to extract pesticide residues from different matrices, such as maize grains, potato, carrot, soil, tomato, lettuce, and yacare eggs. However, the amount of the sample, the organic extractor volume, the freezing time, and the need for an additional cleaning step, after partitioning at low temperature, aroused the interest in simplifying the method by reducing the amounts normally used and applying it in a complex matrix such as the cajá-manga pulp. The cajá-manga has a pulp rich in carbohydrates, sugars, proteins, fibers, volatiles (terpenes), and minerals, besides an intense yellow coloration. Allied to this fact, no methodology for the pesticide residue determination is available to monitor the potential cajá-manga contamination, despite the increasing consumption of the fruit. After optimization and validation, it was possible to obtain an efficient methodology, with good precision and accuracy, based on solid-liquid extraction with low-temperature partitioning, for the determination of pesticide residues in the cajá-manga pulp. The SLE-LTP method proposed reduced the extractor organic volume by at least 25%, the freezing time by half, and the sample mass by at least 75% when compared with previous works. The extraction method did not require an additional step for the cleaning of the extract, even though the cajá-manga was a matrix with a high content of soluble substances and intense color. The quantification performed by GC-FID makes the methodology more accessible, cleaner, and less expensive, making it possible to determine the pesticides below the MRLs established by Brazilian legislation.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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