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Screening of Tropical Fruit Volatile Compounds Using Solid-Phase Microextraction (SPME) Fibers and Internally Cooled SPME Fiber

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In this study, the optimization and comparison of an internally cooled fiber [cold fiber with polydimethylsiloxane (PDMS) loading] and several commercial solid-phase microextraction (SPME) fibers for the extraction of volatile compounds from tropical fruits were performed. Automated headspace solid-phase microextraction (HS-SPME) using commercial fibers and an internally cooled SPME fiber device coupled to gas chromatography−mass spectrometry (GC-MS) was used to identify the volatile compounds of five tropical fruits. Pulps of yellow passion fruit (*Passiflora edulis*), cashew (*Anacardium occidentale*), tamarind (*Tamarindus indica* L.), acerola (*Malphigia glabra* L.), and guava (*Psidium guajava* L.) were sampled. The extraction conditions were optimized using two experimental designs (full factorial design and Doehlert matrix) to analyze the main and secondary effects. The volatile compounds tentatively identified included alcohols, esters, carbonyl compounds, and terpernes. It was found that the cold fiber was the most appropriate fiber for the purpose of extracting volatile compounds from the five fruit pulps studied.

KEYWORDS: Fruits; volatile compounds; experimental design; solid-phase microextraction, cold fiber

INTRODUCTION

Aroma is one of the most important attributes that affects the consumption of fruit from the tropics and subtropics. Because these fruits are often inexpensive and extremely rich in vitamins, their popularity has increased, especially in Europe and the United States. In Brazil, tropical fruits are eaten fresh locally during the whole year and are exported to other countries normally in the form of frozen pulp (*1*).

Acerola pulp is very juicy and cooling and possesses a fruity and sweet flavor, but the fruit is principally known for its amount of vitamin C. Yellow passion fruit possesses a floral, estery aroma with an exotic tropical sulfury note. Special nutrition interest has been given to cashew fruit because of its good characteristics for industrialization owing to its fleshy pulp, soft peel, lack of seeds, high sugar content, and strong exotic flavor. Tamarind fruit is high in sugar and minerals, with a pleasant acid taste and rich aroma. Varieties of guava fruit can differ widely in flavor and seediness. The better varieties are soft when ripe and creamy in texture with a rind that softens to be fully edible. The sweet, musky odor is pungent and penetrating. The seeds are numerous but small and, in good varieties, fully edible.

The main volatile compounds identified in yellow passion fruit belong to the esters (*2*−*4*). Prior studies that used the solid-phase extraction technique to isolate the volatile compounds from the cashew fruit indicate that the most intense (and common) compounds identified in this fruit were methyl and ethyl esters (*5*). Phenylacetaldehyde and furfural are cited as the most common compounds of tamarind fruit (*6*, *7*) while for the acerola the esters were predominant (*8*, *9*). The aroma of guava is composed of a large number of ester and terpenoid compounds (*10*−*14*).

Recently, for the purposes of determining fruit aromas, the solid-phase microextraction (SPME) technique has been applied as an alternative sample preparation strategy, to overcome the problems associated with conventional sampling methodologies, such as elevation costs, time-consumption, and the use of large volumes of organic solvents. In addition, the SPME procedure will more closely reflect the true flavor profile of the fruit pulp than those that might be generated by distillation and solvent extraction processes. Among the SPME fibers commercially available, those fibers that contain liquid (PDMS) and solid [carboxen (CAR) and/or divinylbenzene (DVB)] components have been chosen for the extraction of volatile fruit pulp compounds due to their high sensitivity (*15*−*19*). However, because a high desorption temperature is required, the formation of artifacts is often unavoidable with these coatings. Investigations of artifact formation during the analysis of volatile sulfur compounds (*20*) and volatile amines (*21*) in air by CAR/PDMS have been reported. Thus, the development of new fibers would allow for improved extraction efficiency and low desorption temperatures (*20*). Alternatively, the use of an absorbent coating (in place of an adsorbent coating), which requires a low desorption temperature, would also help to reduce the formation

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of artifacts. A PDMS coating would also be a potential solution, but this fiber exhibits low sensitivity for volatile compounds.

The low sensitivity of the PDMS fiber can be overcome by increasing the analytes' distribution coefficients between the extraction phase and the sample matrix. This strategy can be achieved by cooling the fiber coating.

Recently, an internally cooled SPME device was developed by Zhang and Pawliszyn (*22*) and applied to extract benzene, toluene, ethylbenzene, and xilene from solid samples. Subsequently, the cold fiber device was miniaturized and automated by Chen and Pawliszyn (*23*). The same miniaturized and automated cold fiber system was used for Ghiasvand et al. (*24*) to determine polycyclic aromatic hydrocarbons from sand and sediment. With the cold fiber method, the fiber maintained at low temperature is suspended in the headspace of the sample, which is heated to higher temperatures. Heating the sample to an elevated temperature increases the release of analytes from the matrix, which, therefore, increases their concentration in the headspace. A simultaneous use of lower fiber temperature overcomes the exothermic consequences of SPME and thus increases the partition coefficient between the analytes and the fiber coating. On the basis of the same theory, Chia et al. (*25*) developed a simple device to determine polychlorodibenzo-*p*-dioxins and polychlorodibenzofurans in contaminated soil samples. However, the PDMS fiber was cooled using chilled alcohol during the extraction procedure. Refrigeration of the SPME fiber to cool the fiber was also used by Achten et al. (*26*) to detect methyl *tert*-butyl ether in water. The authors cooled the CAR/PDMS fiber at 0 °C using a cryostat.

In spite of the substantial growth in applications of the SPME procedure to analyze volatile aromas and the fact that SPME analysis is drastically affected by several factors, very little effort has been made to date to optimize the SPME procedure. Generally, "one factor at a time" or "univariate" experiments have been conducted in most of the published studies to determine the optimum analysis conditions for SPME. In this case, the interactions among the factors involved in the extraction procedure are thus overlooked. In contrast, the response surface methodology and/or factorial design have been recently used to determine the best operational conditions for the extraction of volatile compounds from Evodia species fruits (*17*), avocado puree (*18*), and banana fruit (*27*).

In this paper, we report for the first time the use of an automated cold fiber headspace solid-phase microextraction (CF-HS-SPME) device as a powerful tool for direct extraction of volatile compounds from some fruit pulps. Furthermore, no references have been found to use the HS-SPME, including cold fiber, to describe the volatile compounds of yellow passion fruit, cashew, tamarind, and acerola. Additionally, the application of a factorial design and a Doehlert matrix to investigate those conditions that influence the efficiency of HS-SPME of volatile compounds from five tropical fruit pulps is presented. To date, there has been no report of the use of Doehlert matrix to optimize the analysis conditions for headspace volatile compounds in fruit pulps using SPME.

MATERIALS AND METHODS

Fruit Pulp Samples. The samples used in this work consisted of frozen integral pulps (yellow passion fruit, cashew, tamarind, acerola, and guava) packed in polyethylene bags and they were purchased from Brasfrut Frutos do Brasil LTDA (Feira de Santana, Bahia, Brazil).

HS-SPME. The HS-SPME procedures were performed using a CTC CombiPAL autosampler (Zwingen, Switzerland) and the associated Cycle Composer software (ver 1.4.0). The PAL was equipped with a SPME fiber/syringe holder, a temperature-controlled six-vial agitator tray, and a temperature-controlled Needle Heater port. The silica fibers and automatic SPME holder were purchased from Supelco (Bellefonte, PA). Five fibers were used for screening the aroma from fruit pulps: divinylbenzene-carboxen-polydimethylsiloxane (DVB/CAR/PDMS, 50/30 μm), carboxen-polydimethylsiloxane (CAR/PDMS, 75 μm), polydimethylsiloxane (PDMS, 100 μm), polyacrylate (PA, 85 μm), and cold fiber. The coating of all fibers was 1 cm long, and prior to GC-MS analysis, each fiber was conditioned in the Needle Heater according to the instructions provided by the manufacturer. The details about the design of the in-house cold fiber device can be found in the works published by Chen and Pawliszyn (*23*) and by Ghiasvand et al. (*24*). A PDMS liquid polymer tubing with a thickness of 340 μm and a length of 1 cm was used as the coating.

For each extraction, 0.55 ± 0.03 g portions of pulp samples were hermetically sealed in 10 mL screw-top clear vials with polypropylene hole caps and PTFE/silicone septa (Supelco). The samples were equilibrated during the incubation time (selected according to experimental design optimization procedure) in a temperature-controlled six-vial agitator tray at the appropriate temperature (selected according to the experimental design optimization procedure, described below). Subsequently, the SPME device was automatically inserted into the sealed vial through the septum and the fiber was exposed to the sample headspace for the specified extraction time. The agitator tray was turned on or off during the incubation and extraction procedure depending on the experimental design. For cold fiber extraction, no agitation was used. In a preliminary work, it was observed that DVB/CAR/PDMS and CAR/PDMS fibers presented better efficiency of extraction of volatile compounds from guava fruit pulp than the others commercial fibers selected in this study. Thus, the optimization of the commercial SPME procedure was pursued using a DVB/CAR/PDMS coating with a 50/30 μm thickness to extract the volatile compounds from guava fruit pulp.

Following the sampling procedure, the SPME fiber was immediately inserted into the GC injector and the fiber was thermally desorbed for 3 min at 250 °C. Before each sampling procedure, each fiber was automatically reconditioned for 7 min in the Needle Heater port at 250 °C. This reconditioning procedure was enough to guarantee no peaks in blank runs, and it was a good compromise between the chromatography runs and the extraction procedures.

Optimization Strategy. The optimization procedure was achieved with a two-level full factorial and a Doehlert design. All experiments were done in duplicate. The four main variables (factors) in this optimization study of the commercial SPME procedure were extraction/incubation temperature, incubation time, extraction time, and sample agitation. Sample and coating temperatures were optimized only for the extraction performed with the cold fiber. For both optimization designs, the two-level full factorial and the Doehlert matrix, the sum of the peak areas were used as the response for optimization of the computer programs. The experimental data were processed using Statistica computer program.

Gas Chromatography (GC). Because the identities of the compounds were not necessary to optimize the SPME condition and because of the better stability and easy operation of the flame ionization detector (FID), the first step of this work was performed on a Varian 3800 GC instrument coupled to a FID and equipped with a Star Chromatography Workstation (version 5.5.1). A CP-Sil 8 fused silica capillary column (30 m × 0.25 mm i.d. × 0.25 μm phase thickness) from Varian was used for the GC separation of the volatile compounds from guava fruit. The temperature program used included the following settings: the initial temperature of 40 °C was held for 2 min and then increased to 200 °C at 5 °C min^{-1}; this temperature was held for 2 min and then increased to 260 °C at 30 °C min^{-1}. The injector and detector temperatures were 250 and 300 °C, respectively. Helium was used as the carrier gas at a flow rate of 1 mL min^{-1}. For the CF-HS-SPME procedure, to introduce the needle of the cold fiber device into the injector, the holes of GC septum nut and septum support were enlarged to accommodate a 17-gauge needle, and a 2 mm GC liner was used. The samples were injected in the splitless mode and opened after 3 min.

Gas Chromatography−Mass Spectrometry (GC-MS). The volatile compounds extracted by HS-SPME and CF-HS-SPME procedures from fruit pulps were tentatively identified using an Agilent 6890A

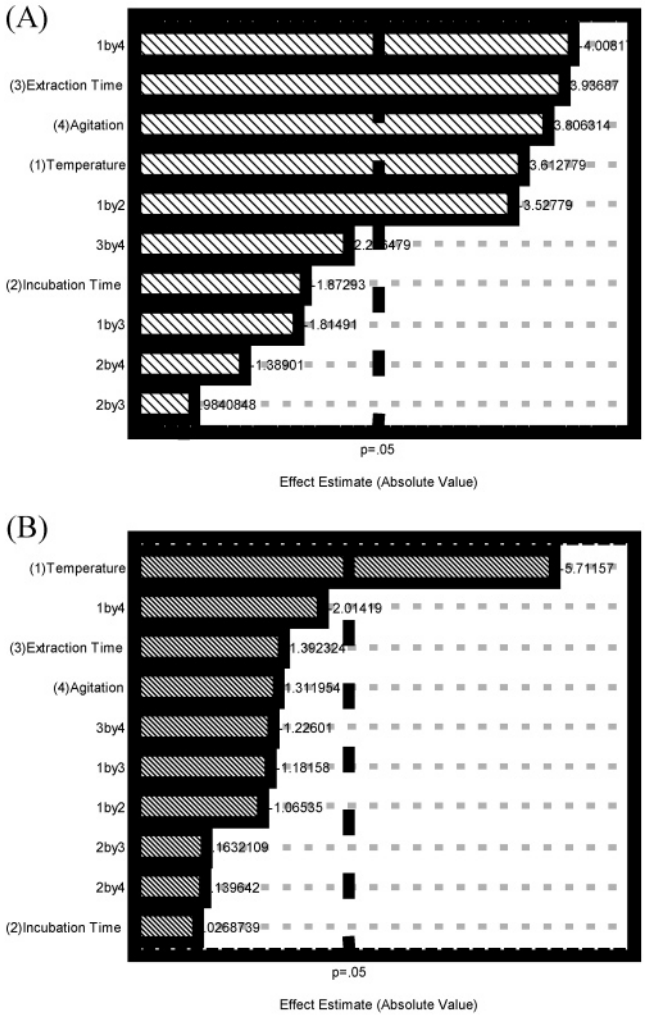

Figure 1. Pareto charts for (**A**) total area for all compounds eluted of the GC analysis of the HS-SPME of guava fruit and (**B**) total area for the compounds eluted until 22 min of the GC analysis of the HS-SPME of guava fruit.

gas chromatograph fitted with an ATAS GL Optic 3 injector and coupled to an Agilent 5973 mass selective detector (Palo Alto, CA). A Varian VF-5MS (5% diphenyl−95% polymethylsiloxane) capillary column (30 m × 0.25 mm × 0.25 μm) was used for the GC separation. Helium was used as the carrier gas at a flow rate of 1 mL min^{-1}. Because different liners used for regular SPME and cold fiber injections could cause different peak shapes, a cryotrap system was used in the beginning of the column to retain the analytes desorbed from the fibers for subsequent chromatographic separation. This procedure allowed a direct comparison between the chromatograms obtained for HS-SPME and CF-HS-SPME procedures. Thus, the temperature of the first 15 cm of the chromatography column was fixed at 0 °C for 5 min and then increased to 250 °C at 25 °C s^{-1}. The injections were performed in the splitless mode, which was opened after 8 min. The oven temperature program was the same as described previously under the GC section. The quadrupole mass detector was operated at 150 °C in the electron impact mode at 70 eV. The ion source temperature was set at 230 °C, and the transfer line was set at 280 °C. The mass acquisition range was 40−400 *m*/*z*. The peaks were identified on the basis of their fragmentation patterns using the Identification System (AMDIS) version 1.01 and the NIST Mass Spectral Search Program version 1.6d (NIST, Washington, DC). In addition, the compounds were tentatively identified by comparing the experimental retention indices with the theoretical ones, which were obtained from the literature (*8*, *10*, *11*, *19*, *28*).

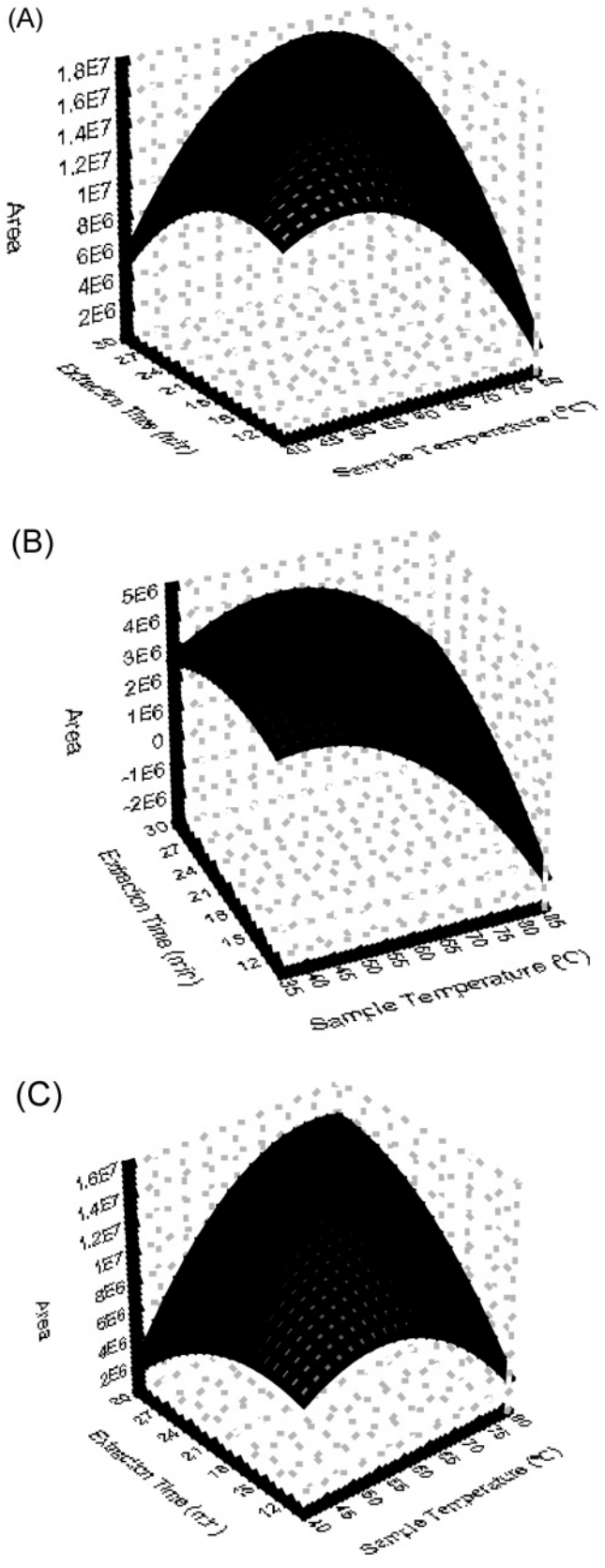

Figure 2. Response surface plot for (**A**) total peak area for all compounds eluted vs sample temperature (°C) and extraction time (min), (**B**) total peak area for the compounds eluted until 22 min vs sample temperature (°C) and extraction time (min), and (**C**) total peak area for the compounds eluted after 22 min vs sample temperature (°C) and extraction time (min).

RESULTS AND DISCUSSION

Experimental Design. The influence of the fiber coating on the HS-SPME extraction of volatile compounds has previously been studied (*29*), and it has been found that the selection of the most appropriate SPME fiber depends on the target compounds and the matrix studied. The authors observed that the most effective fiber for HS-SPME of volatile compounds is composed of liquid (PDMS) and solid (DVB and/or CAR) components. Augusto et al. (*16*) concluded that the two fiber coatings work in a complementary way to screen volatile compounds from some fruit pulps and suggested that CAR/PDMS was the most efficient coating for the extraction of lighter compounds. Bearing in mind that one of the goals of this work was to screen the volatile compounds of fruit pulps, the HS-SPME procedure was optimized using a DVB/CAR/PDMS fiber. The guava fruit pulp was selected as the matrix for this optimization step. Then, the same extraction condition optimized to extract volatile compounds from guava fruit pulp using DVB/CAR/PDMS fiber was used to screen the volatile compounds from other fruit pulps. In addition, other three different commercial fiber coatings were used to screen the volatile compounds using the optimized condition obtained for DVB/CAR/PDMS fiber. For the cold fiber device, the same fruit pulp was used as the study matrix in order to optimize the experimental conditions, including the sample and coating temperatures.

Full Factorial. The experimental variables included in the factorial design, such as the sample temperature and the extraction time, were chosen by considering that these variables greatly influence the vapor pressure and equilibrium of the aroma compounds in the headspace of the sample. In addition, the agitation of the fiber was considered because it could promote a reduction in the analysis time.

In this study, the experimental factors that were evaluated included the incubation time (10−20 min), the extraction time (10−30 min), the sample temperature (40−80 °C), and the sample agitation (250−500 rpm). The study design attempted to cover a wide range of experimental conditions. A two-level full factorial design 2^4 with a central point (C) and 17 runs in total was carried out in duplicate to determine the influence of the selected factors and their interactions on the HS-SPME system. The response, based on the sum of the peak areas, is one of the most useful parameters for the optimization of the SPME conditions (*17*) and therefore used as the end point in these studies to evaluate the significance of each of the aforementioned factors. An analysis of variance (ANOVA) was performed to determine whether the experimental factors studied were significant (at a p value of 0.05) on the performance of the HS-SPME system. The main effects and their interactions are presented in the Pareto chart shown in **Figure 1A**.

In accordance with **Figure 1A**, the following factors were highly significant: extraction time, sample temperature, and sample agitation. The results obtained in this study indicate that by increasing these factors, the analytical signal will also increase. These results were expected except for the positive effect of the sample temperature. Thus, it is possible to attribute the high efficiency of the SPME fiber because of the increase in the concentration of the less volatile compounds in the headspace (due to the higher extraction temperature) than the extraction of the highly volatile compounds to the fiber.

These findings are further supported by the fact that SPME is based on an exothermic process and the extraction of compounds should decrease at high temperatures. Pellati et al. (*17*) made the same conclusions in their optimization work. In addition, the Pareto chart shows significant interactions between

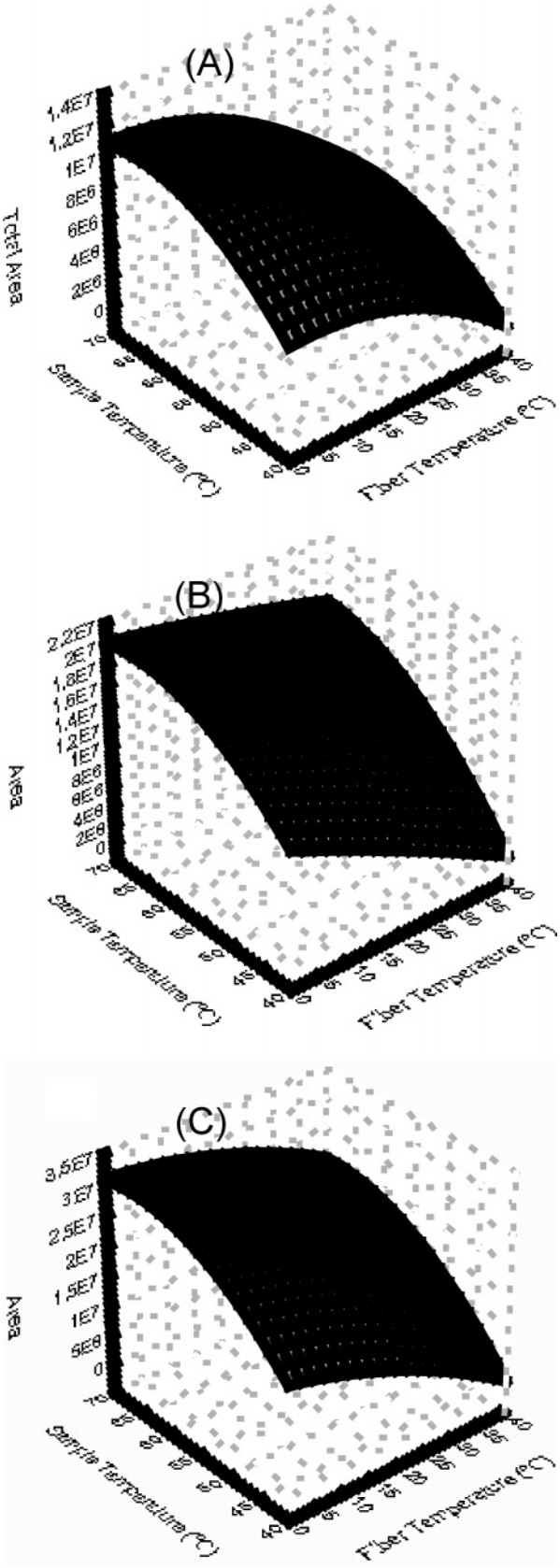

Figure 3. Response surface plot for (**A**) total peak area for the compounds eluted until 22 min vs sample temperature (°C) and fiber temperature (°C), (**B**) total peak area for the compounds eluted after 22 min vs sample temperature (°C) and fiber temperature (°C), and (**C**) total peak area for all compounds eluted vs sample temperature (°C) and fiber temperature (°C).

the sample temperature, the sample agitation, and the incubation time. The negative value for these interactions indicates an opposite effect on the analytical response.

To consider the effect of the concentration of less volatile compounds on the HS-SPME procedure, the sum of the peak areas was used for those peaks that eluted before 22 min of extraction time (150 °C). The result of this study is presented in the Pareto chart (**Figure 1B**), indicating that the sample temperature is highly significant, but the negative value suggests that the analytical signal increases with the decrease in the sample temperature. The influence of the sample temperature on the extraction of compounds that possess different volatilities is clear. A high sample temperature improves the extraction of less volatile compounds, while a low temperature enhances the extraction of more volatile compounds. This result is in agreement with the fact that SPME is based on an exothermic process, and it supports the conclusion that the concentration of less volatile compounds can significantly influence the result of the extraction optimization.

Doehlert Design. The results obtained by the factorial design demonstrate that when considering both volatile and nonvolatile analytes, sample temperature, sample agitation, and extraction time are conditions that require optimization. However, the factorial study also demonstrates that the sample temperature is the only factor that requires optimization when working exclusively with highly volatile analytes.

In both cases, the incubation time was not significant (at the 0.05 level) and was therefore fixed at 10 min. The sample agitation was fixed at 500 rpm, which is the maximum agitation speed allowed by the CombPAL autosampler. The significant variables (sample temperature and extraction time) were optimized using a Doehlert matrix system and the sum of the peak areas for all of the eluted compounds and for those compounds that were eluted prior to and after 22 min. In these three cases, seven experiments were carried out and all experiments were done in duplicate. The sum of the peak areas obtained in these experiments was used to inform the Doehlert matrix and to obtain the surface responses that are presented in **Figure 2**. These results indicate that maximum values were reached for both the sample temperature and the extraction time on the surface response plot. The optimization study illustrated that the best global response was reached when the sample temperature was around 80 °C and the extraction time was 30 min (**Figure 2A**). The maximum stationary points for the sample temperature and the extraction time, when considering the highly volatile compounds, were about 60 °C and 25 min, respectively (**Figure 2B**). On the other hand, when the less volatile compounds (eluted after 22 min) were considered, the response surface (**Figure 2C**) indicated a sample temperature and an extraction time higher than, respectively, 80 °C and 30 min to obtain maximum extraction efficiency. Because of the limited thermal stability of the target analytes in the study matrices, the values chosen for further experimental work were sample temperature of 60 °C, extraction time of 25 min, incubation time of 10 min, and sample agitation of 500 rpm.

The optimization of the sample and coating temperatures for the CF-HS-SPME procedure was also performed using the Doehlert matrix. The same optimum extraction and incubation times for the HS-SPME procedure (25 and 10 min, respectively) were used to allow for the direct comparison of the sensitivity of the different fibers. The sum of the peak areas for the eluted compounds and for those compounds that were eluted prior to and after 22 min was used to inform the Doehlert matrix and obtain the surface responses presented in **Figure 3**.

Table 1. Volatile Compounds of Yellow Passion Fruit Pulp

				SPME fibers			
compounds[a]	RI_{exp}[b]	RI_{ref}[c]	CF	CAR/PDMS	DVB/CAR/PDMS	PDMS	PA
alcohols							
(*Z*)-3-hexenol	790	827	×	×			
1-hexanol	828	847	×	×	×		
1-octanol	1068	1054	×	×	×	×	×
aldehydes							
benzaldehyde	959	923	×	×	×		
nonanal	1098	1089	×				
decanal	1199	1186	×				
undecanal	1298	1290	×				
esters							
ethyl butanoate	778	781	×	×	×		
ethyl pentanoate	871	881	×				
buthyl butanoate	990	978		×	×		
ethyl hexanoate	994	981	×	×	×	×	×
(*Z*)-3-hexenyl acetate	999	984	×	×			
hexyl acetate	1007	993	×	×	×	×	
isobutyl hexanoate	1144	1140			×		
benzyl acetate	1160	1144	×	×	×		
2-methyl hexylpropanoate	1183	1135	×			×	×
hexyl butanoate	1185	1177		×			
ethyl octanoate	1190	1181	×	×	×	×	×
2-methyl hexylbutanoate	1227		×		×	×	
isopentyl hexanoate	1242	1238	×	×	×		
pentyl hexanoate	1280			×	×		
3-hexenyl hexanoate	1371	1364	×	×	×	×	×
hexyl hexanoate	1376	1371	×	×	×	×	×
ethyl-(*E*)-cinnamate	1463	1430		×	×		
terpenic compounds							
α-pinene	878	925		×	×		
1*R*-α-pinene	922		×	×	×		
β-myrcene	982	981	×	×	×	×	×
β-pinene	980	965		×			
α-terpinene	1011	1006	×	×	×		
limonene	1025	1023	×	×	×	×	×
β-ocimene	1031	1025	×	×	×		
α-ocimene	1041	1039	×	×	×	×	×
γ-terpinene	1051	1046	×			×	
3-carene	1054	1001	×		×		×
terpinolene	1081	1076		×	×		
linalool	1097	1083	×	×	×		×
α-terpinol	1193	1171	×	×	×	×	×
caryophyllene	1413	1410		×			
β-ionone	1425	1447		×	×		
α-caryophyllene	1449	1419		×			
(*E*,*E*)-α-farnesene	1469	1455	×			×	
others							
geranyl acetone	1437	1434	×	×	×	×	×
total of compounds			31	32	29	15	13

[a] Tentative identification. [b] RI_{exp}, linear retention index on VF-5MS obtained experimentally. [c] RI_{ref}, linear retention index from literature.

The findings from this study suggest that there is a relationship between the sample and the fiber temperatures. It was found that the analytical signal increases with an increase in the sample temperature and a corresponding decrease in the fiber temperature (**Figure 3**). This behavior is more pronounced for higher volatile compounds (**Figure 3A**) than for less volatile compounds (**Figure 3B**). However, **Figure 3B** suggests that cold fiber is an excellent analytical tool to extract less volatile compounds as well. A compromise between the extraction of higher volatile compounds and the less volatile compounds using a CF-HS-SPME procedure is shown in **Figure 3C**. This study demonstrates that the sensitivity of the method is significantly improved with the use of lower fiber temperatures and higher

Table 2. Volatile Compounds of Cashew Fruit Pulp

				SPME fibers			
compounds[a]	RI_{exp}[b]	RI_{ref}[c]	CF	CAR/PDMS	DVB/CAR/PDMS	PDMS	PA
alcohols							
(Z)-3-hexenol	790	827	×	×			
aldehydes							
benzaldehyde	959	923	×	×			
decanal	1199	1186	×				
esters							
ethyl butanoate	778	771	×				
ethyl 3-methylbutanoate	832	824	×	×	×	×	
ethyl 2-methylbutanoate	753	829	×				
ethyl pentanoate	871	881	×				
ethyl 2-methyl-2-butenoate	1123	1229	×	×	×		
3-methyl propyl butanoate	1094		×				
ethyl hexanoate	994	981	×	×	×	×	×
Isobutyl hexanoate	1144	1140	×	×	×		
ethyl benzoate	1206	1160	×	×	×		×
ethyl octanoate	1190	1181	×	×	×	×	×
(Z)-3-hexenyl isovalerate	1421	1223	×		×	×	
ethyl decanoate	1351	1382	×	×	×	×	
ethyl (E)-cinnamate	1463	1430	×		×		×
terpenic compounds							
limonene	1025	1023	×	×	×	×	
α-ocimene	1041	1039	×		×	×	
α-copaene	1393	1376	×	×	×	×	
β-caryophyllene	1312	1399		×			
caryophyllene	1413	1410	×	×	×	×	
α-caryophyllene	1449	1419		×	×		
others							
acetophenone	1119	1048	×				
benzophenone	1788	1604					×
γ-dodelactone	1689	1647	×		×	×	×
total of compounds			22	14	15	10	6

[a] Tentative identification. [b] RI_{exp}, linear retention index on VF-5MS obtained experimentally. [c] RI_{ref}, linear retention index from literature.

Table 3. Volatile Compounds of Tamarind Fruit Pulp

				SPME fibers			
compounds[a]	RI_{exp}[b]	RI_{ref}[c]	CF	CAR/PDMS	DVB/CAR/PDMS	PDMS	PA
alcohols							
1-hexanol	828	847		×			
1-heptanol	962	949	×				
1-octanol	1068	1054	×				
nonanol	1264	1161	×				
aldehydes							
octanal	997	981	×		×		
nonanal	1098	1089	×	×	×	×	×
(E)-2-nonenal	1152	1135	×				
decanal	1199	1186	×		×		×
(E)-2-decenal	1254	1242	×	×		×	
undecanal	1295	1290	×				
2-undecanal	1353	1350	×				
benzeneacetaldehyde	1140		×				
esters							
ethyl hexanoate	914	981	×	×	×		
(Z)-3-hexenyl acetate	999	984		×			
hexyl acetate	1007	993	×	×			
methyl salicylate	1140	1181	×				
ethyl octanoate	1190	1181		×			
butyl palmitate	1881						×
geranylacetone	1487	1434	×				
terpenic compounds							
limonene	1025	1023	×	×			
α-copaene	1393	1386		×			
isocaryophyllene	1394			×			
caryophyllene	1413	1410	×	×			×
others							
methyl pentenone	978			×			
furfural	736	795	×				
2-pentyl furan	979	983	×				
benzophenone	1788	1604					×
total of compounds			19	12	4	2	5

[a] Tentative identification. [b] RI_{exp}, linear retention index on VF-5MS obtained experimentally. [c] RI_{ref}, linear retention index from literature.

sample temperatures. A sample temperature of 60 °C and a fiber temperature of 0 °C were chosen as the optimum values for further experimental work. The sample temperature of 60 °C was chosen because of the limited stability of the analytes in the matrices and to allow for the comparison of the commercial SPME fibers and cold fiber device.

Fiber temperatures below 0 °C could freeze the fiber because of the water present in the samples and could also decrease the amount of analytes extracted. It is evident that with the use of a lower fiber temperature, the extraction of a volatile with lower volatility as well as high volatility is improved. Normally, there is a high concentration of highly volatile compounds in the headspace, and because of their small distribution constants, only a small amount is transferred onto the fiber. Conversely, although less volatile compounds exhibit high distribution coefficients, they are present at lower levels in the headspace; therefore, the associated extraction efficiencies are low. Thus, it was found that the simultaneous use of a high sample temperature and low fiber temperature accelerates the mass transfer rate (especially for less volatile compounds) and increases the distribution coefficient (especially for highly volatile compounds), thus increasing the amount of analytes extracted by the cold fiber.

The repeatability of the extractions with the optimized experimental conditions was evaluated with seven extractions of yellow passion fruit (cold fiber) and cashew fruit (DVB/CAR/PDMS fiber). The relative standard deviation (RSD%) for the volatile compounds from these fruits and for the sum of the peak areas of all compounds identified was below 10%, indicating a good performance of the method developed for the extraction of volatile compounds of fruit pulps.

Analysis of Volatile Compounds of Fruit Pulps. The compounds tentatively identified in the yellow passion, cashew, tamarind, acerola, and guava are listed in **Tables 1**−**5**, respectively. It was found that no peaks appeared in the blank runs, which were conducted between extractions. This illustrates that there was no carry over or contamination effects that could cause memory effects and misinterpretation of the findings.

The HS-SPME procedure allowed for the identification of 42 compounds in the yellow passion fruit aroma (**Table 1**). Most of these compounds were tentatively identified as terpenes and esters, found in large numbers and exhibiting intense peak areas. **Table 2** shows that 25 compounds were tentatively identified in the cashew aroma, being the most intense methyl and ethyl esters. In this study, 27 volatile compounds were tentatively identified in the tamarind aroma (**Table 3**), including phenylacetaldehyde and furfural. Aldehyde and ester families were most commonly tentatively identified in the tamarind aroma in this study. In the acerola aroma (**Table 4**), 23 substances were identified and the esters were predominant. **Table 5** illustrates that 33 compounds were tentatively identified in the guava aroma; a large number of them were ester and terpenoid compounds.

Table 4. Volatile Compounds of Acerola Fruit Pulp

				SPME fibers			
compounds[a]	RI_{exp}[b]	RI_{ref}[c]	CF	CAR/PDMS	DVB/CAR/PDMS	PDMS	PA
alcohols							
(*Z*)-3-hexenol	790	827	×	×			
1-octen-3-ol	970	963	×	×			
aldehydes							
nonanal	1098	1089	×	×	×		
2,6,6-trimethyl-cyclo-hexene-1-carboxaldehyde	1217		×	×	×	×	×
esters							
ethyl acetate	578	581	×				
ethyl butanoate	778	781	×				
ethyl pentanoate	871	881	×				
methyl hexanoate	914	904	×				
ethyl hexanoate	914	981	×	×	×	×	×
ethyl-2-hexenoate	1036	1051	×		×		
ethyl heptanoate	1188	1082	×			×	
hexyl acetate	1007	993		×			
ethyl benzoate	1206	1160	×	×	×		
ethyl octanoate	1190	1181	×	×			×
(*Z*)-3-hexenyl butanoate	1176	1166	×		×		
(*Z*)-ethyl 4-octenoate	1278	1177	×		×	×	
2-methyl hexylpropanoate	1183	1135	×			×	
isopentyl hexanoate	1242	1238	×	×	×	×	
hexyl hexanoate	1376	1371	×		×	×	×
ethyl decanoate	1351	1382	×	×	×	×	
ethyl-2,4-*trans*,*cis*-decadienoate	1553		×		×	×	×
terpenic compounds							
caryophyllene	1413	1410	×	×	×	×	×
others							
2,2,6-trimethyl-cyclo-hexanone	1027		×				
total of compounds			22	11	12	10	6

[a] Tentative identification. [b] RI_{exp}, linear retention index on VF-5MS obtained experimentally. [c] RI_{ref}, linear retention index from literature.

Table 5. Volatile Compounds of Guava Fruit Pulp

				SPME fibers			
compounds[a]	RI_{exp}[b]	RI_{ref}[c]	CF	CAR/PDMS	DVB/CAR/PDMS	PDMS	PA
alcohols							
(*Z*)-3-hexenol	790	827	×	×			
1-hexenol	828	846	×	×	×		
phenyl ethylalcohol	1111	1104		×	×		×
3-phenyl propylalcohol	1228	1218					×
aldehydes							
nonanal	1098	1089	×	×			×
decanal	1199	1186	×			×	
undecanal	1298	1290	×				
geranial	1262	1252	×		×		×
esters							
ethyl butanoate	778	781	×				
ethyl hexanoate	914	981	×	×	×		×
(*Z*)-3-hexenyl acetate	999	984	×	×	×		×
hexyl acetate	1007	993	×	×	×		×
ethyl octanoate	1190	1181	×	×	×	×	×
octyl acetate	1199	1193	×				
3-phenyl propylacetate	1357	1347					×
ethyl decanoate	1351	1382	×	×	×	×	×
cinnamyl acetate	1439	1419	×		×	×	×
terpenic compounds							
1*R*-α-pinene	922		×				
limonene	1025	1023	×	×			×
eucalyptol	1126		×	×	×		×
α-pinene	878	925	×	×	×		×
α-ocimene	1041	1039	×	×	×		×
linalool	1097	1083					×
edulan II	1247	1328	×				
α-copaene	1366	1386		×	×		×
α-cubebene	1469	1426	×		×	×	×
β-caryophyllene	1312	1399	×	×			
caryophyllene	1413	1410	×	×	×	×	×
α-caryophyllene	1449	1419	×	×	×	×	×
β-nerolidol	1540	1553	×			×	×
caryophyllenyl alcohol	1569	1570			×		×
caryophyllene oxide	1574		×		×	×	×
others							
methyl isohexenylketone	978		×	×	×		
total of compounds			27	18	19	9	23

[a] Tentative identification. [b] RI_{exp}, linear retention index on VF-5MS obtained experimentally. [c] RI_{ref}, linear retention index from literature.

Butylated hydroxytoluene, a compound usually used as a food antioxidizer and that could also be derived from the packaging material of frozen pulps, was detected in all of the samples. Augusto et al. (*16*) found that this compound was present in samples of cajá and graviola fruit obtained from De Marchi Ind. e Com. de Frutas LTDA and Brasfrut Frutos do Brasil LTDA, respectively.

Comparison of the Fiber Efficiencies. The performances of each commercially available SPME fiber and the cold fiber used in this study were determined based on the intensity of the response observed. The sum of the identified peak areas in the total ion chromatogram (TIC) obtained for each fiber was normalized in relation to the sum of the identified peak areas in the TIC obtained for the cold fiber device. The extraction efficiency was calculated for each fruit pulp, and the results are presented in **Figure 4**. In this study, each extraction was done in triplicate and the repeatability (RSD%) was lower than 10%. The amount of compounds extracted by the cold fiber device was greater than the amount extracted by the commercial SPME fibers.

Among the commercial SPME fibers, it was observed that the CAR/PDMS fiber exhibits better extraction efficiency for highly volatile compounds. This fiber is covered with a porous solid coating, which suggests that the analyte extraction occurs via adsorption, which is particularly efficient for volatile compounds. Conversely, for the PDMS fiber, which represents a nonpolar coating, it was observed that it exhibited better efficiency for the less volatile compounds, as compared to CAR/PDMS fiber, likely because the extraction process occurs via absorption. Because of the improved extraction efficiency of the CAR/PDMS fiber for the more volatile compounds, these compounds exhibit larger peak areas than the less volatile compounds extracted with the PDMS fiber.

The second best commercial fiber that was evaluated was the CAR/DVB/PDMS fiber (**Figure 4**), except for the analysis of the volatile compounds from the cashew fruit. This fiber contains a meso-macroporous structure and, therefore, contains a range of pore sizes, as compared with the CAR/PDMS fiber. Furthermore, the combination of the DVB and the CAR increases both the porosity distribution and the polarity of the fiber, improving the retention of the analytes on the fiber as compared to a coating that only consists of PDMS. The absence of highly volatile compounds in the cashew fruit aroma explains improved extraction efficiency, which was observed with the CAR/DVB/PDMS fiber vs the CAR/PDMS fiber.

In the case of the cold fiber device, this study illustrated excellent extraction efficiencies for all classes of compounds

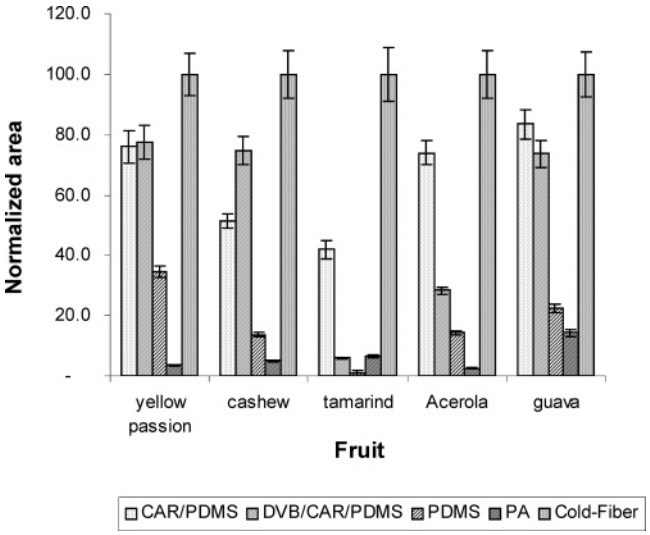

Figure 4. Normalized extraction efficiencies measured for PDMS, CAR/PDMS, DVB/CAR/PDMS, PA, and cold fiber.

studied (**Figure 4**), thus supporting the conclusion that the cold fiber approach provides the best extraction efficiency for the purposes of this research.

The PA fiber, which is moderately polar, demonstrated better extraction efficiency than the cold fiber for the extraction of terpenic alcohol (caryophyllenyl alcohol) and 3-phenyl propyl alcohol, found in the volatile compounds of guava fruit. However, other moderately polar compounds were more efficiently extracted with the cold fiber approach.

The proposed automated CF-HS-SPME approach offers significant performance to extract volatile compounds from fruit pulps. Future works will include the development of multicoated cold fibers and additional applications for analysis of food, plants, and drugs.

ABBREVIATIONS USED

PDMS, polydimethylsiloxane; SPME, solid-phase microextraction; HS-SPME, headspace solid-phase microextraction; GC, gas chromatography; GC-MS, gas chromatography−mass spectrometry; CAR, carboxen; DVB, divinylbenzene; CF-HS-SPME, cold fiber headspace solid-phase microextraction; DVB/CAR/PDMS, divinylbenzene-carboxen-polydimethylsiloxane; CAR/PDMS, carboxen-polydimethylsiloxane; PA, polyacrylate; FID, flame ionization detector; RSD%, relative standard deviation; TIC, total ion chromatogram.

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