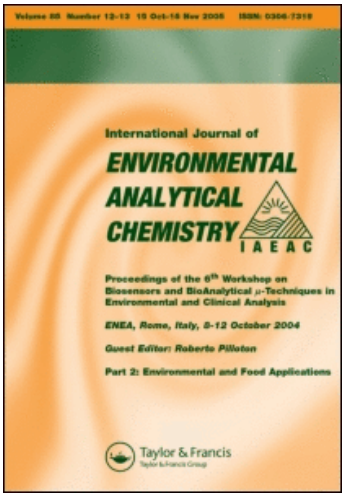

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SPME ANALYSIS OF POTENTIAL ATTRACTANTS FOR PALM WEEVILS

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Solid-phase microextraction was applied for the analysis of a mixture of potential attractants for palm weevils, constituted by seven organic compounds. Various fibers, coated with different sorbent phases, were used in this study and the best results were obtained with the polydimethylsiloxane (PDMS)/divinylbenzene (DVB) and the Carboxen/PDMS fibers. A waiting time of 5 min before sampling was adequate for sample homogenisation and a sampling time of 30 min was used to obtain good extraction efficiencies. A complete desorption of the analytes into the injection port of the gas chromatograph was achieved with an injection time of 1 min. The detection limit of the method ranged from 0.29 to 156 ng/ml for the different components of the mixture, with a lower detection limit for the compounds with higher affinity for the fiber coating. This method was used in the analysis of volatiles released from a diffuser filled with the attractant mixture.

Keywords: SPME; calibration factors; volatiles; fibers

INTRODUCTION

Some weevils of the Rhynchophorus family are important pests of commercial and ornamental palms, as well as other related plants such as sugar cane and banana. The traditional method of controlling these pests is the use of poisoned traps with pieces of coconut, pineapple or banana fruits to attract and eliminate these weevils[1–3]. Nevertheless, plant tissues have a short half-live for attracting weevils and mixtures of synthetic organic compounds have been developed lately for the control of these pests. These mixtures act in synergism with the corresponding insect pheromones, enhancing pheromone attraction and increasing the efficiency of the traps[4–7]. These synthetic compounds, named kairomones,

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are formed by several fermentation compounds of plants with attractant properties for Rhynchophorinae, being important the presence of ethyl acetate as a principal component, alone or in mixtures with ethanol, and a low proportion of other compounds such as alcohols, esters, monoterpenes, or aromatic chemicals.

Solid-phase microextraction (SPME) is a recent technique for the analysis of volatile organic compounds in different matrices, like air, water, soil, foods, insects or human fluids[8–14]. This technique does not require the use of solvents for the extraction of organic compounds and it is fast and sensitive. Therefore, SPME can be used for the analysis of these compounds as an alternative to the more time consuming conventional methods.

Since the SPME technique was commercially available in early nineties, the number of commercial fibers has notably increased. There are, at present, seven fibers available, which consist in a fused silica fiber covered by different polymeric phases such as polydimethylsiloxane (PDMS) or polyacrylate (PA). These polymeric phases can also be mixed with divinylbenzene (DVB) or with carbon-based coatings, like Carboxen.

SPME fibers are not equally sensitive for all compounds and their relative efficiency depends on the compound affinity for the corresponding fiber coating, as well as on other parameters, such as air concentration, temperature and sampling and desorption times[15]. Most of the published papers on SPME analyses are based on the use of PDMS fibers or, in certain cases, PA fibers, and only some works compare the results obtained with both fibers [12, 16–21]. The use of fibers coated with mixed phases is scarce in the literature, although some papers have recently been published[8, 22–26]

The aim of this work is to study the SPME analysis of a mixture of compounds, potential attractants for Rhynchophorinae, using different commercial fibers and gas chromatography with flame ionisation detection (FID). Several compounds, among those reported as potential attractants for palm weevils, have been selected for this study[4–7, 27]. The attractant mixture was formed by ethyl acetate, ethyl propionate, isopentyl acetate, ethyl octanoate, 1-pentanol, phenol and menthone. In addition, this methodology was applied to the analysis of volatiles released from a diffuser filled with the attractant mixture.

EXPERIMENTAL

Chemicals and sample preparation

Ethyl acetate and 1-pentanol were obtained from Panreac (Barcelona, Spain). Ethyl propionate, isopentyl acetate, phenol, menthone and ethyl octanoate were

supplied by Sigma-Aldrich Quimica (Madrid, Spain). Ethyl tiglate was obtained from Lancaster Synthesis (Strasbourg, France).

A mixture of the seven organic compounds was prepared by dilution of the minor components with ethyl acetate to obtain the following composition (v/v): ethyl acetate, 92%; ethyl propionate, 5.4%; 1-pentanol, 0.5%; isopentyl acetate, 1.8%; phenol, 0.08%; menthone, 0.11% and ethyl octanoate, 0.11%.

SPME procedure

SPME fibers coated with 100 μm PDMS, 65 μm PDMS/DVB, 75 μm Carboxen/PDMS, 65 μm Carbowax/DVB (CW/DVB) or 85 μm PA were supplied by Supelco (Madrid, Spain). Table I shows some characteristics of these fibers. The fibers were housed in their manual holders and they were conditioned, as recommended by the manufacturer, in the injection port of a gas chromatograph during 0.5–2 h at 250–300 °C, according to the fiber coating.

TABLE I Type of fiber, application and maximum and working temperatures

Type of fiber	*Application*	*Maximum temperature*	*Working desorption temperature*
100 μm PDMS	Volatiles	280 °C	250 °C
65 μm PDMS/DVB	Volatiles, amines and nitroaromatic compounds	270 °C	250 °C
75 μm Carboxen/PDMS	Low molecular weight compounds	320 °C	270 °C
65 μm CW/DVB	Alcohols and polar compounds	265 °C	250 °C
85 μm PA	polar semivolatiles	320 °C	270 °C

To evaluate the SPME fibers and the analytical parameters, 4 oz wide mouth jars (145 ml of real volume), having screw-top lids with Teflon-lined septa, were employed. Analysis by SPME was carried out at a selected temperature of 27 ± 1°C for the different evaluation studies. In general, 2 μl of the mixture were placed in the jar and the mixture was magnetically stirred. A homogenisation time of 45 min was initially used for the evaluation of fibers, but 5 min of homogenisation was established afterwards. Sampling of the compounds was done by inserting the sheathed fiber through the cap and exposing the corresponding fiber for 30 min. The fiber was then retracted into the needle and transferred to the injection port of the gas chromatograph where the compounds were desorbed for 1 min.

Analysis of volatiles released from the diffuser was performed by placing the diffuser inside a flask (3.7 l of real volume) with a stirring bar and a small tripod for supporting the diffuser. The assays were carried out in an incubator chamber at 27 °C and the diffuser was left inside the flask for 30 min before SPME sampling with PDMS/DVB and Carboxen/PDMS fibers. The diffuser used was a patented system[28] consisting in a plastic flask with a wick connected to a calibrated perforated cap. The attractant mixture contained in the diffuser was released at 1.0 ± 0.11 g per day.

The linearity of the detector response was tested by injecting 2 µl of ethyl acetate solutions of the corresponding compounds, over the range 0.1 – 8 mg/ml for ethyl propionate, pentanol and isopentyl acetate, and 0.02 – 1.3 mg/ml for phenol, menthone and ethyl octanoate. All calibration curves were based on at least five points and the linear regression coefficients obtained were equal or higher than 0.994.

Statistically significant differences were established by subjecting data to an ANOVA test at $p < 0.001$ level.

Analysis of the liquid attractant mixture

The concentration of the compounds in the mixture contained in the diffuser was determined, at different times, using ethyl tiglate as internal standard. The calibration curves were based on at least five determinations. The linear regression coefficients obtained were equal or higher than 0.993.

Apparatus

A Perkin-Elmer 8500 gas chromatograph, equipped with a flame ionisation detector (FID), was used. A fused-silica semicapillary column, BP-1 (25 m × 0.53 mm I.D., 1 µm film thickness) bonded phase, was used with helium as carrier gas at 3 ml/min. The oven temperature was maintained at 50 °C for 3 min, programmed at 10 °C/min to 100 °C and then programmed to 220 °C at 20 °C/min and held 1 min. Injector temperatures were 270 °C for PA and Carboxen/PDMS fibers and 250 °C for PDMS, PDMS/DVB and CW/DVB fibers. The detector temperature was 225 °C.

RESULTS AND DISCUSSION

Evaluation of fibers

SPME extraction of the selected compounds was studied using the commercial fibers indicated above. Figure 1 shows the peak areas obtained by GC-FID in this

study. Carboxen/PDMS was the fiber with the highest efficiency for ethyl acetate and CW/DVB the one with the lowest, although in general, the extraction efficiency for ethyl acetate was low, taking into account the high proportion (92%) of this compound in the mixture. Carboxen/PDMS was also the best fiber for the extraction of ethyl propionate, pentanol and isopentyl acetate. The non polar character of PDMS made this fiber to have a bad extraction efficiency for pentanol. This fact was more pronounced for phenol, being PDMS unable to extract detectable amounts of this compound in our conditions and, therefore, this fiber is not suitable for phenol analysis, as indicated in previous works[16, 18]. Our study shows that phenol can be analysed by using the other four fibers with similar results. PDMS/DVB, followed by Carboxen/PDMS, showed the highest efficiencies for menthone and ethyl octanoate analysis.

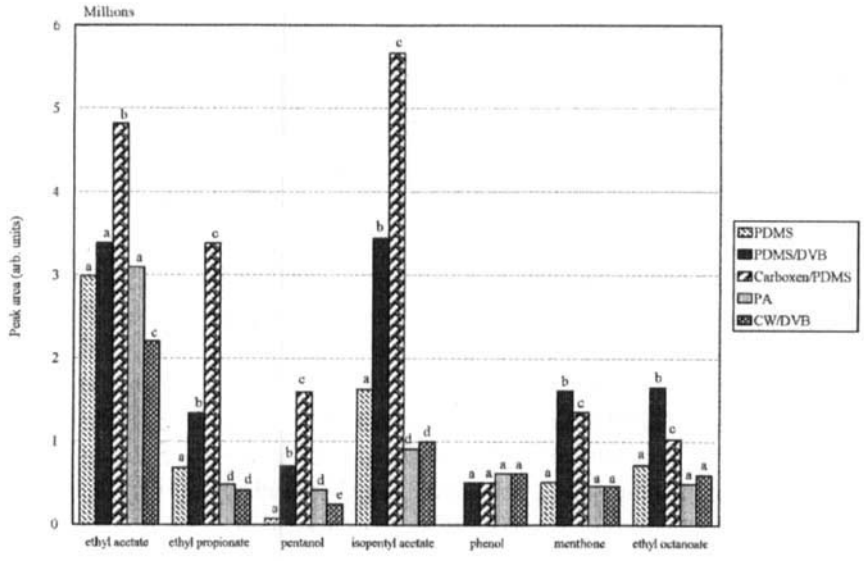

FIGURE 1 Extraction efficiencies of SPME fibers for sampling the mixture of potential attractants (ethyl acetate 92%, ethyl propionate 5.4%, 1-pentanol 0.5%, isopentyl acetate 1.8%, phenol 0.08%, menthone 0.11% and ethyl octanoate 0.11%). Statistically significant differences were established by subjecting data to an ANOVA test. Peak areas with different letters are significantly different at $p < 0.001$ level, within each compound. Values are the mean of four replicates

Calibration factor

The calibration factor (K) indicates the affinity of the fiber for a compound and can be defined as the amount of compound absorbed by the fiber at equilibrium,

or at a defined sampling time if equilibrium is not reached, divided by its concentration in the headspace[29]. This factor can be calculated by the equation:

$$K = n_f \cdot V_j / n_0 - n_f$$

where n_0 is the initial amount of analyte, n_f is the amount absorbed by the fiber and V_j is the volume of the jar. Table II shows the calibration factors obtained for the studied compounds with the different fibers, together with their standard deviations. K values for ethyl acetate were not calculated because this compound was used as a solvent of the external standard mixture and its concentration was out of the calibration range. Among the other studied compounds, only K values for ethyl octanoate (42.7 ml) and isopentyl acetate (2.3 ml) on a PDMS fiber, at 25 °C, were found in the available literature[29]. The calibration factor obtained for these compounds in our conditions (27 °C) on the same fiber were 21.3 ml for ethyl octanoate, lower than the one reported, and 2.8 ml for isopentyl acetate, which is similar to the value reported. Differences in K values may be explained by the influence of the experimental conditions, like temperature and mixture composition, in the calibration factor. Thus, a decrease of the K value in the range from 10 to 45% has been described with a 10 °C increase in temperature[8, 29]. Regarding the influence of the mixture composition, a decrease of the K value has been observed in the SPME analysis of multiple component mixtures[23, 29, 30]. This effect depends on the fiber type, thus fibers based on an absorption extractive mechanism (PDMS and PA) are less influenced by the mixture composition than mixed-phase fibers based on an adsorption extractive mechanism (PDMS/DVB, CW/DVB and Carboxen/PDMS)[30].

In general, these results confirm the higher affinity obtained with Carboxen/PDMS and PDMS/DVB fibers in the SPME analysis of this mixture. Therefore, these fibers will be used in the study of the influence of different analytical conditions in the SPME procedure, in the determination of the detection limits and in the analysis of volatiles released from a diffuser.

Sampling and equilibration time

The effect of the homogenisation time before SPME sampling was studied using jars containing 2 µl of the compounds mixture that was stirred for 5, 10 and 45 min, before SPME sampling with a PDMS/DVB fiber. The results obtained (data not shown) pointed out that the peak areas of the studied compounds were similar at all the times assayed, indicating that 5 min are sufficient for homogenisation before SPME sampling. Homogenisation times of about one hour have been reported previously for headspace analysis of organic compounds[29]. Nevertheless, considering that the volume of our organic mixture deposited in the jar is

low (2 μl) and that the components of the mixture are very volatile compounds, the homogenisation in our conditions can be notably shortened, as it has been evidenced in this assay, and 5 min of homogenisation was established in our case.

The effect of the sampling time on the extraction efficiency was studied with the selected fibers PDMS/DVB and Carboxen/PDMS using 5, 10, 15, 30, 45 and 60 min of sampling. Similar results were obtained with both coating types. Figure 2 shows changes in peak areas with the sampling time on a PDMS/DVB fiber for the compounds studied. These results evidenced that, for most compounds, 30 min of SPME sampling is enough for reaching the equilibrium of the fiber. Some compounds, such as ethyl acetate, ethyl propionate, pentanol and isopentyl acetate, reached the equilibrium very rapidly (5 min), while others like menthone, phenol and ethyl octanoate needed about 30 min for reaching equilibrium. Therefore, a sampling time of 30 min can be considered acceptable for SPME analysis of the attractant mixture studied. This time of sampling for organic volatiles is in agreement with previous works[25, 29], and only when compounds having high molecular weights are analysed this sampling time is usually increased.

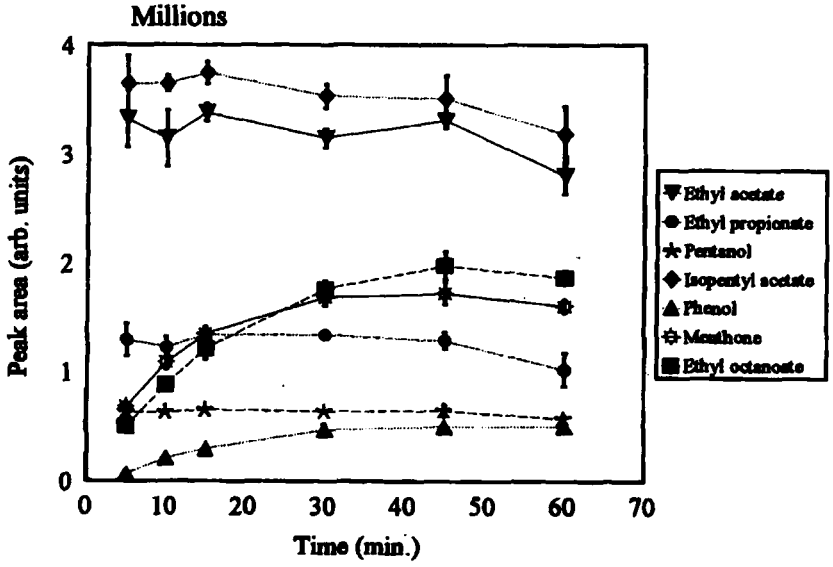

FIGURE 2 Effect of the extraction time on peak areas using the PDMS/DVB fiber. Values are the mean of three replicates

Desorption time

The effect of time on the complete desorption of these compounds from the fiber in the gas chromatograph injector was studied with the selected fibers at desorption temperatures that were 250 °C for PDMS/DVB and 270 °C for Carboxen/PDMS, which are lower than their maximum recommended temperatures (270 °C and 320 °C, respectively). Times of 0.1, 0.25, 0.5, 0.75, 1 and 2 min were selected for this study.

The results obtained showed that after 1 min a complete desorption was achieved for all compounds. Desorption from PDMS/DVB is very rapid, as all compounds were desorbed 0.25 min after injection. Carboxen/PDMS fiber has a behaviour somewhat different with compounds of higher molecular weight, like ethyl octanoate, which showed an increase of the amount desorbed with time, although 1 min is adequate for a complete desorption. Figure 3 depicts representative time-course curves of the desorption of isopentyl acetate and ethyl octanoate from a Carboxen/PDMS fiber.

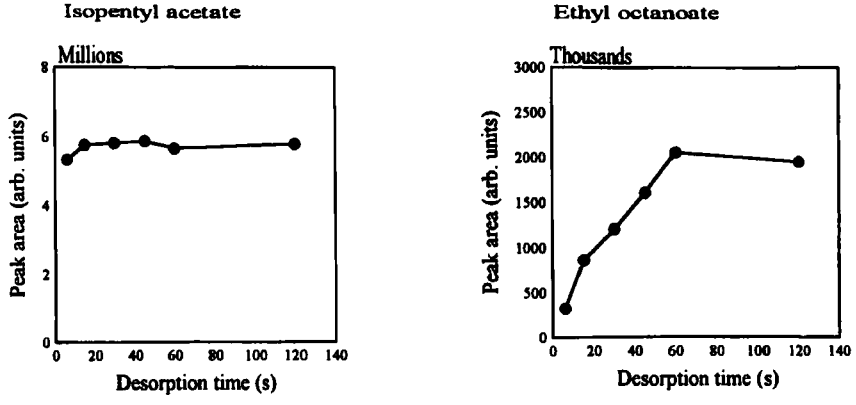

FIGURE 3 Chromatographic response for isopentyl acetate and ethyl octanoate, expressed by peak areas, as a function of the desorption time obtained with the Carboxen/PDMS fiber

Detection limit

The limit of detection for these compounds was studied with the fibers PDMS/DVB and Carboxen/PDMS. The detection limit of the method was considered to be the analyte concentration producing a signal to noise response equal or higher than 3. Table III presents the limits of detection obtained for the studied compounds. Detection limits of low molecular weight compounds are lower for the Carboxen/PDMS fiber, being detection limits similar for the other com-

pounds. In general, the lowest detection limits are obtained with menthone, ethyl octanoate and phenol, which were the compounds with the highest calibration factors for these fibers. The value obtained for phenol is somewhat higher than expected, according to its calibration factor, probably due to the broad chromatographic peak obtained. The main component of the mixture, ethyl acetate, was used as a solvent and it was not considered in the detection limit study.

TABLE II Calibration factors (K) and standard deviations obtained with different fibers by SPME[a]

Compounds	*PDMS* *K (ml) SD%*	*PDMS/DVB* *K (ml) SD%*	*Carboxen/PDMS* *K (ml) SD%*	*PA* *K (ml) SD%*	*CW/DVB* *K (ml) SD%*
Ethyl propionate	0.24 ± 0.02	0.48 ± 0.01	1.26 ± 0.03	0.16 ± 0.01	0.13 ± 0.01
1-Pentanol	4.78 ± 0.15	7.93 ± 0.30	12.25 ± 0.52	6.57 ± 0.22	5.79 ± 0.17
Isopentyl acetate	2.79 ± 0.20	4.64 ± 0.38	6.98 ± 1.04	2.07 ± 0.11	2.16 ± 0.07
Phenol	-----	67.68 ± 12.44	81.72 ± 6.69	111.31 ± 26.23	114.36 ± 40.10
Menthone	11.90 ± 3.48	53.89 ± 7.14	42.25 ± 5.04	10.50 ± 2.21	10.64 ± 1.75
Ethyl octanoate	21.35 ± 5.79	62.14 ± 9.04	32.92 ± 3.13	13.89 ± 2.18	17.40 ± 2.92

a. Results shown are mean of four replicates

TABLE III Detection limits obtained for the studied compounds

	Detection limit (ng/ml)	
	PDMS/DVB fiber	*Carboxen/PDMS fiber*
Ethyl propionate	156	26.0
1-Pentanol	20.5	6.8
Isopentyl acetate	9.3	6.8
Phenol	1.8	1.8
Menthone	0.29	0.32
Ethyl octanoate	0.32	0.32

Volatiles released from the diffuser

The liquid mixture inside the diffuser was analysed at different times along the assay. The analysis of the mixture showed that the concentration of ethyl acetate, ethyl propionate and isopentyl acetate slightly decreased, while menthone and ethyl octanoate concentrations increased with time, being these changes more evident after 15 days of diffusion. SPME diffusion analysis of the mixture was carried out at different times along the assay using PDMS/DVB and Carboxen/PDMS fibers. Figure 4 shows representative chromatograms obtained in

the SPME analysis of the volatiles released from the diffuser after 5 and 25 days of diffusion, with a Carboxen/PDMS fiber. The results obtained with both fibers showed a decrease in the ethyl acetate, ethyl propionate, pentanol and isopentyl acetate concentrations, while menthone and ethyl octanoate concentrations increased with the diffusion time. These results are in agreement with those obtained in the analysis of the liquid mixture and indicate a modification of the mixture composition, showing an increase in concentration of the less volatile compounds along the diffusion assay.

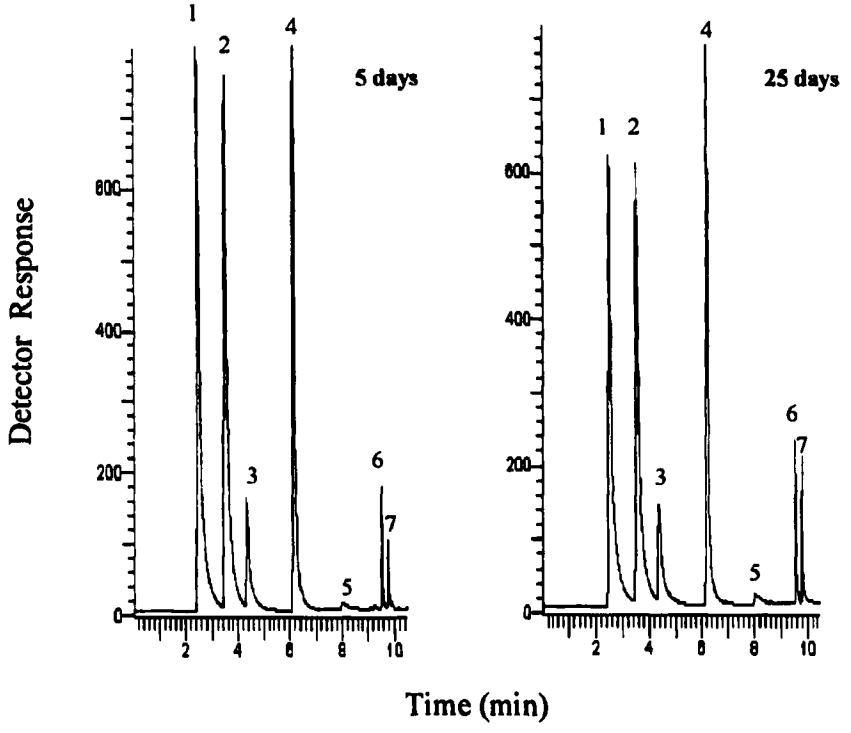

FIGURE 4 Representative chromatograms obtained by SPME analysis with a Carboxen/PDMS fiber of the volatiles released from the diffuser after 5 and 25 days of diffusion. Peak labels: 1= ethyl acetate, 2= ethyl propionate, 3= 1-pentanol, 4= isopentyl acetate, 5= phenol, 6= menthone and 7= ethyl octanoate

CONCLUSIONS

The comparative SPME study shows that PDMS/DVB and Carboxen/PDMS are the fibers more adequate for the analysis of the studied mixture, potentially

attractive for palm weevils. The low affinity of the PDMS coating for phenol makes this fiber inadequate for this analysis.

A waiting time of 5 min before sampling is enough to achieve a homogeneous concentration of the studied compounds in the jar, and a sampling time of 30 min is adequate for obtaining good extraction efficiencies with the assayed fibers. A desorption time of 1 min in the injection port of the gas chromatograph allows the complete desorption of the components of the mixture.

The detection limits obtained were in the range 0.29 – 156 ng/ml, being these limits lower for the compounds with higher calibration factors and Carboxen/PDMS the fiber with the highest sensitivity.

The described SPME method is rapid, simple and sensitive enough for the analysis of the studied potential attractants of palm weevils.

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