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Optimization of the extraction conditions of the volatile compounds from chili peppers by headspace solid phase micro-extraction

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ABSTRACT

A method involving headspace-solid phase micro-extraction (HS-SPME), gas chromatography with flame ionization detection (GC-FID) and gas chromatography with mass spectrometry (GC–MS) was developed and optimized to investigate the volatile composition of *Capsicum* chili peppers. Five SPME fibers were tested for extraction: carboxen/polydimethylsiloxane (CAR/PDMS-75 μm), polydimethylsiloxane (PDMS-100 μm), divinylbenzene/polydimethylsiloxane (DVB/PDMS-65 μm), carbowax/divinylbenzene (CW/DVB-70 μm), and divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS-50/30 μm), the last of which was shown to be the most efficient fiber to trap the volatile compounds. Optimization of the extraction conditions was carried out using multivariate strategies such as factorial design and response surface methodology. Eighty three compounds were identified by GC–MS when using the optimized extraction conditions, the majority of which were esters.

1. Introduction

Peppers, as also sweet peppers, are the fruits of annual plants belonging to the family *Solanaceae* and genus *Capsicum*. This genus has more than 200 species cultivated in various locations in our planet [1]. The interest in their cultivation is due to their use as seasonings in culinary preparations, mainly because of their characteristics of pungency, aroma and color [2,3]. In addition, the food industry employs them widely as coloring and flavoring agents in sauces, soups, processed meats, lunches, sweetmeats and alcoholic beverages [4]. Due to this extensive use, the sensory characteristics provided by the various *Capsicum* fruits are an important factor in the quality of the foods to which they are added. The most important quality factors of the *Capsicum* are their pungency and color, and an increasing volume of research on the evaluation of food quality has concentrated on the characterization of the volatiles in order to understand the aroma of foods [5–7]. Although some papers found in the literature have reported a complex chemical composition for the volatiles of some types of *Capsicum*, identifying hundreds of components, it is common knowledge that the real importance of these components for the aroma is little known [8–10]. It should also be emphasized that the constituents of this volatile fraction can present considerable modifications according to the variety being studied, the location where it is cultivated, the processing procedure and/or the degree of maturation. Thus work related to the characterization of the volatile fraction from peppers constitutes a subject of continued interest to researchers in a variety of countries [3,4,11].

The analytical work involved in characterizing the volatile fraction of peppers can be summarized as the isolation of the volatile compounds, their chromatographic separation and subsequent identification and quantification. In the extraction stage, methodologies such as simultaneous steam-distillation-solvent extraction (SDE), purge and trap and solid phase micro-extraction (SPME) can be used [3,8,12,13].

SPME shows some advantages over the other techniques, such as simplicity, speed, the possibility of working with small amounts of sample, the absence of solvents, adequate sensitivity and low cost. In addition the use of this technique without the lengthy use of organic solvents or high temperatures in the extraction and concentration stages decreases the possibility of forming artifacts in the fraction extracted [14].

Considering that SPME is a technique based on physicochemical processes of equilibrium between the matrix and the headspace, and between the headspace and the material coating the fiber, the success of its use depends on factors such as the chemical nature of the compounds to be extracted, the correct choice of a coating material for the fiber, the temperature

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Table 1
Factors, levels and experimental domain of the conditions applied to optimize the extraction by HS-SPME.

Variables	Coded variables				
	$-\alpha$[a]	−1	0	1	α[a]
Extraction temperature (T, °C)	12	20	40	60	68
Extraction time (*t*.ext., min)	52	60	80	100	108

[a] $\alpha = 1.4142$.

used during extraction and the extraction time to the headspace [15].

In cases such as this, where many factors influence the response of the system, optimization of the extraction procedure can be carried out using multivariate statistical tools. These provide secure information concerning the best analytical conditions, the existence or otherwise of experimental errors, as well as showing any interactions that might exist between the factors involved. While traditional methods of optimization experiments, where only one variable is analyzed at a time, leaving the others fixed, require a large number of experiments, and do not allow to investigate possible interactions between variables, and not explore fully the solution space for optimization [16].

The objective of the present work was the multivariate optimization of the extraction conditions to obtain the volatile fraction from the malagueta chili pepper (*Capsicum frutescens*) using headspace solid phase micro-extraction (HS-SPME), to be applied in the characterization of the volatiles from various species of *Capsicum* by gas chromatography coupled with mass spectrometry (GC–MS).

2. Materials and methods

2.1. Samples

The samples of malagueta chili peppers used in this study (about 2.0 kg) were obtained from the Agronomic Institute (IAC) in the city of Campinas, SP, Brazil. The genotypes selected for the study were obtained from the germ plasma bank of the Vegetable Sector of IAC, Campinas. The botanical identification of the plants was carried out by Dra. Arlete Marchi Tavares de Melo, a research worker at the IAC, Campinas. The samples used for optimization were in the physiological mature state (maximum size development, but still immature). They were harvested in the morning and immediately transported to the laboratory for analysis.

2.2. Sample preparation and SPME procedures

For the analyses, 100 g aliquots of whole pepper fruits were first ground in a blender, and 1.00 g of ground sample then weighed into 15.0 mL SPME flasks complete with screw-top caps and PTFE/silicone septa (Supelco, Bellefonte, PA, USA). The following SPME fibers (Supelco,Bellefonte, PA, USA) were used in this study: 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS), 65 μm divinylbenzene/polydimethylsiloxane (DVB/PDMS), 70 μm carbowax/divinylbenzene (CW/DVB), 75 μm carboxen/polydimethylsiloxane (CAR/PDMS) and, 100 μm polydimethylsiloxane (PDMS). These were duly conditioned before use according to the manufacturer's instructions. In the preliminary selection, all the fibers were tested so as to select the one presenting the best capacity to extract the pepper volatiles. In this step, all the fibers were exposed to the sample headspace under the following conditions: equilibrium time of 15 min, extraction time of 30 min, extraction temperature of 40 °C (conditions arbitrarily established by the authors in the choice-of-fiber step); after extraction, the fibers were introduced into the gas chromatograph injector for desorption of the analytes at a temperature of 250 °C in the splitless mode for a period of 1.0 min. After the extraction and desorption procedures, each of the fibers was reconditioned at 250 °C for 15 min, with the exception of CW/DVB, which was reconditioned at 220 °C following the manufacturer's recommendations. The fiber reconditioning procedure was carried out to guarantee the absence of peaks in the run blanks and the good quality of the

Table 2
Experimental conditions and values for the response (total area) obtained for the CCRD in the stage of optimizing the conditions for the extraction of the volatile compounds from immature malagueta peppers (*Capsicum frutescens*) by HS-SPME.

Experiment number	T (°C)	Extraction temperature	*t*.ext. (min)	Extraction time	Response (total area[a])
1	−1	20	−1	60	3.11E+07
2	1	60	−1	60	5.82E+07
3	−1	20	1	100	5.33E+07
4	1	60	1	100	6.38E+07
5	−1.41	12	0	80	4.10E+07
6	1.41	68	0	80	6.82E+07
7	0	40	−1.41	52	5.90E+07
8	0	40	1.41	108	5.62E+07
9	0	40	0	80	7.33E+07
10	0	40	0	80	7.20E+07
11	0	40	0	80	7.09E+07
12	0	40	0	80	7.01E+07

[a] Total area expressed in arbitrary units.

Table 3
Analysis of variance by the minimum squares method for the parameters of extraction time and temperature for the volatile compounds of malagueta peppers (*Capsicum frutescens*) by HS-SPME.

Sources of variation	Sum of the squares	Degrees of liberty	Mean of the squares	$F_{calculated}$	$F_{tabulated}$	$F_{calculated}/F_{tabulated}$
Regression	1.71E+15	5	3.42E+14	11.85	4.39	2.70
Residues	1.73E+14	6	2.89E+13			
Lack of fit	1.67E+14	3	5.58E+13	28.06	9.28	3.02
Pure error	5.96E+12	3	1.99E+12			
Total	1.89E+15	11				
R^2	0.908					

% Variation explained: 90.81. Maximum % variation explained: 99.68.

Table 4
Volatile compounds identified in the immature malagueta pepper (*Capsicum frutescens*) by HP-SPME and GC/MS.

Compounds	$LTPRI_{Calc.}$	$LTPRI_{Lit.}$	Δ	SPME fibers				
				DVB/CAR/PDMS	CAR/PDMS	PDMS	DVB/PDMS	CW
Alcohols								
2-Propanol[b]	474	482	8	x	x			
1-Pentanol[b]	765	761	−4	x	x			
4-Methyl-pentanol[b]	833	831	−2	x	x	x		
(Z)-4-hexenol[b]	853	857	4	x	x			
1-Hexanol[a]	866	860	−6	x	x	x	x	x
(E)-2-hexen-1-ol[a]	866	868	2	x	x			
1-Octanol[a]	1066	1057	−9	x	x	x	x	
4-Butoxy-1-butanol[b]	1139	1135	−4	x	x	x	x	x
2-Decanol[b]	1179	1178	−1	x	x	x	x	
(E)-2-(E)-4-nonadiene-1-ol	1196	1200	4	x	x	x	x	x
1-Decanol[a]	1260	1260	0	x	x	x	x	
(Z)-6-nonene-1-ol acetate[b]	1294	1290	−4	x	x	x	x	x
10-Undecene-1-ol[b]	1346	1347	1	x	x			
(E)-2-undecene-1-ol[b]	1355	1355	0	x		x	x	x
2,2-Dimethyl-1-decanol[b]	1363	1372	9	x	x	x	x	
1-Dodecanol[a]	1562	1564	2	x		x	x	x
1-Tridecanol[a]	1563	1572	9	x	x	x	x	
(E)-3-pentadecene-2-ol[b]	1677	1683	6	x	x	x		x
Aldehydes and ketones								
2-Methyl-butanal[a]	649	643	−6	x	x			
Hexanal[a]	801	806	5	x	x			
(E)-2-hexenal[a]	851	850	−1	x	x	x	x	
Heptanal[a]	872	873	1	x	x	x		
5-Methyl-5-octene-2-one[b]	1040	1037	−3	x	x	x	x	
2-Nonanone[a]	1055	1052	−3	x	x	x	x	
(Z)-2-decenal[b]	1218	1212	−6	x	x	x	x	x
Dodecanal[a]	1392	1390	−2	x	x			
(E)-2-dodecanal[b]	1447	1442	−5	x		x	x	
(Z)-2-dodecanal[b]	1449	1447	−2	x	x	x	x	
Mirac aldedo[b]	1485	1488	3	x		x	x	
Tridecanal[b]	1493	1494	1	x	x	x	x	
2(E)-tridecenal[b]	1543	1549	6	x	x	x	x	
Tetradecanal[b]	1592	1599	7	x	x		x	
Esters								
Ethyl hexanoate[a]	968	978	10	x			x	
Hexyl acetate[b]	978	984	6	x	x	x	x	
Butyl 2,2-dimethyl propanoate[b]	1003	999	−4	x	x		x	
Butyl isovalerate[b]	1007	1005	−2	x	x	x	x	
Isopentyl isobutyrate[b]	1013	1014	1	x	x		x	
Isoamyl pyruvate[b]	1050	1051	1	x	x			
Prenyl isobutyrate[b]	1060	1050	−10	x	x	x	x	
Pentyl butyrate[b]	1094	1095	1	x	x	x	x	
2-Methylbutyl 2-methylbutanoate[b]	1100	1104	4	x	x	x	x	
2-Methylbutyl isovalerate[b]	1106	1109	3	x		x	x	
Hexyl isobutyrate[b]	1113	1118	5	x	x	x	x	
7-Methyl-4-octyl acetate[b]	1148	1154	6	x	x	x	x	
Hexyl butanoate[b]	1157	1155	−2	x	x	x	x	x
(E)-3-hexenyl butyrate[b]	1187	1191	4	x		x		
Hexyl 2,2-dimethylpropanoate[b]	1201	1197	−4	x		x		
Hexyl 3-methylbutanoate[b]	1208	1202	−6	x	x	x	x	x
2-Methylbutyl hexanoate[b]	1215	1218	3	x	x	x		
(Z)-3-hexenyl isopentanoate[b]	1231	1226	−5	x	x	x	x	x
(Z)-3-hexenyl-2-methyl butanoate[b]	1232	1231	−1	x	x	x	x	
2-Methyl-hexyl butanoate[b]	1237	1239	2	x	x	x	x	x
3-Methyl-hexyl butanoate[b]	1242	1243	1	x	x	x	x	x
Heptyl isobutanoate[b]	1247	1248	1	x	x	x		
3-Methyl-2-butenyl hexanoate[b]	1263	1273	10	x	x	x	x	
(Z)-3-hexenyl pentanoate[b]	1284	1282	−2	x	x	x	x	
Hexyl pentanoate[b]	1289	1282	−7	x	x	x	x	
Butyl isohexyl carbonate[b]	1303	1294	−9	x		x		x
Heptyl 2,2-dimethylpropanoate[b]	1306	1297	−9	x	x	x	x	
Heptyl 2-methylbutyrate[b]	1311	1317	6	x	x	x		
2-Methylpentyl hexanoate[b]	1316	1317	1	x	x	x	x	
Methyl geranate[a]	1330	1322	−8	x	x	x	x	
2-Ethyl hexyl 2,2-dimethylpropanoate	1335	1332	−3	x		x	x	x
Alyl heptyl carbonate[b]	1340	1348	8	x	x	x	x	x
(E)-5-decenyl acetate[b]	1387	1389	2	x	x	x	x	x
Benzyl 2-methylbutanoate[b]	1394	1394	0	x	x			
Octyl pyvalate[b]	1402	1396	−6	x		x	x	
Alyl decanoate[b]	1463	1471	8	x	x	x	x	

Table 4 (*Continued*)

Compounds	$LTPRI_{Calc.}$	$LTPRI_{Lit.}$	Δ	SPME fibers				
				DVB/CAR/PDMS	CAR/PDMS	PDMS	DVB/PDMS	CW
Terpenes								
(E)-β-ocimene[a]	1047	1041	−6	x	x	x	x	
α-Copaene[a]	1373	1375	2	x		x	x	
β-Cariophyllene[a]	1415	1410	−5	x	x			
α-Ionone[a]	1428	1421	−7	x	x			
Cadinadiene[b]	1440	1440	0	x		x	x	
(E)-β-pharnesene[a]	1456	1452	−4	x		x	x	
α-Selinene[a]	1475	1474	−1	x		x	x	x
β-(E)-bergamotene[a]	1480	1483	3	x	x	x	x	
β-Ionone[a]	1486	1480	−6	x	x		x	
Δ-Cadinene[a]	1521	1518	−3	x	x			
Alkanes								
Undecane[a]	1103	1100	−3	x	x		x	
(Z)-3-tetradecene[b]	1421	1421	0	x	x	x	x	
1-Pentadecene[a]	1490	1489	−1	x				
Pentadecane[a]	1499	1500	1	x	x	x	x	
1-Hexadecene[a]	1581	1589	8	x		x	x	
Hexadecane[a]	1598	1600	2	x	x	x	x	
(Z)-7-hexadecene[b]	1614	1620	6	x	x		x	
Heptadecane[a]	1698	1700	2	x	x	x	x	
Total number of identified compounds				83	69	65	63	18

$LTPRI_{Calc.}$ = retention indices obtained using a 5% phenyl/95% dimethylpolysiloxane capillary column. $LTPRI_{Lit.}$ = retention indices obtained using a 5% phenyl/95% dimethylpolysiloxane capillary column. Δ = difference between the calculated retention indices and those in the literature; $LTPRI_{Lit.} - LTPRI_{Calc.}$

[a] The reliability of the identification proposal is indicated by mass spectrum and linear retention index agreed with standards.

[b] The reliability of the identification proposal is indicated by mass spectrum and linear retention index agreed with literature data.

SPME extraction and chromatographic procedures. All the fibers were tested in triplicate and the results presented represent the mean values obtained.

2.3. Gas chromatography (GC-FID)

Since knowledge of the identity of the compounds is not necessary for the optimization step of the SPME conditions, and due to the excellent stability and ease of operation of the flame ionization detector (FID), this first step of the work was carried out using a Varian 3800 GC equipped with a FID detector and Star Chromatography Workstation (version 4.5). An SPB-5 (5% phenyl/95% dimethylpolysiloxane) (30 m × 0.25 mm i.d. × 0.25 μm of film thickness) fused silica capillary column from Supelco (Bellefonte, PA, USA) was used to separate the volatile compounds of the sample. The following instrumental conditions were used: injector in the splitless mode for 1.0 min at 250 °C, stripping gas: hydrogen at 1.0 mL min^{-1}; oven temperature gradient: 40 °C initially, increasing to 240 °C at 3 °C min^{-1} and remaining at this temperature for a further 4 min; detector temperature of 250 °C.

2.4. Gas chromatography/mass spectrometry (GC–MS)

The GC–MS analyses were carried out using a Shimadzu (Japan) model GC-17A/QP-5000 equipment under the following experimental conditions: HP-5 MS (5% phenyl/95% dimethylpolysiloxane) (30 m × 0.25 mm i.d. × 0.25 μm) fused silica capillary column from J&W Scientific (USA); injector: splitless mode for 1.0 min at 250 °C; stripping gas of helium at 1.0 mL min^{-1}, oven temperature gradient: 40 °C initially, increasing to 240 °C at 3 °C min^{-1} and remaining at this temperature for a further 4 min; interface temperature: 240 °C, electron ionization source at +70 eV; mass analyzer of the simple quadrupole type monitoring the range from 35 to 350 *m*/*z*. A mixture of aliphatic hydrocarbons (C_6–C_{20}) (PolyScience, IL, USA)

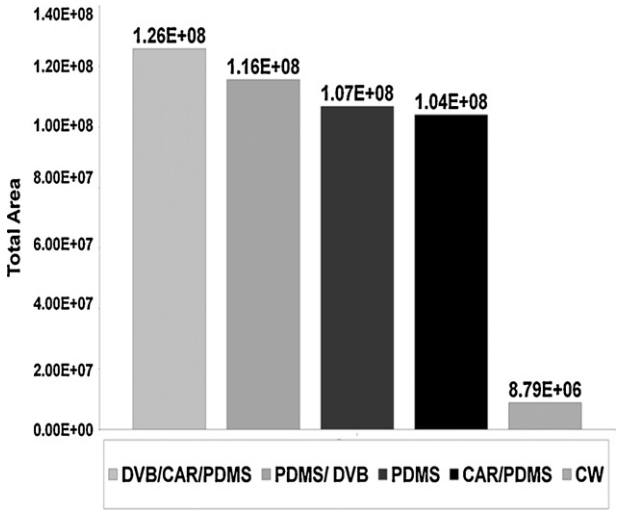

Fig. 1. Efficiency of the SPME fiber coatings in the extraction of volatile compounds from malagueta chili peppers by HS-SPME. The results are the means of triplicates of the total areas obtained on GC-FID chromatograms.

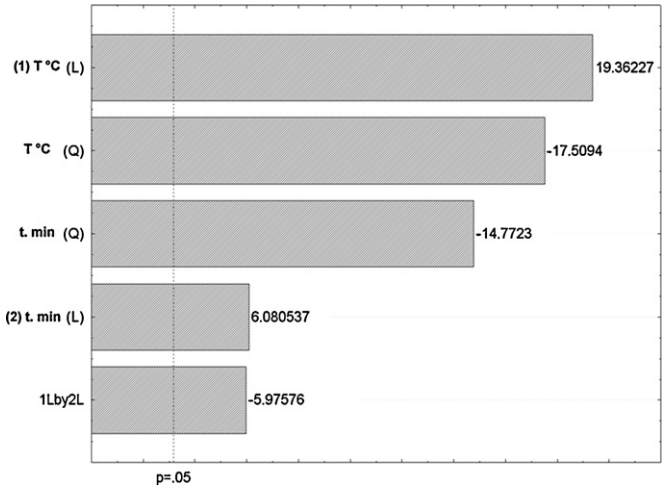

Fig. 2. Pareto diagram of the effects of the variables studied. Response: total area, 2 factors, 1 block, 12 experiments, pure error = 1.99E+12.

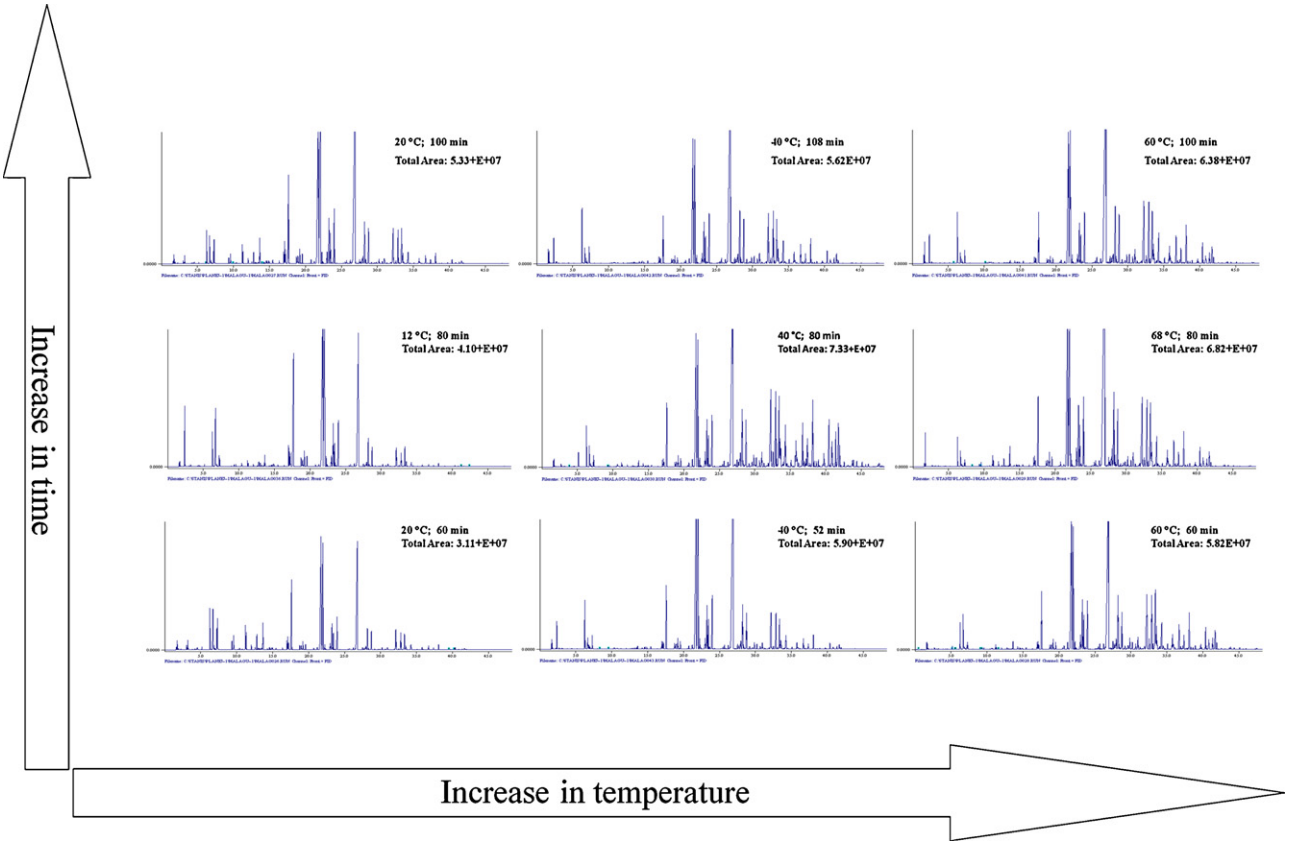

Fig. 3. Chromatograms of the headspace of immature malagueta chili peppers obtained using HS-SPME for each experiment of the experimental design; the arrows indicate the direction of the increases in levels of the time and temperature factors.

was placed in a SPME flask and submitted to extraction and injected under the same conditions as the sample, in order to calculate the Van den Dool & Kratz retention indices (LTPRI – linear temperature programmed retention index) of the volatile compounds. A tentative identification of the components was carried out by comparing the LTPRI and mass spectra obtained for the sample with those found in the literature (Adams [15] and NIST, 2005), with a similarity of at least 85% for the mass spectra and maximum variation of ±10 for the LTPRI. The positive identification of some compounds was obtained by comparison of their mass spectrum and LTPRI with those of reference standards.

2.5. Optimization strategy

Optimization of the HS-SPME conditions was carried out using a 2^2 factorial central compound rotational design (CCRD), with four axial points ($\alpha = 1.4142$) and four central points [16]. For optimization of the HS-SPME, the variables chosen were the temperature (T, °C) and extraction time (t, min), and the levels of each variable can be seen in Table 1. The other parameters, such as amount of sample, headspace volume and equilibrium time were arbitrarily fixed by the authors. Twelve experiments were carried out at random. The software Statistica v. 7 (Statsoft Inc., Tulsa, OK, USA) was used for the statistical analyses.

3. Results and discussion

Fig. 1 shows the results for total area obtained for each of the five SPME fibers tested with respect to their capacity to extract the volatile fraction of the malagueta chili pepper. Each fiber was exposed to the headspace under the same conditions of equilibrium time, extraction time and temperature, and although the extraction conditions were the same, the differences in the areas obtained revealed the behavior of each type of coating used for each fiber tested.

Although the means of the total areas obtained for the different fibers did not present significant statistical differences (Tukey at $p < 0.05$), the fiber DVB/CAR/PDMS was chosen, since it presented a greater number of peaks on the chromatograms, probably because it was composed of three types of coating material, thus uniting the advantages of three coating materials in a single fiber. In addition, there are reports in the literature of the use of DVB/CAR/PDMS in studies on fresh fruit aromas (volatiles and semi-volatiles) as an adequate coating for the capture of compounds related to aroma [17].

Fig. 2 shows the Pareto diagram summarizing the results obtained using the experimental design with fiber DVB/CAR/PDMS. It can be seen that all the factors evaluated were significant at 95% of confidence. Based on this information these factors were used for the experimental design so as to obtain the best conditions for the extraction temperature and extraction time. Experiments were also carried out at the central point ($n = 4$) with the objective of estimating the pure error and detecting any possible lack of fit of the model.

Table 2 shows the responses for the total areas of the chromatograms obtained in this stage of the experiment, while Fig. 3 shows the chromatographic profiles obtained at the experimental levels of the factors tested in the CCRD. The distribution of the chromatograms in this figure followed the design, with the minimum and maximum points of each parameter at the extremities and the axial points between them. The chromatogram in the middle refers to one of the four repetitions at the central point. This figure shows an increase in the areas of the peaks under the conditions used at the central point of the design, that is, using a temperature of 40 °C with

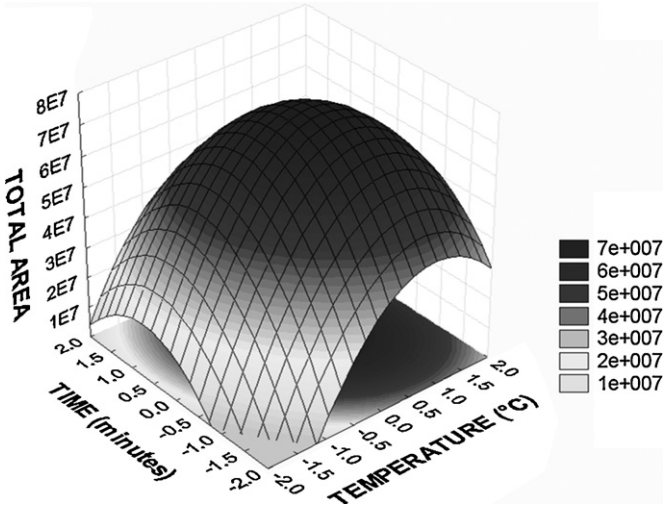

Fig. 4. Response surface obtained using the quadratic model: $y = 7.16E{+}07 + 9.53E{+}06 \times T - 9.65E{+}06 \times T^2 - 3.00E{+}06 \times t - 8.15E{+}06 \times t^2 - 4.15E{+}06\ {}^*T{}^*t$, in the optimization of the conditions (temperature T, °C and time t, min) for extraction of the volatiles from the malagueta chili pepper (*Capsicum frutescens*) by HS-SPME. The values to T, °C and t, min represent in the picture are coded value; real values can be seen in Table 2.

an extraction time of 80 min. The fact that a chromatogram richer in terms of the number of compounds and area was obtained at a temperature not above 40 °C is an advantage, since various studies have reported the possibility of degradations and the formation of artifacts at higher temperatures [15]. In addition the optimum extraction time of 80 min was possibly influenced by the coating material of the fiber, since the solid coatings of CAR and DVB present adsorption mechanisms that are normally slower than the partition observed for the liquid PDMS film [15,17].

The mathematical model describing the response surface for the CCRD was validated using the analysis of variance (ANOVA), since the results for the regression are more adequate to provide evidence of the existence or otherwise of a lack of fit of the model and decide if it is possible to make predictions based on the latter [16].

The ANOVA results can be seen in Table 3. The statistical significance of the regression of the ratio between the mean of the squares of the regression and the quadratic mean of the residues (MQ_R/MQ_r), or $F_{calculated}$, was 11.85; and when compared at a level of significance of 95% with the value for $F_{tabulated(5,6,95\%)}$, which, in this case, was 4.39, it was shown that $F_{calculated} > F_{tabulated}$ by about 2.7 times, indicating the existence of an adequate correlation between the variables studied.

However, on analyzing the statistical significance of the lack of fit of the model, given by the ratio between the quadratic mean of the lack of fit and the quadratic mean of the pure error (MQ_{faj}/MQ_{ep}), which, for a good fit of the model should present $F_{calculated} < F_{tabulated}$, it was found that the model obtained presented a lack of fit with respect to the experimental results, making it impossible to predict the responses (values of the total area) for other extraction times and temperatures, based on the model obtained. Although a lack of fit was indeed found, the experimental results obtained were real and valid, and thus the optimal conditions found for the extraction time and temperature could be used, since they provided the greatest values for the total area of the pepper chromatograms. The relative standard deviation (% SD) for the values of total area obtained at the central point of the CCRD was only 1.97%, indicating adequate repeatability of the method when developed under this condition.

Fig. 4 shows the response surface obtained in the CCRD. The optimal values found for extraction time and temperature were 80 min and 40 °C. Although CCRD has indicated for optimal time using SPME a longer period than the total time used in chromatographic analysis, this is not very far from that employed for the analysis of volatiles in *Capsicum* for other not optimized methods of extraction by simultaneous steam distillation solvent extraction (SDE), headspace dynamic using Tenax TA and SPME [4,6–8,10].

Table 4 shows the results obtained in the comparison of the extraction efficiency of each fiber, tested by GC–MS under the conditions described for the central point of the CCRD for immature malagueta chili peppers.

Various classes of chemicals groups were found in the volatiles of the peppers, such as esters, alcohols, aldehydes, terpenes, alkanes and ketones. The fiber that extracted the greatest number of compounds was DVB/CAR/PDMS, followed by CAR/PDMS, PDMS, DVB/PDMS and CW.

Considering the polarity of the coating materials, PDMS is the most apolar followed by the mixtures CAR/PDMS, DVB/CAR/PDMS, DVB/PDMS and CW. The latter has a polar nature, a fact probably related to its capacity to extract compounds of a more polar nature, such as alcohols, aldehydes and esters. On the other hand, the intermediate polarity of DVB/CAR/PDMS, associated with the mixed nature of this coating (existence of meso-macropores resulting from the rugosity of the liquid PDMS film associated with the solid pores of CAR and DVB), is probably related to its capacity to extract a greater number of volatiles as compared to the others [15,17].

In all, 83 compounds were tentatively identified in the volatile fraction of the sample. Although many of these have been described in other vegetable species, none were previously published for malagueta chili pepper [4,6,7,9,11].

4. Conclusions

The headspace solid phase micro-extraction methodology was shown to be efficient in extracting the volatiles from immature malagueta chili peppers, showing good repeatability. The multivariate optimization of the extraction conditions allowed for the fixing of the best extraction time and temperature. The results for the tentative optimization by GC–MS of the volatiles obtained under the optimized extraction conditions, showed a complex chemical composition with 83 components, predominantly esters.

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