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Evaluation of solid-phase micro-extraction coupled to gas chromatography–mass spectrometry for the headspace analysis of volatile compounds in cocoa products

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Abstract

The aroma profile of cocoa products was investigated by headspace solid-phase micro-extraction (HS-SPME) combined with gas chromatography–mass spectrometry (GC–MS). SPME fibers coated with 100 μm polydimethylsiloxane coating (PDMS), 65 μm polydimethylsiloxane/divinylbenzene coating (PDMS-DVB), 75 μm carboxen/polydimethylsiloxane coating (CAR-PDMS) and 50/30 μm divinylbenzene/carboxen on polydimethylsiloxane on a StableFlex fiber (DVB/CAR-PDMS) were evaluated. Several extraction times and temperature conditions were also tested to achieve optimum recovery. Suspensions of the samples in distilled water or in brine (25% NaCl in distilled water) were investigated to examine their effect on the composition of the headspace. The SPME fiber coated with 50/30 μm DVB/CAR-PDMS afforded the highest extraction efficiency, particularly when the samples were extracted at 60 °C for 15 min under dry conditions with toluene as an internal standard. Forty-five compounds were extracted and tentatively identified, most of which have previously been reported as odor-active compounds. The method developed allows sensitive and representative analysis of cocoa products with high reproducibility. Further research is ongoing to study chocolate making processes using this method for the quantitative analysis of volatile compounds contributing to the flavor/odor profile.

Keywords: Headspace solid-phase micro-extraction (HS-SPME); Gas chromatography–mass spectrometry (GC–MS); Chocolate; Cocoa; Volatile compounds

1. Introduction

Chocolate and cocoa are amongst the most popular flavors worldwide. Unlike many other natural flavors that have one principal compound responsible for their flavor [e.g. vanilla (vanillin), banana (amyl acetate), peach (γ-undecalactone) or almond (benzaldehyde)], chocolate and cocoa flavors reside in their volatile fraction, which is composed of a complex mixture of up to 500 compounds [1] with new research continuously increasing this number [2,3]. The complexity of this flavor is obvious and thus far it has not been possible for the chemist to duplicate the flavor. It has been known for some time that the compounds that give cocoa and chocolate their flavors are N- and O-containing molecules such as pyrazines, pyrroles, furans, aldehydes and amides, which are mainly generated during the Maillard reaction [4].

The analysis of volatile fractions of cocoa and chocolate is essential to study their aromatic profile. In general, the volatile fractions have been recovered by steam distillation or solvent extraction and analyzed by GC [5]. Considering the limitations of these sampling techniques, headspace solid-phase micro-extraction (HS-SPME) emerges as an attractive alternative [6]. The technique has been reported to be relatively cheap,

Abbreviations: SPME, solid-phase micro-extraction; GC–MS, gas chromatography–mass spectrometry; PDMS, polydimethylsiloxane; DVB, divinylbenzene; CAR, carboxen; HS, headspace; NCP, natural cocoa powder; CCP, conched chocolate powder; RI, retention index.

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solventless, fast and reproducible. SPME seems particularly appealing since it also eliminates problems associated with chemically and thermally unstable samples where generation of artifacts can be problematic as in the case of chocolate and cocoa. SPME, and HS-SPME in particular, in combination with GC and GC–MS, have been largely used in food [7,8], environmental [9] and biomedical analyses [10].

Counet et al. [11] used HS-SPME-GC–MS to analyze the flavor of 8 cocoa liquors and tentatively identified 43 compounds, of which 5 had never been associated with chocolate flavor. In each case, 2 g of the cocoa liquor was adsorbed onto a CAR-PDMS fiber for 20 min at 25 °C and deorbed for 5 min at 250 °C in the GC injector. Pini et al. [12] assessed HS-SPME-GC-FID for the analysis of the alkylpyrazine content in single-roasted cocoa liquor. The optimum recovery was obtained using the DVB/CAR-PDMS fiber when the extraction was performed at 60 °C for 45 min, following a 15 min equilibration. In related studies, they had also analyzed the pyrazine content of cocoa mass [13] and cocoa nibs [14] using a CAR-PDMS fiber under similar conditions.

The objective of this study was to investigate the use of HS-SPME-GC–MS to analyze cocoa products, such as natural cocoa powder (NCP) and conched chocolate powder (CCP). NCP is a product obtained from cocoa liquor which has been pressed hydraulically to reduce the fat content and milled into a powder [15]. CCP is a new product obtained by a similar process but using chocolate. The chocolate is prepared following the traditional process (mixing, refining and conching), then pressed hydraulically to reduce the fat content and ground into a fine powder.

In the present study, four types of SPME fibers, coated with different stationary phases (PDMS, PDMS-DVB, CAR-PDMS and DVB/CAR-PDMS), were used to examine their extraction efficiencies and compared for sensitivity. Conditions that might affect the SPME procedure, such as extraction time and temperature, were also investigated to determine the analytical performance of the selected fiber coatings.

2. Experimental

2.1. Materials

Non-alkalized natural cocoa powder (NCP) and conched chocolate powder (CCP), from West African origin, were obtained from The Chocolate Powder Company (Solihull, United Kingdom). The fat content of the NCP was 10–12%. The fat content of the CCP was 10–12% and the cocoa content was 69%. The NCP and CCP were stored in hermetic boxes at room temperature.

Analytical grade 2-methylpropanal, 3-methylbutanal, 2-methylbutanal, 2-phenyl-5-methyl-2-hexanal, 2,3-dimethylpyrazine, trimethylpyrazine, tetramethylpyrazine were purchased from Alfa Aesar (Heysham, United Kingdom) and acetone, benzyl alcohol, benzaldehyde, vanillin, caffeine and 1-(2-furanylmethyl)-1*H*-pyrrole from Sigma–Aldrich (Dorset, United Kingdom).

2.2. SPME extraction

Volatiles from the NCP and CCP samples were extracted using four fibers: 100 μm polydimethylsiloxane coating (PDMS), 65 μm polydimethylsiloxane/divinylbenzene coating (PDMS-DVB), 75 μm carboxen/polydimethylsiloxane coating (CAR-PDMS) and 50/30 μm divinylbenzene/carboxen on polydimethylsiloxane on a StableFlex fiber (DVB/CAR-PDMS). These fibers were purchased from Supelco (Bellefonte, PA, USA). Before extraction, the fibers were conditioned according to the supplier's instructions. Extractions were carried out in 10 mL PTFE/Silicone septa vials (Supelco, Bellefonte, PA, USA). Every septum was predrilled with a regular needle before sampling with the SPME fiber.

2.2.1. Selection of fiber coating

The sample (2.0 g) was placed in the vial and conditioned for 10 min at the extraction temperature, 60 °C. The extraction time was 15 min. The SPME fiber was then exposed to the headspace. The desorption time was 5 min and the temperature in the GC liner was 250 °C.

2.2.2. Selection of extraction time and temperature

The sample (2.0 g) was placed in the vial and conditioned for 10 min at the extraction temperature. The fiber was exposed for 1, 5, 10, 15 and 30 min to the headspace of the vial at four different temperatures, 25, 40, 60 and 80 °C. The desorption time was 5 min and the temperature in the GC liner was 250 °C.

2.2.3. Dry, wet and brine conditions

The sample (1.0 g) was placed in a vial (dry conditions). In a second vial the sample (1.0 g) was mixed with 4 mL of distilled water. The effect of the ionic strength was studied by substituting the water by brine (25% of NaCl in distilled water) in a third vial. In all cases the headspace extraction was carried out using the optimized conditions (T_{ext}: 60 °C; t_{ext}: 15 min). The desorption time was 5 min and the temperature in the GC liner was 250 °C.

2.2.4. Quantitative determination

The sample (2.0 g) was placed in the vial and toluene (50 μg) was added. The vial was conditioned for 10 min at the extraction temperature (60 °C). The fiber was exposed for 15 min to the headspace of the vial at the same temperature. The desorption time was 5 min and the temperature in the GC liner was 250 °C. The standard curves were obtained using 2-methylpropanal (slope: 2.5715; intercept: 0.0011; R^2: 0.9997), 3-methylbutanal (slope: 4.1238; intercept: −0.0019; R^2: 0.9997), 2-methylbutanal (slope: 16.79; intercept: −0.0149; R^2: 0.9996), 2,3-dimethylpyrazine (slope: 2.1598; intercept: 0.0175; R^2: 0.9990), trimethylpyrazine (slope: 3.7317; intercept: 0.0342; R^2: 0.9900) and tetramethylpyrazine (slope: 6.567; intercept: 0.00758; R^2: 0.9817).

2.3. Gas chromatography–mass spectrometry (GC–MS)

The analysis of volatiles extracted by HS-SPME was carried out using a Varian CP-3800 Gas Chromatograph linked to a Varian 1200L Quadrupole MS/MS. The GC was equipped with a CTC CombiPAL autosampler upgraded with a SPME analysis kit. The injector port has a deactivated glass SPME liner supplied by Supelco. The GC was fitted with a 30 m capillary column with a 0.25 mm i.d. and 1 μm FD, Factor 4 VF-5ms purchased from Varian. After absorption, the SPME fibers were introduced into the injector port of the GC (in splitless mode at 250 °C for 5 min). The volatiles extracted by the fibers were thermally desorbed and introduced in the capillary column. The GC was setup with a constant flow of 1.0 mL/min (helium), the oven temperature was programmed to start at 30 °C (5 min)–(10 °C/min)–200 °C–(25 °C/min)–280 °C (5 min). The transfer line was at 280 °C. The MS was setup with the source at 280 °C, where electronic ionization energy was −70 eV and with a 1200 V in the detector. One mass spectra scan every second was acquired. Three identical samples were prepared for each analysis. The average of their results is reported. The compounds were identified by a combination the US national Institute of Standards and Technology (NIST) 98 library of mass spectra and gas chromatographic retention indexes reported of standard compounds. Under these conditions, no sample carry-over was observed as confirmed by blank runs conducted between each analysis.

3. Results and discussion

3.1. Selection of fiber coatings

Fiber type selection is very important to define the optimum extraction conditions from the headspace. Several types of coating fibers, with a range of polarities and mechanisms, are commercially available for the analysis of volatiles of food samples [7]. PDMS-supported fibers are the best as they result in optimal long-term stability and overall performance, including chromatography, extraction characteristics and analyte recovery. These fibers are also very durable and usually retain their performance for up to 100 analytical cycles. The non-polar PDMS fiber is recommended by the supplier for the extraction of non-polar analytes, such as volatile flavor compounds but can also be applied to more-polar compounds. Mixed fiber coatings, containing DVB or CAR, increase retention capacity due to a mutually potentiating effect on extraction and distribution of the stationary phase. PDMS-DVB and CAR-PDMS have been used for the extraction of volatile low-molecular mass and polar analytes. The dual-coated fiber DVB/CAR-PDMS comprises a layer of PDMS-DVB over a layer of CAR-PDMS and is recommended for flavor and odor extraction (volatiles and semi-volatiles). The natural cocoa powder (NCP) and conched chocolate powder (CCP) were sampled using these four SPME fibers and analyzed by GC–MS, as described above. The objective was to establish which fibers reliably achieved the highest performance for the recovery of flavor compounds.

Altogether 42 compounds were extracted and identified on the basis of their mass spectra (MS) and retention indexes (RI) (Table 1). In general, the four fibers extracted compounds with RI from less than 500 (RI: 495 for acetone) to almost 1800 (RI: 1783 for caffeine). These included 9 aldehydes, 14 heterocyclic compounds, 5 ketones, 4 alcohols, 2 esters, 5 acids and 3 terpenes. Inspection of the total peak area for each fiber clearly showed that the most efficient fiber is CAR-PDMS, extracting around five times more than the PDMS-DVB and DVB/CAR-PDMS fibers and 25 times more than the PDMS fiber. Extraction with the latter fiber (total peak area = 5000–6000) was very weak compared to the other three fibers (total peak area > 200,000). Indeed very few peaks were observed on the chromatogram, with some undetected by the MS, making this fiber unsuitable for our study. The CAR-PDMS fiber presented the highest extraction (total peak area > 1,000,000) and proved to be very efficient at extracting volatiles, providing wide peaks at the beginning of the chromatogram (RI < 950). A split injection might lead to sharper peaks. However even under splitless conditions we were unable to extract most of the semi-volatiles, making this fiber undesirable for our study. Finally the PDMS-DVB and DVB/CAR-PDMS fibers resulted in similar extractions (total peak area = 200,000–300,000); the peaks were sharp and well distributed throughout the chromatogram. Both fibers would be suitable.

A number of compounds have been identified by gas chromatography-olfactometry (GC-O) as key odorant compounds in dark chocolate [2]. 2-Methylpropanal, 3-methylpropanal and 2-methylbutanal have been related to chocolate odors while trimethylpyrazine, 2,5-dimethylpyrazine and acetylpyrrole have been attributed cocoa odors. Methylpyrazine, 2,3-dimethylpyrazine, 2-ethyl-6-methylpyrazine and benzaldehyde added a hazelnut/almond note to the aroma profile of chocolate; ethylpyrazine, 3-ethyl-2,5-dimethylpyrazine and tetramethylpyrazine a roasty note. These compounds were tentatively extracted by all four fibers (Figs. 1 and 2).

In general, the PDMS fiber proved very inefficient at extracting key odorant compounds. The CAR-PDMS fiber was found to be the best fiber for the extraction of volatiles (RI < 950) while the PDMS-DVB was best at extracting semi-volatiles (RI > 950). In their study, Counet et al. [11] tentatively identified 43 flavor compounds using the CAR-PDMS fiber, only 22 of which were also extracted in our study. We attribute this difference to the matrix of the cocoa samples which has been shown to affect the composition of the headspace. Counet analyzed cocoa liquors which can contain up to 40% fat, whereas our study analyzed cocoa powders containing 10–12% fat. Interestingly, the DVB/CAR-PDMS seemed to extract both volatiles and semi-volatiles with the same efficiency. This dual-coated fiber proved to be the best fiber for the analysis of odorant compounds for both NCP and CCP because it combined the characteristics of the CAR-PDMS fiber with the addition of the properties of PDMS-DVB. The CAR layer allowed the fiber to extract the low molecular weight analytes (RI < 950), but with less efficiency than the CAR-PDMS, presumably because the layer of CAR is thinner in the dual-

Table 1
Effect of fiber type on the peak area ($\times 10^8$ area units) of natural cocoa powder (NCP) and conched chocolate powder (CCP) using HS-SPME-GC–MS[a]

No.	Compound[b]	RI	PDMS		DVB-PDMS		CAR-PDMS		DVB/CAR-PDMS	
			NCP	CCP	NCP	CCP	NCP	CCP	NCP	CCP
Volatile aldehydes and ketones										
1	Acetone[b]	495	65 ± 11	22 ± 8	1246 ± 11	243 ± 61	33,050 ± 3275	6311 ± 945	3073 ± 458	1660 ± 355
2	Methyl acetate[c]	521	18 ± 2	ND	456 ± 2	49 ± 23	14,080 ± 1648	982 ± 145	1416 ± 342	332 ± 82
3	2-Methylpropanal[b]	550	69 ± 12	66 ± 14	916 ± 12	661 ± 128	40,910 ± 5224	15,550 ± 2513	914 ± 245	1719 ± 320
4	2,3-Butanedione[c]	581	1783 ± 251	1292 ± 185	757 ± 251	562 ± 152	13,760 ± 2360	15,970 ± 2458	1908 ± 265	2925 ± 236
5	2-Butanone[c]	586	ND	ND	813 ± 145	224 ± 49	37,620 ± 5124	10,060 ± 1420	3470 ± 521	1567 ± 251
7	3-Methylbutanal[b]	653	139 ± 32	169 ± 29	3401 ± 242	3091 ± 423	77,690 ± 9423	86,430 ± 11,045	4439 ± 623	7179 ± 1366
8	2-Methylbutanal[b]	662	69 ± 14	97 ± 21	1361 ± 142	1476 ± 252	41,520 ± 6210	59,890 ± 8541	2241 ± 252	4231 ± 651
9	Pentanal[c]	696	ND	ND	304 ± 64	86 ± 26	7509 ± 845	3940 ± 687	906 ± 260	544 ± 124
10	3-Hydroxy-2-butanone[c]	707	ND	63 ± 10	1550 ± 194	1610 ± 210	19,490 ± 2180	49,900 ± 8511	1560 ± 310	4926 ± 845
12	35-Dimethyl-dihydro-furan-2-one[c]	766	11 ± 4	ND	610 ± 154	59 ± 24	5700 ± 945	2344 ± 322	583 ± 240	240 ± 62
15	Hexanal[c]	802	ND	ND	403 ± 94	57 ± 21	1221 ± 182	ND	607 ± 124	322 ± 58
Acids and alcohols										
6	Acetic acid[c]	630	1612 ± 211	1519 ± 261	29,290 ± 3250	21,460 ± 2854	389,700 ± 40,211	509,000 ± 61,525	78,310 ± 11,254	134,000 ± 21,804
11	Dimethylpropanedioic acid[c]	755	ND	ND	4875 ± 954	3125 ± 487	33,710 ± 4127	ND	2400 ± 325	3256 ± 812
13	2,3-Butanediol[c,d]	782	560 ± 102	670 ± 123	55,860 ± 5821	60,960 ± 6221	170,000 ± 22,943	276,500 ± 36,102	33,990 ± 5442	61,060 ± 12,209
14	2,3-Butanediol[c,d]	792	306 ± 51	314 ± 62	32,670 ± 4151	28,590 ± 3562	89,220 ± 12,452	123,300 ± 19,450	11,990 ± 2112	22,210 ± 3251
17	3-Methyl-butanoic acid[c]	837	185 ± 42	ND	16,180 ± 1822	15,420 ± 2511	35,600 ± 3254	92,010 ± 14,520	9667 ± 1225	14,860 ± 2524
18	2-Methyl-butanoic acid[c]	847	ND	ND	3491 ± 385	3.091 ± 520	7457 ± 1346	15,680 ± 2364	2146 ± 363	2666 ± 326
19	2-Furanmethanol[c]	854	6 ± 3	ND	439 ± 84	203 ± 38	3288 ± 651	3245 ± 458	996 ± 251	860 ± 152
30	Benzyl alcohol[b]	1048	ND	ND	256 ± 62	586 ± 154	234 ± 74	871 ± 210	118 ± 22	344 ± 54
35	Phenylethyl alcohol[c]	1133	78 ± 44	44 ± 9	6248 ± 1114	11,850 ± 1514	3158 ± 845	6628 ± 1125	5645 ± 945	7342 ± 945
36	Benzoic acid[c]	1157	17 ± 5	12 ± 4	157 ± 34	456 ± 86	106 ± 32	796 ± 124	189 ± 34	464 ± 214
39	Isopentyl benzoate[c]	1404	45 ± 11	ND	831 ± 214	166 ± 53	30 ± 15	ND	226 ± 14	61 ± 24
Pyrazines										
16	Methylpyrazine[c]	832	30 ± 13	18 ± 6	1117 ± 224	551 ± 94	17,480 ± 3254	19,690 ± 3458	3243 ± 432	4249 ± 832
20	2,5-Dimethylpyrazine[c]	921	146 ± 44	116 ± 34	8254 ± 1244	5817 ± 854	30,760 ± 1346	40,710 ± 8245	6987 ± 732	12,760 ± 1532
21	2,3-Dimethylpyrazine[b]	928	14 ± 6	ND	396 ± 85	764 ± 152	705 ± 251	ND	351 ± 122	1175 ± 232
22	Ethylpyrazine[c]	926	ND	ND	ND	ND	603 ± 124	259 ± 51	428 ± 74	1429 ± 645
27	Trimethylpyrazine[b]	1011	75 ± 21	123 ± 42	3517 ± 655	4936 ± 866	2578 ± 345	8396 ± 1257	1693 ± 645	4206 ± 532
28	2-Ethyl-6-methylpyrazine[c]	1013	2 ± 1	ND	143 ± 44	243 ± 62	106 ± 31	850 ± 53	101 ± 32	1489 ± 445
33	3-Ethyl-2,5-dimethylpyrazine[c]	1086	3 ± 1	ND	202 ± 57	824 ± 122	136 ± 42	651 ± 132	160 ± 35	690 ± 232
34	Tetramethylpyrazine[b]	1094	169 ± 54	105 ± 37	2648 ± 454	2484 ± 482	1150 ± 185	1846 ± 324	1714 ± 382	1829 ± 352
Semi-volatile aldehydes and ketones										
24	Benzaldehyde[b]	981	75 ± 34	54 ± 25	6138 ± 1032	6617 ± 1102	19,790 ± 3232	34,290 ± 4251	11,790 ± 1845	17,010 ± 2842
29	1*H*-Pyrrole-2-carboxaldehyde[c]	1018	7 ± 4	ND	424 ± 82	654 ± 148	777 ± 151	2653 ± 450	255 ± 82	624 ± 255
31	Benzeneacetaldehyde[c]	1062	33 ± 15	58 ± 34	1567 ± 284	13,780 ± 2152	164 ± 21	626 ± 122	1192 ± 355	5198 ± 1252
32	2-Acetylpyrrole[c]	1072	19 ± 6	19 ± 8	1479 ± 314	4235 ± 651	1829 ± 251	6870 ± 853	925 ± 132	2561 ± 512
37	3,5-Dihydroxy-6-methyl-2,3-dihydro-pyran-4-one[c]	1164	39 ± 21	ND	1341 ± 253	2628 ± 541	ND	ND	339 ± 82	734 ± 252
38	3,5-Dimethyl-benzaldehyde[c]	1250	ND	ND	391 ± 132	147 ± 25	47 ± 25	ND	161 ± 25	69 ± 18
40	Vanillin[b]	1419	7 ± 4	451 ± 148	699 ± 157	11,180 ± 2104	22 ± 9	777 ± 178	135 ± 34	1661 ± 258
Terpenes and others										
23	α-Pinene[c]	949	66 ± 28	ND	7478 ± 1612	ND	20,440 ± 3512	69 ± 22	6259 ± 845	ND
25	β-Pinene[c]	999	ND	ND	544 ± 126	ND	1273 ± 210	ND	287 ± 42	ND
26	35-Dimethyl-octane[c]	1007	ND	ND	4701 ± 745	ND	ND	ND	1340 ± 281	ND
41	8-Hydroxy-3-methyl-iso-chroman-1-one[c]	1565	ND	ND	106 ± 34	112 ± 35	ND	ND	22 ± 12	24 ± 18
42	Caffeine[b]	1783	170 ± 46	101 ± 54	348 ± 72	594 ± 142	ND	ND	107 ± 32	109 ± 34
Total			5816	5311	203,606	209,591	1,122,912	1,397,093	204,283	328,585

ND: compound not detected.
[a] All runs preformed with extraction at 60 °C for 15 min under dry conditions.
[b] Compound identified by GC–MS and RI using authentic compounds.
[c] Compound tentatively identified by GC–MS and RI using NIST98 database.
[d] Diastereoisomers.

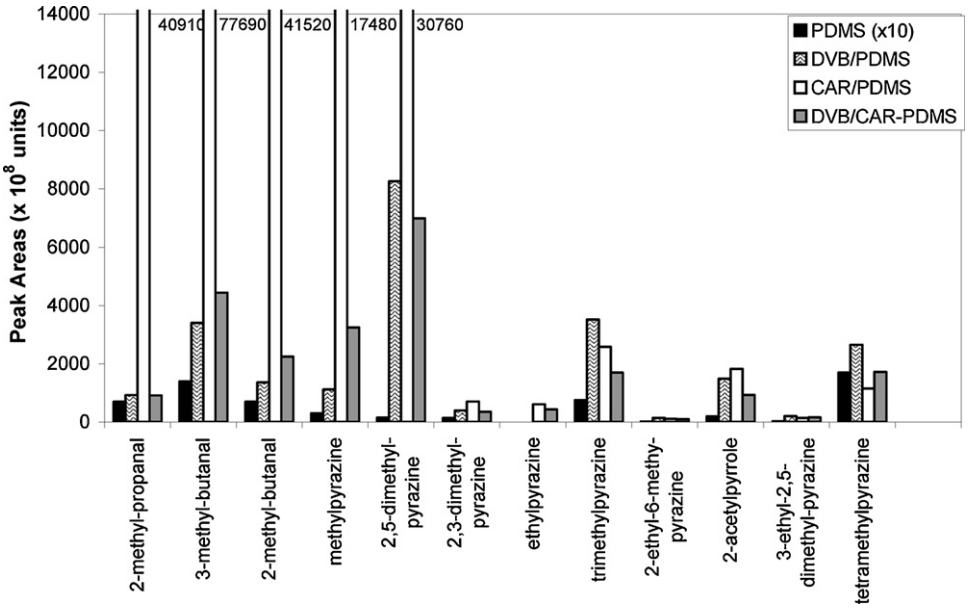

Fig. 1. Peak areas (in 10^8 area counts) of key odorant compounds identified by GC–MS extracted from NCP with four types of SPME fibers at 60 °C for 15 min.

coated fiber (75 μm in CAR-PDMS versus 30 μm in DVB/CAR-PDMS).

Furthermore, the DVB/CAR-PDMS fiber afforded high-resolution chromatograms when compared to the other fibers' chromatograms. We therefore decided to use this fiber to characterize the cocoa and chocolate products and establish their aroma and volatile profiles.

3.2. Selection of extraction time and temperature

Temperature and time of extraction are two important factors that control sample recovery by the fibers [16]. Consequently, these parameters were studied in order to establish the optimum extraction conditions. Time affects the mass transfer of the analytes onto the fiber; optimum time is required for the fiber to reach its equilibrium. Temperature directly affects how fast this equilibrium is reached, favoring the diffusion and it also has an influence on the composition of the volatile phase, increasing the ratio of compounds with low vapor pressure (semi-volatiles). A 3D representation of the extracted amount (total peak area) versus extraction times and temperatures (Fig. 3) clearly shows that the equilibrium is reached within 15 min (total peak area remains constant after that time).

The extraction was investigated at four different temperatures (25, 40, 60 and 80 °C) and the compounds extracted were divided into five main compound family: pyrazines, volatile aldehy-

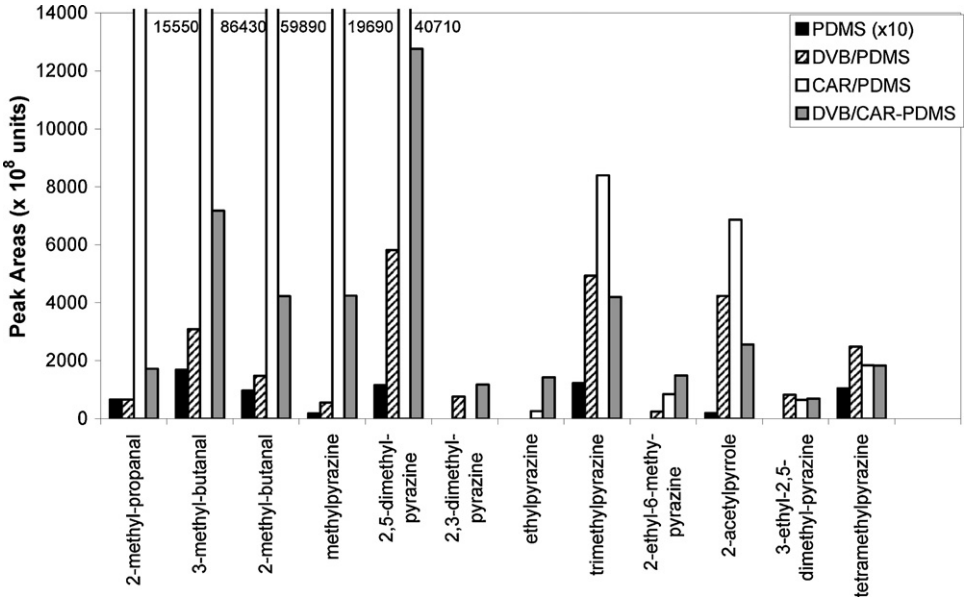

Fig. 2. Peak areas (in 10^8 area counts) of key odorant compounds identified by GC–MS extracted from CCP with four types of SPME fibers at 60 °C for 15 min.

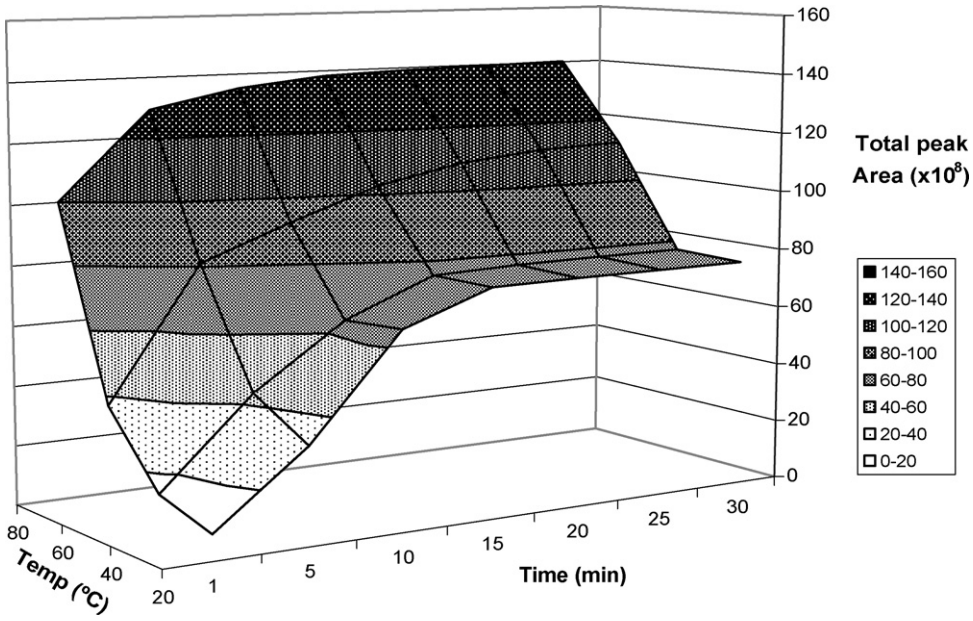

Fig. 3. Effect of extraction time and temperature on the total peak area (*x*-axis: time in min; *y*-axis: total peak area expressed in total ion counts; *z*-axis: temperature in °C). CAR/DVB-PDMS fiber was used.

des/ketones, semi-volatile aldehydes/ketones, acids/alcohols, and terpenes and others (Fig. 4).

In general, the amount extracted increases exponentially with the temperature of extraction, allowing optimum extraction at 80 °C. Nonetheless this temperature was discarded since chemical changes are likely to occur in the sample at such high temperature. The "acid and alcohol" fraction are better extracted at higher temperatures. The "pyrazine" and the "semi-volatile aldehyde and ketone" groups behave similarly at low temperatures (weak peaks) and from 60 °C their extraction efficiency is improved. Volatile compounds have a higher vapor pressure at room temperature; at 25 °C their concentration in the gas phase is higher than at higher temperatures when the presence of semi-volatiles starts to became important. On the other hand, there is a dramatic decrease in the "volatile aldehyde and ketone" family of compounds (30% at 25 °C) with increase of the temperature (only 5% at 60 °C). Peaks for 2-methylpropanal, 2-butanone or 3-methylbutanal almost disappear at 40 °C.

Augusto et al. [12] reported that the optimum extraction conditions were obtained when the cocoa liquor was extracted at 60 °C with 15 min of headspace equilibration and 45 min extraction while de Brito et al. [13] reported the extraction of cocoa mass under the same conditions. In their cases, the matrix is high in fat content (50% fat), while our cocoas are low in fat. The fat content affects the release of volatiles thus the extraction efficiency. In general, the extraction of compounds reached equilibrium within 15 min (Fig. 3). Looking at the families of compounds, the volatiles tended to be extracted within 10 min

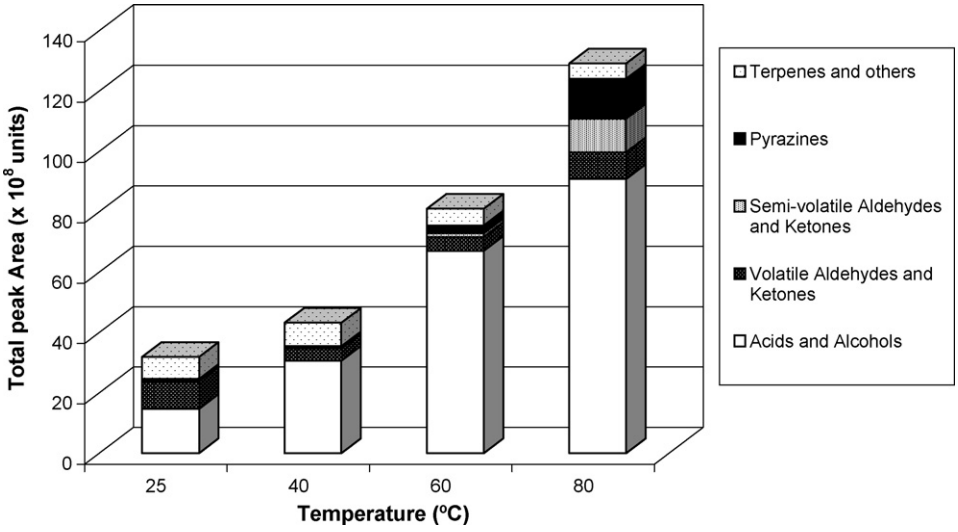

Fig. 4. Effect of extraction temperature on the extraction efficiency by main compound families (*x*-axis: temperature in °C; *y*-axis: total peak area expressed in total ion counts). DVB/CAR-PDMS fiber was used, and 5 min for extraction time.

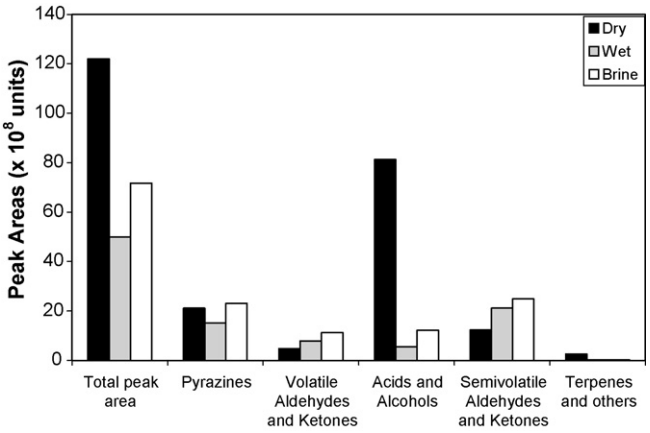

Fig. 5. Effect of dry, wet and brine conditions on the headspace compounds in NCP by SPME. CAR/DVB-PDMS fiber was used (sampling at 60 °C for 15 min).

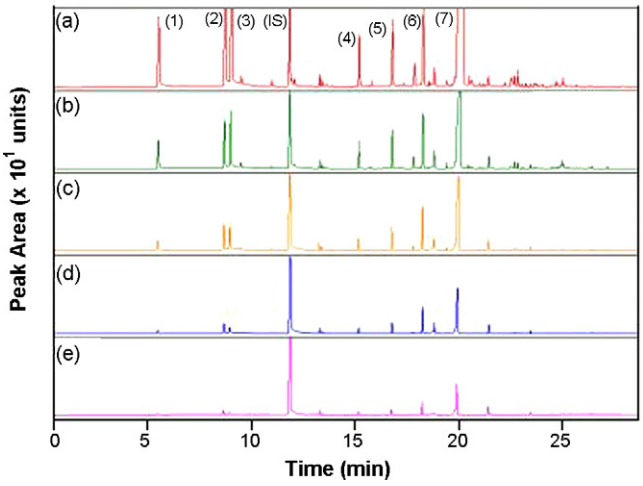

Fig. 6. HS-SPME-GC–MS chromatograms of standard mixture (Table 2) spiked with 50 μg of toluene (IS): (a) ratios as described in Table 2; (b) 1/3 dilution of (a); (c) 1/3 dilution of (b); (d) 1/3 dilution of (c); (d) 1/3 dilution of (c); peak identification: (1) 2-methylpropanal; (2) 3-methylbutanal; (3) 2-methylbutanal; (4) 2,3-dimethylpyrazine; (5) trimethylpyrazine; (6) tetramethylpyrazine; (7) 1-furfurylpyrrole. Peak area scale is the same for the five chromatograms.

but after 20 min, a decrease in the amount of volatile extracted was noticed. On the other hand, semi-volatiles took a bit longer to be extracted, i.e. 30 min. A consensus was reached to extract for 15 min which we consider to be the equilibrium for the competition between volatiles and semi-volatiles.

Taking these results into account (chromatograms available in Supporting information), the optimum conditions were chosen to be 60 °C for the extraction temperature and 15 min for the extraction time, preceded by 10 min of conditioning at the same temperature.

3.3. Study in wet, dry and brine conditions

In order to examine water and salt influence on the composition of the headspace, samples were analyzed under dry, wet and brine conditions.

In the absence of water, the amount of compounds extracted by the fiber was higher than under wet conditions (Fig. 5). Increasing the ionic strength by adding brine to the sample resulted in better extraction than in plain water, as recently reported by Pini et al. [12]. Comparing the chemical families, the largest difference was found in the "acid and alcohol" group, being the least well extracted. These results can be explained by the fact that acids and alcohols are very soluble in water, decreasing their presence in the volatile phase. The "pyrazine" group presented only weak signals in plain water which by increasing the ionic strength of the media increased significantly. The polarity of their nitrogen gives pyrazines an acceptable solubility in water, which in presence of NaCl makes them less soluble in water thus increasing their presence in the volatile fraction.

Table 2
List of compounds and their amounts in the initial solution (water 5 mL)

No.	Compound	Amount (mg)
1	2-methylpropanal	1
2	3-methylbutanal	1
3	2-methylbutanal	0.5
4	2,3-Dimethylpyrazine	1
5	Trimethylpyrazine	0.5
6	Tetramethylpyrazine	0.5
7	1-Furfurylpyrrole	0.5
IS	Toluene	0.05

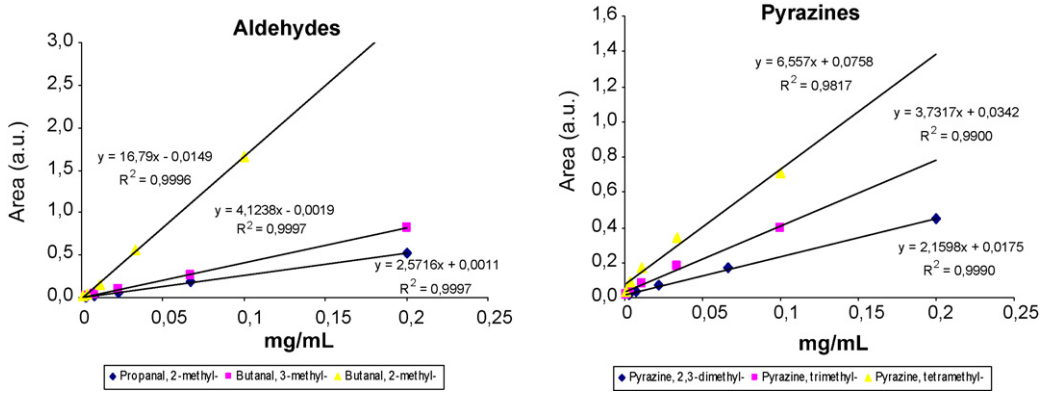

Fig. 7. Calibration curves of some representative standards obtained using SPME.

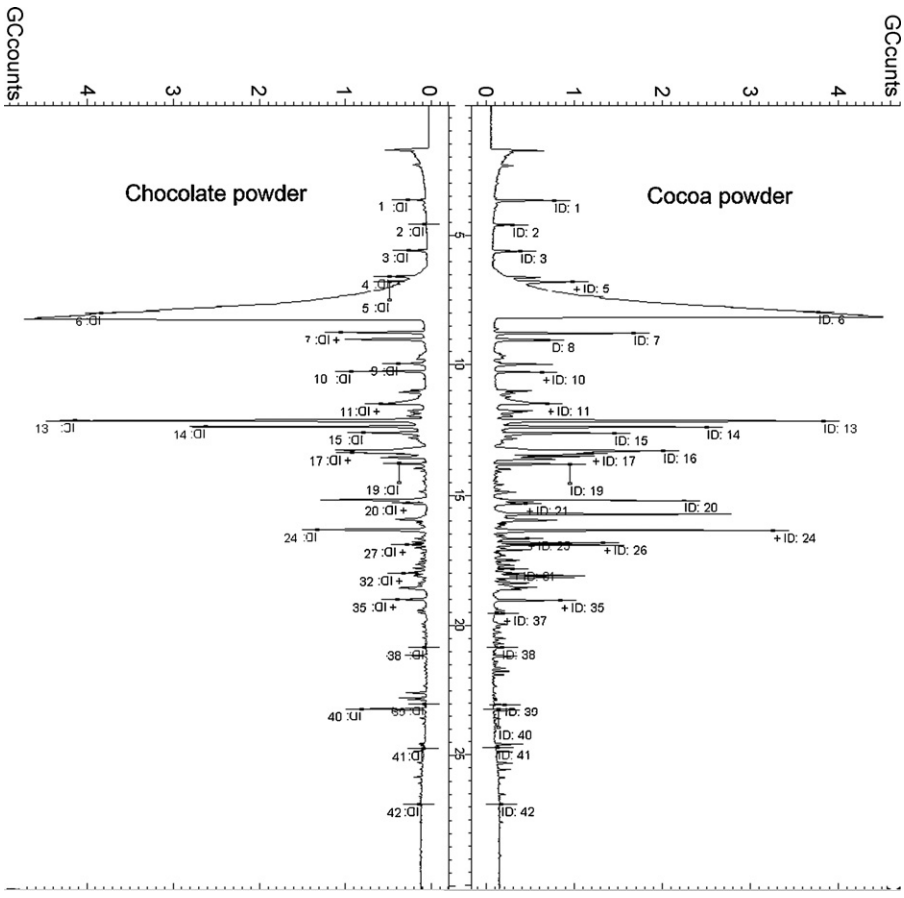

Fig. 8. Typical HS-SPME-GC–MS chromatograms of compounds extracted from chocolate (left) and cocoa (right) powders with DVB/CAR-PDMS fiber. Peak identification (see Table 1).

Three new pyrazines were tentatively identified under wet conditions, 3,5-dimethyl-2-isobutylpyrazine (RI: 1197), 2-isoamyl-6-methylpyrazine (RI: 1258) and 2,6-dimethyl-5-isopentylpyrazine (RI: 1325). These new pyrazines have longer carbon chains, increasing their hydrophobicity. As a consequence, their presence in the volatile phase is higher and can be detected. On the other hand, a few compounds could not be detected under wet conditions such as dimethylpropanedioic acid, 2,3-butanodiol and α-pinene.

3.4. Quantitative determination

Quantitative determination can be carried out by the method of internal standards which requires a calibration curve of each compound. A quantification in equivalents can also be used by using the method of the internal standard, in which ratios between the response of the individual compounds and that of the internal standard can be used (Table 2 and Figs. 6 and 7). In this case, toluene was used as an internal standard. The optimum amount of toluene added to the analyte was adjusted to 50 μg to avoid saturation of the detector and also to prevent it from masking peaks of interest. Subsequent semi-quantitative determination was accomplished with reference to the internal standard.

4. Conclusion

HS-SPME coupled to GC–MS has proven a valuable tool for analysis of volatile and semi-volatile compounds from cocoa and chocolate products. The technique is very sensitive to experimental conditions; fibre coating, temperature, extraction time, ionic conditions were shown to influence the extraction efficiency. Consequently the DVB/CAR-PDMS fibre was found to afford the most efficient extraction of both volatile and semi-volatile compounds from the analyte's headspace. An extraction temperature of 60 °C was found to be optimum to ensure efficient transfer from the headspace to the fibre while avoiding production of unwanted thermal artefacts, while an extraction time of 15 min was deemed sufficient. Although wet and ionic conditions presented slightly higher extraction efficiencies, they did not offer any significant advantage over the dry conditions. Finally quantitation of the volatile and semi-volatile fractions from cocoa and chocolate products was made possible by the method of internal standard and reproducibility was ascertained alongside.

The method is simple, sensitive, reproducible, rapid and low-cost for the evaluation of key odorant compounds found in cocoa and chocolate products (Fig. 8) and the mild conditions used for the analysis allow a good estimation of the aroma profile as

perceived by the human sensory organs (nose and tongue). This SPME method was developed to study small chemical changes that are sensorially detectable in cocoa and chocolate samples since there is an increasing interest to identify chemical modifications in the process leading to the production of cocoa liquor, cocoa powder and chocolate.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2007.08.034.

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