https://dx.doi.org/10.21577/0103-5053.20230125

J. Braz. Chem. Soc., Vol. 34, No. 11, 1707-1716, 2023

Article

Pesticide Determination in Fresh Coconut Water (*Cocos nucifera* Linn.) by GC-MS Using Microwave-Assisted Liquid-Liquid Extraction

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Fresh coconut (*Cocos nucifera*) water, a popular beverage in tropical countries, is often exposed to pesticide contamination due to agricultural practices. Thus, this work proposed the simultaneous determination of 36 pesticides in fresh coconut water samples using liquid-liquid extraction, drying assisted by a household microwave oven, and gas chromatography-mass spectrometry (GC-MS) analysis. Limits of detection varied from 5.4 (disulfoton) to 9.6 ng L^{-1} (parathion-methyl). Nine pesticide residues were detected (dimethoate, γ-HCH, β-HCH, 4,4'-dichlorodiphenyldichloroethylene, endosulfan II, 4,4'-dichlorodiphenyldichloroethane, 4,4'-dichlorodiphenyltrichloroethane, endrin ketone, and methoxychlor) with concentrations from < limit of quantification to 5,768.34 ± 1.64 ng L^{-1}. Dimethoate was quantified in all samples, indicating its recent use. The proposed methodology offers several advantages, including the use of small sample volumes and solvent extraction, which eliminates the need for costly cartridges and reduces waste production. Additionally, the obtained results can be valuable for regulatory agencies, aiding in the mitigation of environmental damage and the protection of human health.

Keywords: liquid-liquid extraction, microwave oven, contamination, beverage, dimethoate

Introduction

The coconut (*Cocos nucifera* Linn.) is an economically recognized plant in the food and cosmetic industry due to its use in a wide variety of by-products including pulp, water, oil, and coconut milk.[1] Coconut water, taken directly from the inner part of the fruit, is widely consumed in tropical countries because it presents attractive sensorial characteristics such as sweet taste and refreshing sensation.[2,3] Also, it is rich in various functional bioactive compounds, including vitamins B and C, minerals, and enzymes, related to anti-inflammatory and antioxidant activities.[4]

Coconut crop is an important agricultural activity for the Brazilian economy, amounting to approximately R$ 1.3 billion in 2021. The country produced 1.64 million metric tons, being the Northeast region responsible for more than 70% of the national production.[5] In this scenario, Bahia State stands out with 552.5 thousand tons (i.e., 30.3%).[1] Frequently, pests attack coconut palms and reduce its productivity and lifespan. Then, pesticides are sprayed directly on foliage or injected into the stem and root systems to prevent or revert the damages, and consequently minimize losses in the coconut production.[6] The United States Environmental Protection Agency (US-EPA) defines pesticide as any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating pests.[7] Therefore, coconut water may be contaminated by residues of pesticides used during the planting, handling, and/or harvesting processes.[8]

Some pesticides are bioaccumulative and can also biomagnify, resulting in their residues persisting in crops and the environment. This not only impacts the quality of water, soil, and atmosphere but also poses negative health risks to humans and animals.[9] Exposure to pesticide residues may cause increased susceptibility to endocrine disrupting effects,[10] teratogenic fetal abnormalities,[11]

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Dedicated to Professor Carol Collins for her valuable contributions to Brazilian analytical chemistry.
Editor handled this article: Andréa R. Chaves (Associate)

occurrences of neurological disorders,[12] psychiatric disorders,[13] and neoplasms.[14]

The Food and Agriculture Organization of the United Nations (FAO) reports the worldwide use of pesticides in agriculture at 2.7 million tonnes (Mt) of active ingredients, while the total trade of formulated products attained approximately 7.2 Mt in 2020, reaching USD 41.1 billion.[15] In 2017, Bahia State used 26.3 thousand tons of active ingredients of pesticides, representing 4.87% of the Brazilian consumption, i.e., the eighth largest consumer.[16] Despite this, Brazilian and international laws do not establish maximum levels for these chemical species in this beverage, although studies confirmed the presence of these contaminants in coconuts produced and marketed in Brazil.[8]

Regarding the literature, gas chromatography coupled to mass spectrometry (GC-MS) is the most widely used technique due to its high selectivity, sensitivity, and low limits of detection for pesticide determination in fresh and processed foods.[17-23] To the best of our knowledge, no works involving the use of a household microwave (which is a cheap and accessible device) for the drying step of thirty-six pesticides in coconut water has been developed. Therefore, this work proposes the simultaneous GC-MS determination of thirty-six pesticides (from the organochlorine, organophosphate, pyrethroid, carbamate, thiocarbamate, and strobilurin classes) in commercial fresh coconut water samples using a simple, fast, and efficient liquid-liquid extraction (LLE) methodology that employs small volumes of samples and solvents, and a drying step assisted by a household microwave oven.

Experimental

Chemicals, reagents and samples

Standard solutions of pesticides were prepared in a hexane:toluene (1:1) solution (both of analytical grade, Sigma-Aldrich, Saint Louis, USA) using a mixture of certified reference standards containing eighteen organochlorine pesticides (at 2,000 μg L^{-1}; EPA 46960-U, Bellefonte, USA) and other eighteen pesticides from the carbamate, thiocarbamate, organophosphate, pyrethroid, and strobilurin classes (at 10,000 μg L^{-1}; AccuStandard, New Haven, USA).

The stock standard solution of the thirty-six analytes were also prepared in hexane:toluene (1:1) at a concentration of 1,000 μg L^{-1}. Then, working solutions were prepared by appropriated dilution of the stock standard solution using the same solvent.

The "green dwarf coconut" variety was selected for validation and method applicability. A total of thirty coconuts were acquired from two farms that supply the product to local markets in Barreiras city, Bahia State, Brazil. All samples were within the 6 to 8 months of maturity. Fresh coconut water samples were then stored in amber glass bottles (100 mL) previously cleaned, and finally frozen at –20 °C until analysis.

Analytical procedure

Sample preparation

Initially, the efficiency of the household microwave oven for the drying step was evaluated. For this, standard solution was dried in different ways: (*i*) 15 min of heating; (*ii*) 5 min of heating and 5 min of cooling; and (*iii*) 3 min heating and 3 min of cooling. After total drying, they were resuspended and injected into the GC-MS. The drying step using a household microwave oven was developed following a previous work.[22] A household microwave oven (Consul, model CMS25ABHNA, 2450 MHz, 1.2 KW) was used.

Based on the best results obtained for the drying step, the experimental conditions for the extraction method were optimized by varying the solvents (hexane and hexane:toluene), the proportion of the hexane:toluene mixtures (1:1 and 7:3), stirring time (20 and 30 min), and stirring speed (200, 600 and 800 rpm). Six tests were then carried out: (*i*) toluene, 20 min of stirring, and speed of 200 rpm; (*ii*) toluene, 30 min of stirring, and speed of 200 rpm; (*iii*) toluene, 30 min of stirring, and speed of 600 rpm; (*iv*) toluene, 30 min of stirring, and speed of 800 rpm; (*v*) hexane:toluene (1:1), stirring for 30 min, speed 800 rpm; (*vi*) hexane:toluene (7:3), 30 min of stirring, and speed of 800 rpm. Magnetic stirring was performed using a Fisatom stirrer (model 753A, São Paulo, Brazil). All tests were performed with spiked samples of fresh coconut water at 1,000 ng L^{-1}. After total drying, they were resuspended and analyzed by GC-MS, followed by the analysis of the standard solution at equivalent concentration.

After optimization, thirty-six pesticides were simultaneously extracted using 10 mL of fresh coconut water and 500 μL of hexane:toluene (7:3) solution into a glass vial. The mixture was then subjected to magnetic stirring for 30 min (800 rpm). The supernatant (400 μL) was collected in an amber vial and dried in a household microwave oven at the minimum power (70 W), with intervals of 3 min between the heating and cooling steps. The cooling step was performed by removing the amber vial from the household microwave and left at room temperature. After complete drying, the samples were resuspended in 200 μL of hexane:toluene (7:3) solution and then subjected to GC-MS analysis.

Chromatographic method

The analytes were determined using a single quadrupole GC-MS-QP2020 NX gas chromatograph-mass spectrometer (Shimadzu, Kyoto, Japan), with a split/splitless injector. For the chromatographic separation, a fused-silica capillary column (DB-5,5% phenyl, 95% methylsiloxane, Agilent Technology, USA) was used as the stationary phase (30 m × 0.25 mm i.d. × 0.25 μm). Helium (purity > 99.999 vol%) was used as the carrier gas at a flow rate of 1 mL min^{-1}.

For the pesticide analysis, the GC oven was initially heated to 60 °C (t = 0 min). After 1 min at this temperature, the oven was heated to 200 °C at a rate of 25 °C min^{-1}. Then, it was heated to 230 °C at 5 °C min^{-1}, and finally to 300 °C, maintaining for 1.5 min. The injector was set at 300 °C in the splitless mode and the injection volume was 1.0 μL. The temperatures of the ion source and interface were set at 300 °C. The mass spectrometer was operated in the electron ionization mode at ionization energy of 1.1 kV.

The analytical method was developed in the scan mode, using a standard solution at 100 μg L^{-1}. Selected ion monitoring (SIM) was used for the quantification of pesticides. The most intense and specific ions were chosen for each analyte (one quantification ion and up to three confirmation ions). The ion ratios from sample extracts were within ± 30% (relative) of the average of the calibration standards.[24] The quantification and confirmation ions were confirmed using the National Institute of Standards and Technology (NIST) database,[25] as indicated in Table 1. All analyzes were carried out in triplicate.

Method validation

The parameters used for the method validation followed the recommendations of the *Codex Alimentarius*,[24] guideline CXG 90-2017: selectivity, calibration, linearity, limit of detection (LOD), limit of quantification (LOQ), intra- and inter-day precision, matrix effect, accuracy (recovery test), and application of the method to real samples of fresh coconut water.

The calibration was performed by constructing analytical curves in the form $y = ax + b$, where y is the area of the peaks, a is the angular coefficient, x is the concentration value, and b is the linear coefficient of regression line. Coefficient of determination (R^2) greater than 0.99 was considered as linearity criterion in the regression of the calibration curves constructed using seven different concentration levels in the range from 10 to 400 ng L^{-1}. The LOD and LOQ were calculated as $(3\times S_b)/a$ and $(10\times S_b)/a$, respectively, where S_b is the standard deviation of the linear coefficient and a is the angular coefficient of the calibration curve. The matrix effect was investigated by comparing the slope of the analytical curve obtained from the analyte standards with the slopes of the analytical curves obtained from the spiked samples. Precision and accuracy were estimated by analyzing fresh coconut water samples spiked with the standard work solution containing all the pesticides, to achieve final concentrations of 32, 200, and 350 ng L^{-1}, in triplicate. Precision was assessed by the coefficient of variation (CV) of nine injections performed at three different times (morning, afternoon, and night) on the same day (intra-day precision) and at the same time by seven consecutive days (inter-day precision). Accuracy was assessed by conducting analyte recovery tests, which involve adding a known quantity of the analyte (32, 200, and 350 ng L^{-1}) to a sample and subsequently determining the percentage of the added amount that was detected. After validation, the analytical methodology was applied to determine the concentration of thirty-six pesticides in fresh coconut water samples.

Results and Discussion

Validation method and optimization of extraction conditions

The selectivity of the method was evaluated by comparing the chromatograms resulting from the analysis of the standard solution containing the pesticides at 50 μg L^{-1} (Figure 1a), extraction of a spiked sample (Figure 1b), and extraction of a non-spiked sample (Figure 1c). These chromatograms were obtained in full scan mode, so at the beginning, it is possible to observe the elution of several compounds. The scan mode allowed for a comprehensive visualization of the compounds present in the matrix. However, confirmation that only the analytes eluted was achieved through validation tests (matrix effect, accuracy) in the SIM mode. Since a blank sample of fresh coconut water was not available, the selectivity test was performed on a real sample that contained pesticides. The selectivity was confirmed through the recovery test, where the area values of the pesticides identified in Figure 1c (non-spiked sample) were compared to the differences observed in Figure 1b (spiked sample) and Figure 1a (analytical standard).

Table 2 shows the analytical validation parameters for the simultaneous determination of thirty-six pesticides in fresh coconut water samples. As observed, the values of R^2 were from 0.9990 to 0.9997 for all pesticides. For the analysis of the commercial fresh coconut water samples, dilutions of the extracts were performed for all concentrations above the working linear range. LOD

Table 1. Experimental parameters selected for the GC-MS analysis of pesticides in fresh coconut water samples

Segment / min	Pesticide	Retention time / min	Quantification ion (*m/z*)	Confirmation ion (*m/z*)
5-8	carbofuran	6.15	164	122; 149
	molinate	7.54	126	55
8.01-9.9	sulfotep	8.40	322	97
	dimethoate	8.56	87	125
	α-HCH	8.74	183	109
	γ-HCH	9.20	219	109
	β-HCH	9.26	183	109
	diazinon	9.28	137	179
	disulfoton	9.47	88	142; 186
	demeton-O	9.47	89	125
	δ-HCH	9.70	183	109
9.91-12.4	parathion-methyl	10.25	109	125; 263
	heptachlor	10.52	272	100; 274
	fenitrothion	10.80	277	109; 125; 260
	malathion	10.92	173	93; 127
	fenthion	11.20	278	109; 125
	chlorpyrifos	11.22	197	97; 314
	aldrin	11.25	66	265
	parathion	11.26	291	97
	heptachlor epoxide	12.13	353	81; 263
12.41-15.45	endosulfan I	13.03	239	195
	4,4'-DDE	13.54	246	318
	dieldrin	13.70	263	79; 108
	endrin	13.70	277	81; 237
	endosulfan II	14.55	195	241
	4,4'-DDD	14.71	235	165
	ethion	14.74	231	153
	endrin aldehyde	15.08	279	173; 345
15.46-18	endosulfan sulfate	15.77	237	276
	4,4'-DDT	15.77	235	165
	endrin ketone	17.17	317	67
	bifenthrin	17.23	181	166
	methoxychlor	17.48	227	196; 212
18.01-28	permethrin 1	20.32	183	165
	permethrin 2	20.55	183	165
	azoxystrobin	25.60	344	288

HCH: hexachlorocyclohexane, DDE: dichlorodiphenyldichloroethylene, DDD: dichlorodiphenyldichloroethane, DDT: dichlorodiphenyltrichloroethane; α: alfa; γ: gama; β: beta; δ: delta.

values varied from 5.4 ng L^{-1} for disulfoton to 9.6 ng L^{-1} for parathion-methyl, while LOQ values varied from 17.8 to 31.8 ng L^{-1} for the same analytes, respectively. For all the analytes, the intra-day precision presented CV values lower than 10%, being the highest one of 7.86% for dimethoate at 350 ng L^{-1}. The inter-day precision, in turn, had the highest CV value of 21.12% for methyl parathion at 32 ng L^{-1}. Both intra- and inter-day precision values were in accordance with the Official Methods of Analysis of AOAC International, which precision varies according to the analyte concentration. At concentrations below or equal to 1,000 ng L^{-1}, the CV should be within 30%.[26]

The accuracy was assessed through a recovery study, which considered the efficiency of the extraction and drying steps. When comparing the results, extraction with toluene for 30 min at 800 rpm, with 3 min of heating followed by

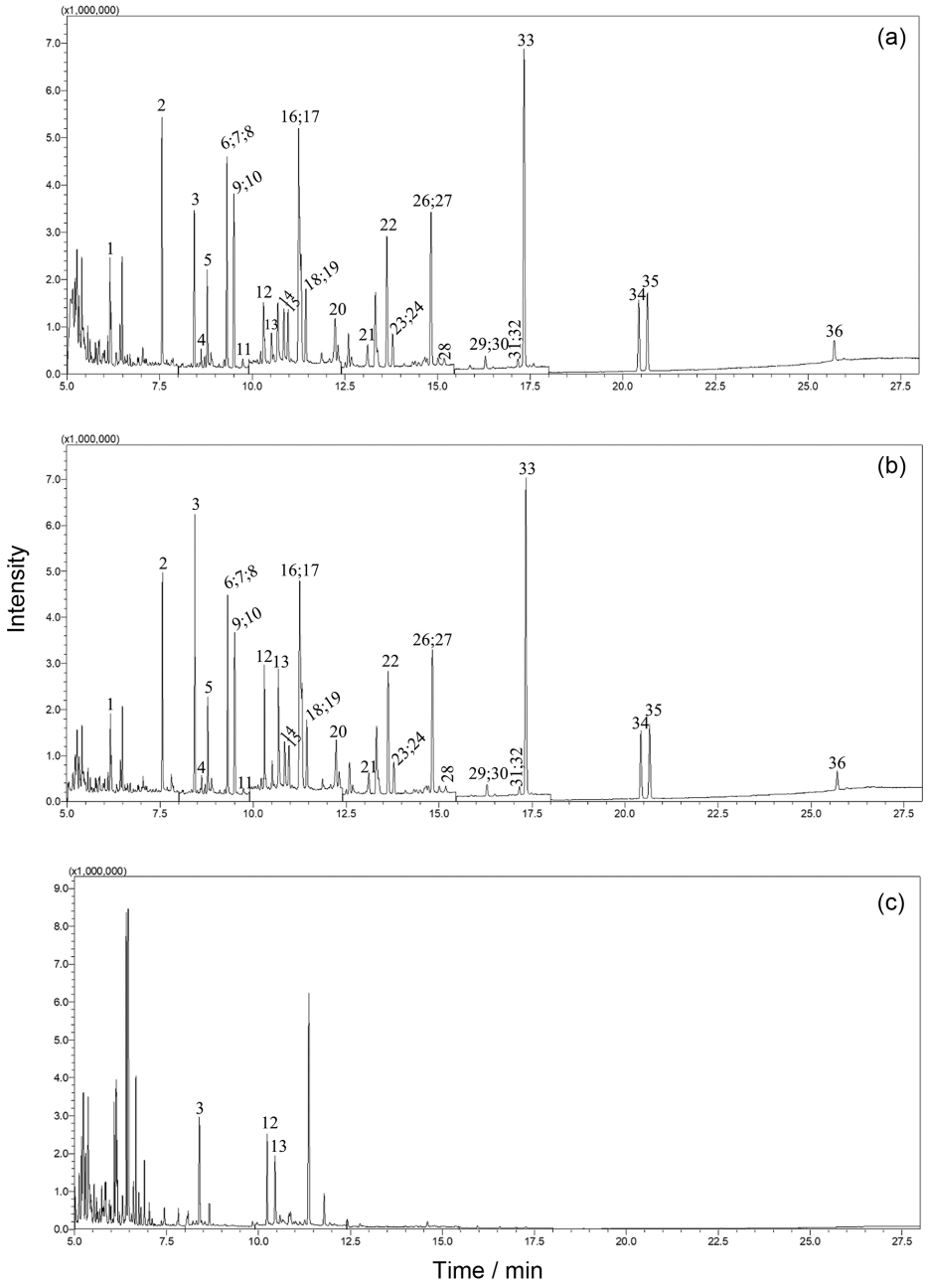

Figure 1. (a) Chromatogram of the analytical standard containing the 36 pesticides (1: carbofuran; 2: molinate; 3: sulfotep; 4: dimethoate; 5: α-HCH; 6: γ-HCH; 7: β-HCH; 8: diazinon; 9: disulfoton; 10: demeton-O; 11: δ-HCH; 12: parathion-methyl; 13: heptachlor; 14: fenitrothion; 15: malathion; 16: fenthion; 17: chlorpyrifos; 18: aldrin; 19: parathion; 20: heptachlor epoxide; 21: endosulfan I; 22: 4,4'-DDE; 23: dieldrin; 24: endrin; 25: endosulfan II; 26: 4,4'-DDD; 27: ethion; 28: endrin aldehyde; 29: endosulfan sulfate; 30: 4,4'-DDT; 31: endrin ketone; 32: bifenthrin; 33: methoxychlor; 34: permethrin 1; 35: permethrin 2; 36: azoxystrobin). (b) Chromatogram of the extraction of 36 pesticides in a spiked sample. (c) Chromatogram of the extraction of 36 pesticides in a non-spiked sample.

3 min of cooling, showed better result. This approach was chosen because the amber glass vial containing the standard solution did not reach a high enough temperature to degrade or completely evaporate the analytes. In other words, there was no significant difference (at a significance level of 0.05) between the peak areas of the standard solution after microwave-assisted drying and a standard solution at the same concentration. Figure 2 shows the recovery results obtained for the optimization of the extraction method using the six different experimental conditions. Comparing the results of tests (*i*) to (*iv*), where the samples were extracted using only toluene, it is possible to conclude that the longer the extraction time and the higher the agitation speed, the better the recovery values for the studied analytes. Moreover, it was verified that the analytes with lower polarity presented low recovery values. Thus, solvent mixtures with

Table 2. Analytical method validation parameters for the determination of the 36 pesticides studied in fresh coconut water samples

Pesticide	Linear range / (ng L^{-1})	R^2	LOD / (ng L^{-1})	LOQ / (ng L^{-1})	Intra-day precision (CV) / %			Inter-day precision (CV) / %			Recovery / %					
											32 ng L^{-1}		200 ng L^{-1}		350 ng L^{-1}	
					32 ng L^{-1}	200 ng L^{-1}	350 ng L^{-1}	32 ng L^{-1}	200 ng L^{-1}	350 ng L^{-1}	Mean	CV	Mean	CV	Mean	CV
Carbofuran	28.2-400	0.9992	8.4	28.2	1.55	0.69	1.22	13.10	12.74	12.11	94.9	6.94	96.7	4.69	99.0	2.88
Molinate	20.6-400	0.9996	6.2	20.6	3.93	1.32	0.75	12.33	11.71	11.40	96.6	10.76	99.6	7.06	103.3	4.08
Sulfotep	22.6-400	0.9995	6.8	22.6	1.74	2.37	0.86	9.36	11.17	8.74	95.3	7.36	97.2	4.96	99.6	3.02
Dimethoate	30.4-400	0.9991	9.0	30.4	1.71	4.22	7.86	11.59	10.05	10.72	97.2	1.20	100.6	1.18	100.9	1.16
α-HCH	28.4-400	0.9992	8.6	28.4	2.21	2.49	0.24	15.51	10.22	11.05	95.8	5.94	97.3	4.08	99.2	2.58
γ-HCH	30.2-400	0.9991	9.0	30.2	3.53	1.58	0.76	8.64	8.66	12.25	91.3	10.4	94.2	6.84	97.8	3.94
β-HCH	25.6-400	0.9993	7.6	25.6	4.82	7.52	0.59	13.66	10.85	11.63	98.2	5.95	99.7	4.10	101.6	2.61
Diazinon	28.6-400	0.9992	8.6	28.6	6.18	1.17	0.74	13.71	8.64	11.33	94.8	5.92	96.3	4.07	98.1	2.57
Disulfoton	17.8-400	0.9997	5.4	17.8	1.37	0.44	0.72	19.98	12.29	13.16	94.0	6.24	95.6	4.26	97.6	2.66
Demeton-O	22.8-400	0.9995	6.8	22.8	1.82	3.02	3.95	20.06	12.59	13.30	84.1	4.48	85.2	3.13	86.5	2.04
δ-HCH	31.2-400	0.9990	9.4	31.2	3.49	1.33	1.44	20.06	13.35	13.96	91.8	4.02	92.8	2.88	93.9	1.96
Parathion-methyl	31.8-400	0.9990	9.6	31.8	1.91	1.69	0.52	21.12	13.05	12.23	110.0	4.12	112.9	3.02	111.0	2.13
Heptachlor	25.6-400	0.9993	7.6	25.6	2.08	2.83	1.08	19.04	14.19	12.11	95.5	3.34	96.2	2.48	97.1	1.78
Fenitrothion	30.2-400	0.9991	9.0	30.2	3.97	1.39	1.63	18.06	13.83	10.05	95.0	7.51	96.9	5.05	99.4	3.06
Malathion	20.2-400	0.9996	6.0	20.2	4.28	2.35	1.44	17.50	10.17	11.04	106.1	5.71	107.5	3.98	109.2	2.59
Fenthion	22.4-400	0.9995	6.8	22.4	2.19	4.25	0.29	20.78	13.26	11.78	72.9	4.18	75.9	2.90	88.2	1.86
Chlorpyrifos	29.8-400	0.9991	9.0	29.8	1.96	3.31	0.01	11.88	13.02	10.83	99.5	4.98	100.7	3.51	102.2	2.32
Aldrin	25.8-400	0.9993	7.8	25.8	6.28	0.38	1.92	13.94	11.73	12.59	94.3	4.93	92.5	3.44	94.0	2.24
Parathion	27.6-400	0.9992	8.2	27.6	4.53	3.86	0.87	4.86	12.85	11.18	104.2	6.46	104.8	4.43	106.8	2.80
Heptachlor epoxide	31.0-400	0.9990	9.2	31.0	2.28	1.54	2.01	5.42	3.23	6.25	89.9	7.59	91.9	5.08	94.4	3.05
Endosulfan I	31.0-400	0.9990	9.4	31.0	4.31	2.04	1.55	6.45	8.02	8.48	99.8	8.06	101.9	5.41	104.6	3.27
4,4'-DDE	21.8-400	0.9995	6.6	21.8	1.81	2.35	1.05	9.85	8.47	12.01	90.3	8.79	92.7	5.82	95.6	3.42
Dieldrin	30.0-400	0.9991	9.0	30.0	1.51	3.68	6.83	8.05	9.29	8.96	106.8	4.11	107.7	3.00	108.8	2.10
Endrin	29.4-400	0.9991	8.8	29.4	2.26	2.50	0.56	6.35	8.83	11.10	91.9	4.73	93.1	3.32	94.5	2.18
Endosulfan II	28.6-400	0.9992	8.6	28.6	3.89	2.21	1.51	4.70	6.31	5.77	80.7	5.77	75.5	3.91	84.1	2.41
4,4'-DDD	31.2-400	0.9990	9.4	31.2	4.88	7.22	1.22	8.13	11.07	11.81	103.6	6.52	105.2	4.47	107.2	2.82
Ethion	30.0-400	0.9991	9.0	30.0	6.01	1.68	1.31	11.33	7.81	12.36	94.2	7.84	96.3	5.25	98.9	3.16
Endrin aldehyde	25.8-400	0.9993	7.8	25.8	2.11	1.31	1.55	9.42	5.09	5.09	86.6	6.32	88.2	4.28	90.3	2.63
Endosulfan sulfate	31.8-400	0.9990	9.6	31.8	2.35	3.39	4.19	5.28	4.29	8.10	96.5	6.76	98.3	4.60	100.4	2.84
4,4'-DDT	26.0-400	0.9993	7.8	26.0	3.62	1.75	1.85	10.23	12.69	12.46	100.6	6.14	102.1	4.23	104.0	2.68
Endrin ketone	19.0-400	0.9996	5.6	19.0	2.25	2.06	1.04	4.83	8.02	1.86	100.2	10.8	117.9	7.15	113.5	4.21
Bifenthrin	30.6-400	0.9990	9.2	30.6	2.73	3.35	1.84	8.21	5.79	5.21	97.9	7.54	99.9	5.08	102.3	3.09
Methoxychlor	30.2-400	0.9991	9.0	30.2	4.21	1.98	2.19	6.46	6.11	5.04	90.4	1.64	90.6	1.40	90.8	1.21
Permethrin 1	29.0-400	0.9991	8.8	29.0	4.24	2.57	1.79	8.64	12.6	9.31	105.5	4.26	106.4	3.09	107.6	2.14
Permethrin 2	30.4-400	0.9991	9.0	30.4	2.61	4.39	0.96	11.12	12.96	9.42	109.0	4.29	109.9	3.12	111.1	2.18
Azoxystrobin	30.0-400	0.9991	9.0	30.0	2.33	3.46	0.61	10.77	6.68	5.49	86.3	3.07	86.9	2.27	87.1	1.62

R^2: coefficient of determination; LOD: limit of detection; LOQ: limit of quantification; CV: coefficient of variation; HCH: hexachlorocyclohexane; DDE: dichlorodiphenyldichloroethylene; DDD: dichlorodiphenyldichloroethane; DDT: dichlorodiphenyltrichloroethane.

different dipole moment (μ) (hexane (μ = 0.00) and toluene (μ = 0.360)) were studied to determine the best proportion that allows an efficient recovery for all thirty-six pesticides.

After optimization, the best condition for simultaneous extraction of the studied analytes was add 500 μL of hexane:toluene (7:3) to 10 mL of sample, under magnetic stirring at 800 rpm for 30 min and drying in household microwave oven with intervals of 3 min between heating and cooling steps. Under this condition, recoveries of the pesticides (Table 2) ranged from 72.9% (fenthion) to 110% (parathion-methyl) at 32 ng L^{-1}, from 75.5% (endosulfan II) to 117.9% (endrin ketone) at 200 ng L^{-1}, and from 84.1% (endosulfan II) to 113.5% (endrin ketone) at 350 ng L^{-1}. All these values are in accordance to the *Codex Alimentarius* guideline,[24] which establishes recovery values in the range 70-120% with CV ≤ 20% in complex matrices.

For the matrix effect study, the parallelism test showed *p*-values ranging from 0.09 to 0.95 ($p > 0.05$). In this sense, the null hypothesis that the slope coefficients are equal is valid for the thirty-six analytes at a significance level of 0.05, i.e., there are no interferences in the analytical instrumental response related to the various components of the matrix.

Regarding the literature, the results obtained in this work for LOQ were significantly lower than those reported in previous studies employing different extraction and detection methods (Table 3). For the accuracy, despite some studies[23] presented better recovery results, they made use of expensive and laborious extraction techniques such as solid-phase microextraction (SPME) and matrix solid-phase dispersion (MSPD). Therefore, the present work stands out because it used small solvent volumes, besides replaced gas purge with drying in a household microwave

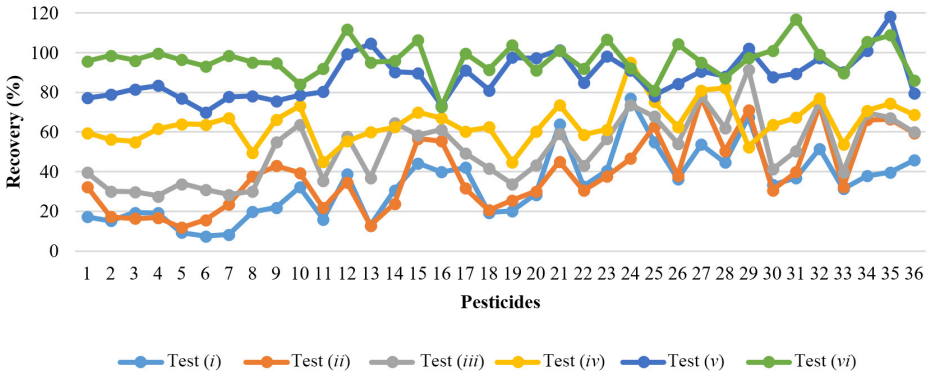

Figure 2. Recovery results obtained for the optimization of the extraction method (1: carbofuran; 2: molinate; 3: sulfotep; 4: dimethoate; 5: α-HCH; 6: γ-HCH; 7: β-HCH; 8: diazinon; 9: disulfoton; 10: demeton-O; 11: δ-HCH; 12: parathion-methyl; 13: heptachlor; 14: fenitrothion; 15: malathion; 16: fenthion; 17: chlorpyrifos; 18: aldrin; 19: parathion; 20: heptachlor epoxide; 21: endosulfan I; 22: 4,4'-DDE; 23: dieldrin; 24: endrin; 25: endosulfan II; 26: 4,4'-DDD; 27: ethion; 28: endrin aldehyde; 29: endosulfan sulfate; 30: 4,4'- DDT; 31: endrin ketone; 32: bifenthrin; 33: methoxychlor; 34: permethrin 1; 35: permethrin 2; 36: azoxystrobin). Test (*i*): toluene, 20 min of stirring, and speed of 200 rpm; test (*ii*): toluene, 30 min of stirring, and speed of 200 rpm; test (*iii*): toluene, 30 min of stirring, and speed of 600 rpm; test (*iv*): toluene, 30 min of stirring, and speed of 800 rpm; test (*v*): hexane:toluene (1:1), stirring for 30 min, speed 800 rpm; test (*vi*): hexane:toluene (7:3), 30 min of stirring, and speed of 800 rpm.

oven, leading to a noteworthy analytical method able to determine simultaneously thirty-six pesticides of different chemical classes at very small concentrations in a complex matrix such as coconut water.

Application in real samples of fresh coconut water

After validation and optimization of the extraction method, the thirty-six pesticides studied were determined in thirty samples of fresh coconut water. Although most of the pesticides have been or are still used in the crops of the studied region, twenty-seven from the thirty-six pesticides were not identified in any of the samples of fresh coconut water analyzed. The concentration of the nine pesticides found in commercial fresh coconut water samples are shown in Table 4.

In fact, only γ-HCH and dimethoate were found in all samples, although only dimethoate (68.90 ± 0.92-5,768.34 ± 1.64 ng L^{-1}) was quantified. Analytes such as 4,4'-DDE, detected in sixteen samples, and endosulfan II, detected in twenty-two samples, presented concentrations below their respective LOQ values, while β-HCH, 4,4'-DDD, 4,4'-DDT, endrin ketone, and methoxychlor had lower detection frequencies. It is worth noting that the use of dimethoate in coconut crop is a common practice in Brazil, which is described by the Brazilian Agricultural Research Corporation (EMBRAPA) as an agent used to mitigate pest action in this crop.[27] It must also be highlighted that dimethoate is resistant to photodegradation and has a half-life lower than two months.[28,29] The results obtained in this work may be associated to recent contamination by dimethoate in the cultivation areas, either by direct application to the coconut crop or indirectly through the mass transport from cultivated areas of other crops. As can be seen in Table 4, the samples from farm 1 (which is irrigated by the Grande's River)

Table 3. Comparison of analytical methods for the simultaneous determination of pesticides in coconut water samples

Analyte	Extraction method	Detection	Recovery		LOQ / (μg L^{-1})	Reference
			Lower fortification value / (μg L^{-1})	Range / %		
10 pesticides	LSLE	LC-MS/MS	10	59 ± 3-172 ± 50	10	8
3 pesticides	MSPD	HPLC-UV	250	70 ± 9.4-116 ± 7.8	40-100	21
19 pesticides	SDME	GC-MS	5	28.3-143	1.21-6.69	22
5 pesticides	LLE	GC-ECD	10	80 ± 7.7-99 ± 4	10-500	23
4 pesticides	LLE	GC-TSD	500	90 ± 9-97 ± 8.4	10-500	23
5 pesticides	SLE	HPLC-UV	500	75 ± 7.3-96 ± 6.9	500-1000	23
36 pesticides	LLE	GC-MS	0.032	72.9 ± 4.2-110 ± 4.1	0.0178-0.0318	this study

LSLE: liquid-solid-liquid extraction, SDME: single drop microextraction, MSPD: matrix solid-phase dispersion, LLE: liquid-liquid extraction, SLE: solid-liquid extraction, LC-MS/MS: liquid chromatography tandem mass spectrometry, GC-MS: gas chromatography mass spectrometry, HPLC-UV: high performance liquid chromatography with ultraviolet detector, GC-ECD: gas chromatography with electron capture detector, GC-TSD: gas chromatography with thermionic specific detector, LOQ: limit of quantification; μ: micro.

Table 4. Concentration of the nine pesticides found in commercial fresh coconut water samples

Sample		Pesticide concentration[a] / (ng L^{-1})								
		Dimethoate	γ-HCH	β-HCH	4,4'-DDE	Endosulfan II	4,4'-DDD	4,4'-DDT	Endrin ketone	Methoxychlor
Farm 1	1	2,796.62 ± 0.98	< LOQ	ND	< LOQ	< LOQ	ND	ND	ND	ND
	2	2,125.50 ± 1.43	< LOQ	ND	< LOQ	< LOQ	ND	ND	ND	ND
	3	2,060.54 ± 1.22	< LOQ	ND	< LOQ	< LOQ	ND	ND	ND	ND
	4	2,971.73 ± 1.09	< LOQ	< LOQ	< LOQ	< LOQ	ND	ND	ND	ND
	5	2,226.48 ± 1.20	< LOQ	ND	< LOQ	< LOQ	ND	ND	ND	ND
	6	2,411.50 ± 2.81	122.77 ± 1.24	< LOQ	< LOQ	< LOQ	ND	ND	ND	ND
	7	2,352.40 ± 0.78	< LOQ	ND	< LOQ	< LOQ	ND	ND	ND	ND
	8	1,801.21 ± 3.11	< LOQ	ND	< LOQ	ND	ND	ND	ND	ND
	9	2,257.48 ± 1.64	< LOQ	< LOQ	< LOQ	ND	ND	ND	ND	ND
	10	4,048.82 ± 0.77	< LOQ	< LOQ	< LOQ	< LOQ	ND	ND	ND	ND
	11	1,495.91 ± 0.52	< LOQ	ND	< LOQ	< LOQ	ND	ND	ND	ND
	12	2,126.73 ± 2.91	< LOQ	ND	< LOQ	ND	ND	ND	ND	ND
	13	2,023.76 ± 0.79	< LOQ	ND	< LOQ	< LOQ	ND	ND	ND	ND
	14	5,768.34 ± 1.64	< LOQ	< LOQ	< LOQ	ND	ND	ND	ND	ND
	15	3,191.12 ± 1.52	< LOQ	ND	< LOQ	< LOQ	< LOQ	ND	< LOQ	< LOQ
Farm 2	16	1,164.62 ± 3.45	< LOQ	ND	< LOQ	< LOQ	ND	< LOQ	ND	< LOQ
	17	613.01 ± 2.11	< LOQ	ND	ND	< LOQ	ND	ND	ND	ND
	18	< LOQ	< LOQ	ND	ND	ND	ND	ND	ND	ND
	19	68.90 ± 0.92	< LOQ	ND	ND	< LOQ	ND	ND	ND	ND
	20	711.97 ± 4.01	< LOQ	ND	ND	< LOQ	ND	ND	ND	ND
	21	660.90 ± 1.83	< LOQ	ND	ND	< LOQ	ND	ND	ND	ND
	22	887.17 ± 2.37	< LOQ	ND	ND	< LOQ	ND	ND	ND	ND
	23	786.03 ± 0.67	< LOQ	ND	ND	< LOQ	ND	ND	ND	ND
	24	1,004.77 ± 2.98	< LOQ	ND	ND	< LOQ	ND	ND	ND	ND
	25	680.87 ± 3.38	< LOQ	ND	ND	ND	ND	ND	ND	ND
	26	674.72 ± 0.44	< LOQ	ND	ND	ND	ND	ND	ND	ND
	27	418.35 ± 1.74	< LOQ	ND	ND	< LOQ	ND	ND	ND	ND
	28	570.26 ± 3.35	< LOQ	ND	ND	< LOQ	ND	ND	ND	ND
	29	818.15 ± 0.54	< LOQ	ND	ND	< LOQ	ND	ND	ND	ND
	30	842.79 ± 4.45	< LOQ	ND	ND	< LOQ	< LOQ	< LOQ	ND	< LOQ

[a]Mean ± standard deviation (n = 3); ND: not detected; < LOQ: below limit of quantification; HCH: hexachlorocyclohexane; DDE: dichlorodiphenyldichloroethylene; DDD: dichlorodiphenyldichloroethane; DDT: dichlorodiphenyltrichloroethane; α: alfa; β: beta.

presented dimethoate concentrations four times higher than those ones from farm 2 (which is irrigated by the Branco River). It is worth to note that nearby farming activities such as soybeans, corn, and cotton use dimethoate, which can be runoff into river during wet season.

Regarding γ-HCH, its values were considerably lower than dimethoate. Although, γ-HCH had its production, use, import, and export banned since 1985 by Ordinance 329 of the Brazilian Ministry of Agriculture[30] and reinforced by Federal Decree No. 5,472/2005, based on the Stockholm Convention.[31] This prohibition is also applied to aldrin, dieldrin, endrin, and heptachlor. The presence of γ-HCH in analyzed samples indicates a recent exposure, since its half-life is just a few days.[32] Although the absence of insecticidal activity of its alpha, beta, gamma, delta, and epsilon isomers, they have half-lives of up to nine years and also induce harmful effects on human health.[33] These compounds can act as endocrine disruptors, causing changes in thyroid hormone levels during pregnancy,[34] increasing the susceptibility to Parkinson's disease,[35] and present carcinogenic activity.[36]

In Brazil, the United States, and the European Union, there is no specific law for the presence of pesticide residues in fresh coconut water. Thus, the results obtained in this study were compared with the established

standards for drinking water. We assumed that the fresh coconut water is mainly ingested from the fruit directly, without any industrial processing. Among the analytes found in the studied samples, the Brazilian regulation establishes the maximum levels only for 4,4'-DDE, 4,4'-DDD, and 4,4'-DDT pesticides, whose the sum of their concentrations must be lower than 1,000 ng L^{-1}.[37] In the United States, there is a maximum level only for the γ and β-HCH at 200 ng L^{-1}.[38] In European Union, it is established a maximum concentration at 100 ng L^{-1} for pesticides regardless of the type, and 500 ng L^{-1} for the total pesticide sum.[39] Therefore, the results obtained in this work were below the limits established by Brazilian and US regulations, but higher than those ones adopted by the European regulation. In addition, the levels of dimethoate alone were higher than the limit allowed for the sum of all pesticides, considering the European regulation.

It is important to highlight that the results obtained in this work contribute to further knowledge about the distribution of the studied pesticides in environmental matrices in the Western region of Bahia, Brazil, such as surface and bottom sediments.[40,41]

Conclusions

This study demonstrated the development and validation of an efficient analytical method for the simultaneous determination of thirty-six pesticides belonging to the chemical classes of organochlorines, organophosphates, pyrethroids, carbamates, thiocarbamate, and strobilurin in fresh coconut water samples using GC-MS. The main advantage of the proposed methodology is the use of a small solvent volume through a liquid-liquid extraction method, and drying of the sample extracts assisted by a household microwave oven. This reduces waste production, besides avoiding the use of expensive extraction cartridges. The application of the method in real samples of fresh coconut water collected in two locations revealed high concentrations of dimethoate, which may be related to the contamination of the irrigation water from the two rivers in the region. Consequently, the proposed methodology has the potential to provide data to pesticide regulatory agencies, and also contribute for mitigating actions to environmental damages and prevention to human health.

Acknowledgments

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-Brasil (CAPES), Fundo de Amparo à Pesquisa do Estado da Bahia (FAPESB), Pró-Reitoria de Pós-Graduação, Pesquisa e Inovação (PROPGPI/UFOB), and Financiadora de Estudos e Projetos (FINEP).

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Submitted: April 15, 2023
Published online: July 31, 2023