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Study of the effect of different fiber coatings and extraction conditions on dry cured ham volatile compounds extracted by solid-phase microextraction (SPME)

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Abstract

Extraction of dry cured ham volatile compounds by solid-phase microextraction (SPME) was optimized. Different fiber coatings (carboxen/polydimethylsiloxane (CAR/PDMS), divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS), polydimethylsiloxane (PDMS), polydimethylsiloxane/divinylbenzene (PDMS/DVB)), times of extraction (15, 30, 60 min) and sample preparation (ground samples and homogenates with NaCl saturated solution) were assayed. CAR/PDMS and DVB/CAR/PDMS fiber coatings extracted more than 100 volatile compounds and showed the highest area counts for most volatile compounds. CAR/PDMS coating extracted better those compounds whose Kovats index (KI) was lower than 980 (on average) and DVB/CAR/PDMS those with higher KI. Fifteen minutes of extraction provided a volatile compound profile with lower area counts for most compounds and qualitatively different to that obtained with 30 and 60 min of extraction. Homogenates gave a different profile compared to ground samples, with lower total counts for most compounds but higher proportion of aldehydes, and presence of several compounds not found in ground samples.

Keywords: Volatile compounds; Solid-phase microextraction; Dry cured ham; Extraction conditions

1. Introduction

Flavor of dry cured ham is a key characteristic for its quality [1]. The importance of flavor for consumer acceptance has resulted in a number of studies about flavor volatile compounds in different types of dry cured ham (Serrano, Iberian, Parma and French as most important), in which volatiles extraction was carried out by different methods, such as vacuum distillation [2], purge and trap [3–5], solid-phase microextraction [6–8] or simultaneous distillation/extraction with organic solvents [9,10].

Solid-phase microextraction (SPME) is a versatile sample preparation technique [11] that has been used, among other applications, to describe the volatile flavor profile of foodstuffs [12]. In comparison to traditional techniques for analyzing volatile constituents of foodstuffs, SPME is inexpensive, solvent free, easy to handle, sensitive, and selective [13]. In addition, SPME allows using mild sampling conditions, such as an extraction temperature below 50 °C, avoiding artifacts formation during sample analysis [14].

The key component of an SPME device is a fused-silica fiber that is coated on the outside with an appropriate stationary phase. Analytes from the sample are directly extracted and concentrated onto the fiber coating. The choice of the fiber coating is a key factor, since the type and amount of compounds that are extracted from the sample depends on the physico-chemical characteristics of the fiber stationary phase and on the film thickness. The affinity of the fiber for an analyte relies on the principle of 'like dissolves like', and coating fibers having different properties or thickness are selected in accordance with target compounds [15]. Non-polar polydimethylsiloxane (PDMS) fiber is preferred for the extraction of non-polar analytes, whereas the more polar polyacrilate (PA) fiber is more appropriate for the extraction

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of polar analytes. Mixed coating fibers, containing divinylbenzene (DVB) copolymers, templated resin (TPR) or carboxen (CAR) increase retention capacity. PDMS/DVB and CAR/DVB can be used for the extraction of low molecular weight volatile and polar analytes [15].

The amount of analyte extracted onto the fiber depends not only on the polarity and thickness of the stationary phase, but also on the extraction conditions and on the concentration of the analyte in the sample [15]. Extraction of analyte is typically improved by agitation, addition of sodium chloride or other salt to the sample, changing the pH, and increasing the temperature [15]. Different temperature and extraction times in the determination of the volatile profile of dry cured ham have been previously studied [6,7].

The optimal time for extraction should be the time to reach equilibrium. But for practical reasons, extraction times shorter than 60 min seem to be enough to analyze ham volatiles [6], as long as the extraction conditions are kept constant [16].

The effect of the way in which dry cured ham samples are prepared to carry out SPME extraction remains unstudied. Some research has been made on slices [6], cubes [8], and ground [17] dry cured ham. However, dry cured ham is a complex matrix whose heterogeneity causes variations in volatile compounds extraction [6]. Sample homogenization might reduce variability and make feasible the use of an internal standard [14]. Moreover, it would also allow the matrix to release intracellular volatile compounds more easily, as a result of the breakdown of muscle fibers and the magnetic stirring of the sample. Agitation in meat homogenates has been used in other studies [18] in order to increase volatile extraction. Sodium chloride addition to the sample improves extraction efficiency and supersaturation with salts is most effective for the extraction of analytes onto the fiber due to the salting-out effect [15]. The effect of the amount of sample and the addition of NaCl and/or $KHCO_3$ has been preliminarily studied in dry cured ham [7].

The aim of this work was to study the effect of fiber coating, extraction time, and homogenization with a sodium chloride solution on the volatile profile of dry cured ham analyzed by SPME, in order to determine the most suitable conditions for further analysis.

2. Materials and methods

2.1. Materials

Samples were taken from slices of a dry cured ham with protected designation of origin (‘Jamón de Teruel’) ripened for 1 year, supplied by a dry cured ham industry. In each slice the semimembranosus muscle was selected for the study in order to minimize sample variability. Samples were kept frozen at −80 °C until the day before analysis, and then they were placed in the refrigerator until the assay the following day.

2.2. Sample preparation

Semimembranosus slices were ground with a commercial grinder. One gram of this ground ham was weighed into a 4 ml vial, which was screw-capped with a laminated Teflon-rubber disk. Dry cured ham homogenates were prepared by homogenizing 5 g of ground semimembranosus slices with 25 ml of distilled water saturated with NaCl. One milliliter of this mixture was placed into 4 ml vial that was also screw-capped with a laminated Teflon-rubber disk. A magnetic stirrer was placed into the homogenates for stirring during extraction.

2.3. SPME sampling

Different SPME fiber coatings and several extraction times were used to study the volatile profile of ground dry cured ham and dry cured ham homogenates. The assayed fibers were carboxen/polydimethylsiloxane (CAR/PDMS) (85 μm thickness), divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) (50/30 μm thickness), polydimethylsiloxane (PDMS) (100 μm thickness), and polydimethylsiloxane/divinylbenzene (PDMS/DVB) (65 μm thickness). Before the SPME fiber was inserted into the vial, the sample was equilibrated for 15 min at the extraction temperature (35 °C). Extraction times studied were 15, 30, and 60 min.

Prior to analysis the SPME fiber was preconditioned in the injection port of the gas chromatograph at the temperature and for the time suggested by the manufacturers. This temperature was the same as that set for the injection port of the gas chromatograph during desorption. Temperatures set for desorption were 280 °C for CAR/PDMS, 270 °C for DVB/CAR/PDMS, 250 °C for PDMS, and 260 °C for PDMS/DVB. Analysis of each tested condition was repeated twice.

2.4. Gas chromatography-mass spectrometry

Analyses were performed using a Hewlett-Packard 6890 series II gas chromatograph coupled to a mass selective detector (Hewlett-Packard HP 5973) (Wilmington, USA). Volatiles were separated using a 5% phenyl-methyl silicone HP-5) bonded-phase fused silica capillary column (Hewlett-Packard, 50 m × 0.32 mm i.d., film thickness 1.05 μm), operating at 6 psi of column head pressure, resulting in a flow of 1.3 ml min^{-1} at 40 °C. The SPME fiber was desorbed and maintained in the injection port at the temperature and for the time suggested by manufacturers. The injection port was in splitless mode. The temperature program was isothermal for 10 min at 40 °C, raised to 200 °C at a rate of 5 °C min^{-1} and then raised to 250 °C at a rate of 20 °C min^{-1} and held for 5 min. *n*-Alkanes (Sigma R 8769, Saint Louis MO, USA) were run under the same chromatographic conditions as the samples to calculate the Kovats indices (KI) of detected compounds. The transfer line to the mass spectrometer was maintained at 280 °C. The mass

spectra were obtained using a mass selective detector by electronic impact at 70 eV, a multiplier voltage of 1756 V, and collecting data at a rate of 1 scan s^{-1} over the *m*/*z* range of 30–550 u.m.a. Compounds were tentatively identified by comparing their mass spectra with those contained in the NIST/EPA/NIH and Wiley libraries and by comparison of their KI with those reported in the literature [19,20]. Results from volatile analyses are provided in total area counts.

3. Results and discussion

Twenty-four different combinations of type of adsorbent fiber, sample preparation, and extraction time were assayed in order to evaluate their effect on dry cured ham volatile compounds extracted by SPME. Extraction temperature was set to 35 °C for all samples. A previous study about extraction of volatile compounds by SPME in dry cured ham showed that several compounds strongly increased in area when extraction was performed at 60 °C but not at 40 °C, probably due to thermal generation of such compounds during extraction [6]. In the present study, fiber exposure was set at 35 °C in order to avoid such type of volatile compounds generation processes, especially lipid oxidation, during extraction.

A total of 112 volatile compounds were tentatively identified in this study, although not all of them were detected with all fibers and conditions tested. Identified compounds belonged to the following chemical groups: acids (11 compounds), alcohols (17 compounds), aldehydes (19 compounds), ketones (23 compounds), esters (3 compounds), sulfur compounds (5 compounds), aliphatic hydrocarbons (20 compounds), aromatic hydrocarbons (4 compounds), terpenes (2 compounds), phenols (1 compound), nitrogen compounds (4 compounds), furans (2 compounds), and ethers (1 compound). These groups of volatile compounds basically agree with those reported for dry cured ham by different authors using SPME [6,8,17,21].

3.1. SPME fiber coating

Total area counts obtained for each studied condition are shown in Table 1. Total ion chromatograms of dry cured ham samples extracted with DVB/CAR/PDMS and CAR/PDMS fibers are shown in Figs. 1 and 2. CAR/PDMS coated fibers extracted the highest total amount of volatile compounds, followed by DVB/CAR/PDMS. The total amount of compounds extracted with PDMS and PDMS/DVB was much lower. In fact, in ground samples and for 30 min of extraction, they extracted 3 and 0.74%, respectively of the amount extracted with CAR/PDMS under the same extraction conditions. Therefore, PDMS and PDMS/DVB fibers were discarded for further detailed consideration about individual compounds due to the low total area counts and the smaller number of compounds extracted. Moreover, the extracted compounds were basically aliphatic hydrocarbons (45.45% in PDMS and 57.47% in PDMS/DVB) (data not shown), which are not really relevant for the aroma of dry cured ham due to their high threshold value [10], whereas other volatile compounds previously pointed out as ripening markers or significant for dry cured ham flavor [5,22] were not extracted. The wider range of compounds and higher total area extracted with the bipolar porous coatings CAR/PDMS and DVB/CAR/PDMS than with PDMS and PDMS/DVB fibers have been previously shown by other authors in several foodstuffs [8,23]. However, in the first published study about SPME extraction of volatile compounds in dry cured ham [6], the number of volatile compounds extracted using a PDMS fibers was higher than in the present study. Nevertheless, it could be a question of different sensitivities of the MS equipments used in the previous study and in the present one, the former allowing the detection of compounds in lower amounts.

Table 2 shows the relative percentages of the different chemical groups extracted by CAR/PDMS and DVB/CAR/PDMS fibers in both ground and homogenized samples and for 15, 30, and 60 min of extraction. DVB/CAR/PDMS fiber favored the extraction of acids whereas the profile of volatile compounds extracted using CAR/PDMS fibers showed much higher proportions of aliphatic hydrocarbons. In ground samples extracted with CAR/PDMS fiber the major group was that of the aliphatic hydrocarbons followed by aldehydes, ketones, alcohols, acids, and sulfur compounds. The rest of chemical families showed less than 1% of chromatographic area. Using the same sampling conditions, the profile us-

Table 1
Total area counts of identified volatile compounds extracted by SPME with different fiber coatings for 15, 30, and 60 min of extraction in both ground and homogenized dry cured ham samples

Sample	Fiber	15 min	30 min	60 min
Ground	CAR/PDMS	3,676,875	4,994,839	7,976,442
	PDMS	111,086	141,663	141,484
	DVB/CAR/PDMS	1,927,467	2,242,177	3,238,448
	PDMS/DVB	44,135	36,974	44,077
Homogenized	CAR/PDMS	1,608,430	2,989,234	4,063,605
	PDMS	111,334	114,730	130,172
	DVB/CAR/PDMS	1,529,064	2,237,297	2,101,916
	PDMS/DVB	26,006	29,404	32,822

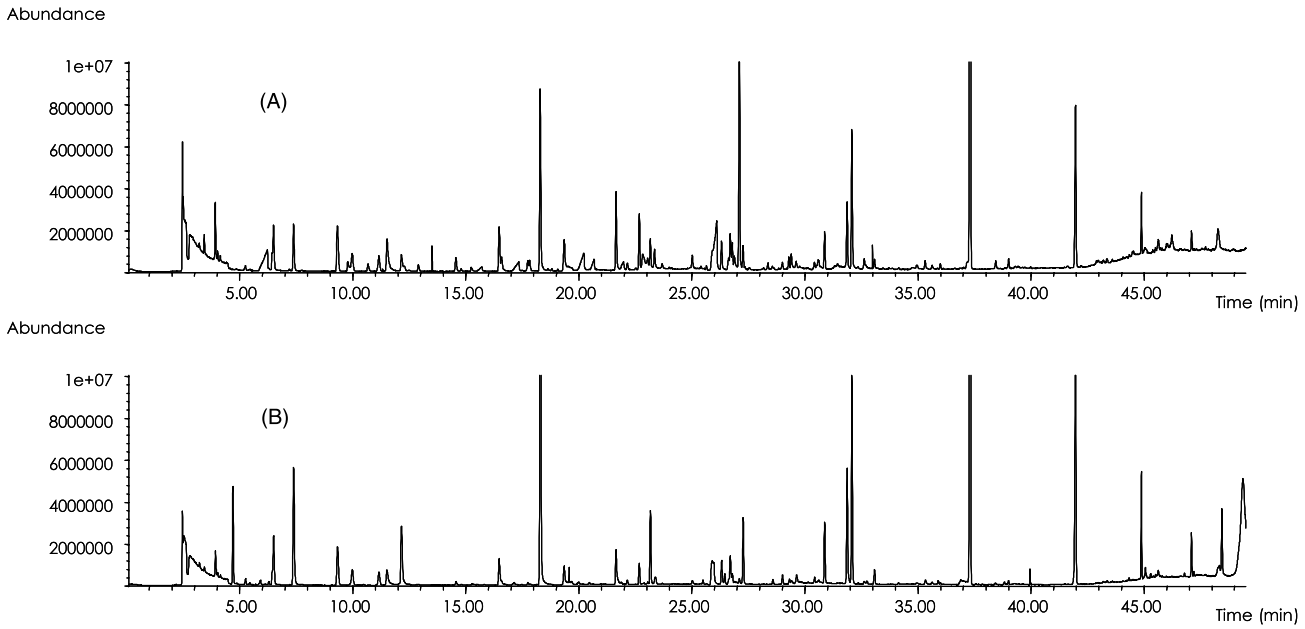

Fig. 1. Total ion chromatograms of dry cured ham volatiles extracted for 30 min with DVB/CAR/PDMS SPME fibers. (A) ground sample; (B) homogenized with ClNa solution sample.

ing a DVB/CAR/PDMS fiber showed a higher percentage of acids, terpenes, phenols, nitrogen compounds, and furans and lower of ketones (most of them of low molecular weight) and of aliphatic hydrocarbons than that obtained using the CAR/PDMS one. These results concerning the type of compounds adsorbed by each kind of fiber were in agreement with previous works [8,24].

Table 3 shows the total area of compounds extracted with CAR/PDMS and DVB/CAR/PDMS fibers in both ground and homogenized samples for 30 min of extraction. For all families of compounds and both in ground and in homogenized samples before CAR/PDMS extracted higher amounts of compounds with lower KI, whereas the amount of compounds with higher KI was greater using the DVB/CAR/PDMS fiber. This agrees with previous available information, in which the CAR/PDMS coating is generally recommended for extracting small analytes [24] whereas the DVB/CAR/PDMS is recommended for larger analytes.

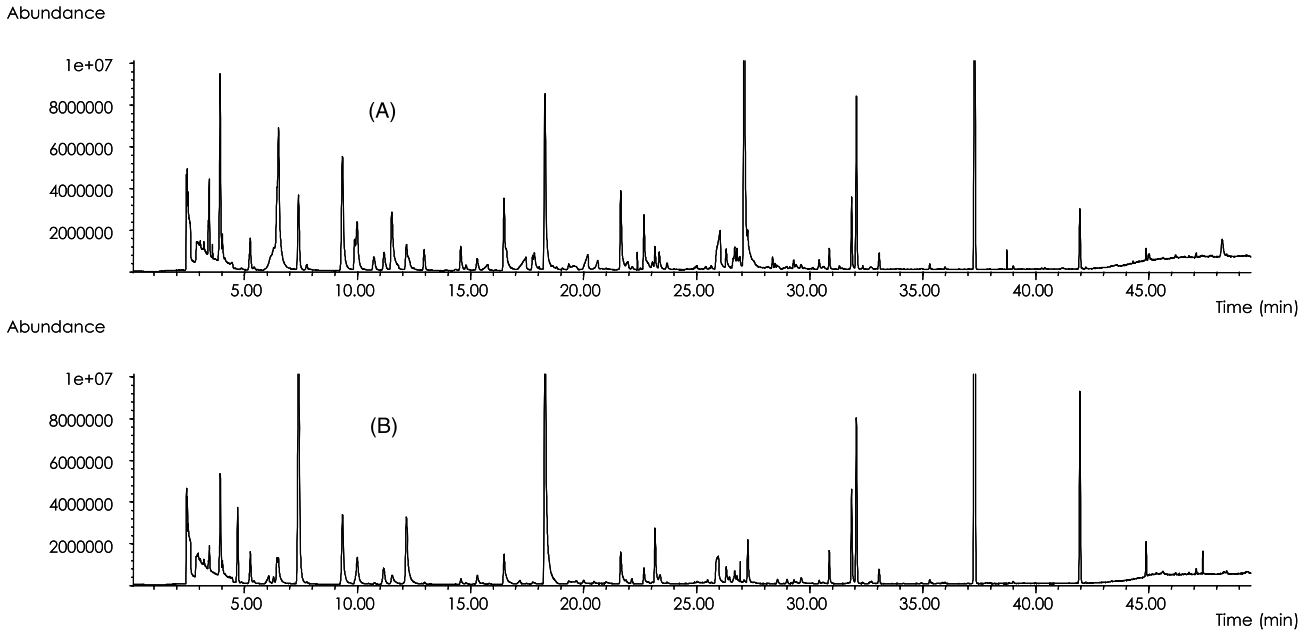

Fig. 2. Total ion chromatograms of dry cured hams volatiles extracted for 30 min with CAR/PDMS SPME fibers. (A) ground sample; (B) homogenized with ClNa solution sample.

Table 2
Proportion of chromatographic area of each chemical family of volatile compounds extracted with CAR/PDMS and DVB/CAR/PDMS fibers for 15, 30, and 60 min of extraction in both ground and homogenized dry cured ham samples

	CAR/PDMS						DVB/CAR/PDMS					
	Ground			Homogenized			Ground			Homogenized		
	15 min	30 min	60 min	15 min	30 min	60 min	15 min	30 min	60 min	15 min	30 min	60 min
Acids	7.81	12.58	13.32	2.34	3.28	4.44	14.64	18.32	20.27	2.70	3.95	7.05
Alcohols	13.27	14.67	13.91	9.87	9.50	11.41	14.60	13.98	14.71	9.29	10.73	12.63
Aldehydes	20.32	24.78	24.04	64.90	62.34	66.20	24.29	25.79	25.83	72.45	70.59	63.37
Ketones	25.40	20.19	19.02	15.16	19.29	12.12	16.26	17.04	18.29	8.81	9.33	10.15
Esters	0.06	0.07	0.07	0.07	0.04	0.05	0.28	0.20	0.12	0.06	0.05	0.07
Sulfur compounds	1.21	1.21	1.09	1.71	1.35	1.32	0.64	0.80	0.72	0.64	0.62	0.57
Aromatic hydrocarbons	0.95	0.67	0.80	0.19	0.14	0.13	0.91	0.92	0.96	0.18	0.17	0.21
Terpens	0.08	0.23	0.20	0.02	0.01	0.01	0.09	1.77	1.93	0.06	0.04	0.05
Phenols	0.07	0.08	0.10	0.01	0.03	0.03	0.21	0.19	0.23	0.04	0.03	0.44
Nitrogen compounds	0.28	0.40	0.51	0.3	0.26	0.30	0.57	0.85	0.99	0.24	0.28	0.45
Aliphatic hydrocarbons	28.88	24.88	25.81	5.30	3.67	3.80	26.84	19.40	15.30	5.26	3.96	4.70
Furans	0.25	0.21	0.23	0.07	0.06	0.12	0.42	0.45	0.46	0.17	0.17	0.19

Values are the percentage of total chromatographic area of each chemical family.

Carboxen is a porous carbon with a high surface area (around 1200 $m^2 g^{-1}$) which has been described as adequate for extraction of low boiling point compounds, although it allows the adsorption of a wide range of compounds due to the presence of different types of pores (micro-, meso-, and macropores). On the other hand, the layer of PDMS-DVB in the DVB/CAR/PDMS fiber provides this type of fibers the ability to extract high boiling point compounds. These characteristics explain the different amounts of compounds with low and high boiling point extracted with each type of fiber observed in this study.

Gianelli et al. [8], found that DVB/CAR/PDMS extracted a higher number of compounds than CAR/PDMS (60 versus 41 compounds, respectively). In our study differences in extracted compounds between both types of fibers were scarce. Using DVB/CAR/PDMS a total of 99 compounds were identified in ground samples, and 87 in homogenates, whereas using CAR/PDMS were 103 and 85, respectively. Strangely, the most noteworthy difference between both types of fibers was the lack of extraction of some of the high KI hydrocarbons with the DVB/CAR/PDMS fibers. Besides, both CAR/PDMS and DVB/CAR/PDMS extracted some of the compounds highlighted as odor active compounds in different types of dry cured ham [21,22,25], such as 2-heptanone, 3-methylbutanal, methanethiol, hexanal, 2-methylpropanal, 1-octen-3-ol or 2-hexenal, although this latter compounds was not detected using the DVB/CAR/PDMS fiber in ground samples. Thus, it seems that both porous fibers tested, CAR/PDMS and DVB/CAR/PDMS, provides a similar volatile compounds profile for dry cured ham, and that both satisfactorily extracts most compounds previously highlighted as odor active compounds in this meat product.

3.2. Extraction time

Extraction time of 15, 30, and 60 min were studied for each type of fiber and each sample preparation. These times were not selected for studying equilibria, since longer equilibration times are required for most volatiles identified [8]. Studied times were chosen for practical purposes, in order to check the shorter extraction time giving rise to a representative volatile profile of dry cured ham.

In general, the longer the extraction time, the higher the amount of total volatile compounds obtained (Table 1). PDMS and PDMS/DVB were affected in a lesser extent than the porous fibers CAR/PDMS and DVB/CAR/PDMS, giving rise with 15 min of extraction to areas around 80% (or even higher) of those obtained with 60 min of extraction (see Table 1). CAR/PDMS fiber showed a marked extraction time effect: 30 min of extraction gave rise to 62 and 73% (ground and homogenized, respectively) of the amount extracted with 60 min. On the other hand, DVB/CAR/PDMS fiber extracted similar amounts for 30 and 60 min of extraction in homogenized samples, but 44% more in 60 min extraction in ground samples. All these results are not surprising, since equilibrium for most compounds takes a longer time than those studied in this work, as it has been observed in other studies [6,8]. For example, Gianelli et al. [8], when comparing these two fibers for the analysis of aldehydes in dry cured ham concluded that equilibrium for these compounds was reached in 3 h of exposure for DVB/CAR/PDMS and in 5 h for CAR/PDMS.

Table 2 shows the pattern of chemical families of compounds extracted at 15, 30, and 60 min of extraction in both ground and homogenized samples for both types of porous fibers under study. For CAR/PDMS fibers and ground samples, 15 min of extraction gave rise to lower proportion of acids and aldehydes and higher of ketones and alyphatic hydrocarbons. The trend was similar for homogenized samples, although the differences were smaller. For DVB/CAR/PDMS fiber, the profile obtained with 15 min of extraction time also showed a lower proportion of acids and higher of aliphatic hydrocarbons. However, aldehydes and ketones showed a different behavior to that found for CAR/PDMS

Table 3
Total area counts of identified dry cured ham volatile compounds extracted for 30 min by SPME with either a CAR/PDMS or a DVB/CAR/PMS coated fiber in ground and homogenized with a saturated sodium chloride solution sample

RT	KI[a]	Rel[b]	Compounds	CAR/PDMS		DVB/CAR/PDMS	
				Ground	Homogenized	Ground	Homogenized
Acids							
6.26	591	MS, KI	Acetic acid	293,759	24,326	108,848	10,688
11.87	693	MS, KI	Propanoic acid	19,005	N.D.	8,294	N.D.
15.71	757	MS, KI	2-Methylpropanoic acid	22,105	1,792	15,057	1,202
17.47	788	MS, KI	Butanoic acid	78,190	10,186	37,665	6,440
20.25	841	MS, KI	3-Methylbutanoic acid	61,687	8,455	58,063	5,575
20.70	851	MS, KI	2-Methylbutanoic acid	24,207	4,271	25,375	3,802
22.01	878	MS, KI	Pentanoic acid	16,416	4,580	13,880	3,377
26.13	976	MS, KI	Hexanoic acid	107,617	40,600	124,067	43,970
32.62	1161	MS, KI	Octanoic acid	3,637	2,381	7,428	5,048
35.62	1264	MS, KI	Nonanoic acid	602	1,047	9,151	6,772
38.44	1364	MS, KI	Decanoic acid	906	262	2,865	1,456
			Total	628,131	97,900	410,693	88,330
Alcohols							
3.44	–	MS	Ethanol	99,990	27,197	18,010	5,364
5.20	548	MS, KI	1-Propanol	13,453	3,903	1,729	766
7.75	622	MS, KI	2-Methyl-1-propanol	10,514	2,095	1,349	542
10.71	673	MS, KI	1-Methoxi-2-propanol	41,894	4,019	14,592	1,479
11.17	681	MS, KI	1-Penten-3-ol	50,139	N.D.	N.D.	N.D.
14.33	734	MS, KI	3-Methyl-3-buten-1-ol	3,373	536	1,247	492
14.55	738	MS, KI	3-Methyl-1-butanol	58,442	12,960	21,342	8,400
14.78	742	MS, KI	2-Methyl-1-butanol	14,814	2,252	3,280	1,567
16.49	770	MS, KI	1-Pentanol	159,339	95,315	65,832	61,068
21.64	870	MS, KI	1-Hexanol	176,373	79,881	105,498	77,584
23.05	900	MS, KI	2-Heptanol	16,870	2,185	8,183	4,630
23.35	907	MS, KI	2-Butoxyethanol	25,656	5,953	19,516	6,008
26.31	980	MS, KI	1-Octen-3-ol	43,495	34,768	33,000	49,445
28.17	1030	MS, KI	2-Ethyl-1-hexanol	3,360	1,509	2,502	3,000
28.56	1041	MS, KI	Benzenemethanol	1,083	N.D.	1,142	N.D.
29.61	1071	MS, KI	1-Octanol	11,232	10,894	10,855	18,588
31.44	1124	MS, KI	Benzeneethanol	2,799	424	5,086	1,208
			Total	732,826	283,891	313,163	240,141
Aromatic hydrocarbons							
16.58	772	MS, KI	Methylbenzene	29,495	4,167	15,783	3,446
21.53	868	MS, KI	Ethylbenzene	910	58	709	193
21.88	875	MS, KI	1,4-Dimethylbenzene	2,034	N.D.	3,083	249
25.56	962	MS, KI	Propylbenzene	973	N.D.	948	N.D.
			Total	33,412	4,225	20,523	3,888
Esters							
4.60	523	MS, KI	Acetic acid methyl ester	1,210	186	663	148
7.20	612	MS, KI	Acetic acid ethyl ester	1,599	904	2,566	904
24.11	926	MS, KI	Hexanoic acid methyl ester	705	N.D.	1,318	N.D.
			Total	3,514	1,090	4,547	1,052
Aldehydes							
2.96	–	MS	Acetaldehyde	2,173	5,657	147	2,116
5.26	550	MS, KI	2-Methylpropanal	64,706	69,631	6,385	10,442
9.34	649	MS, KI	3-Methylbutanal	367,191	217,608	91,266	97,723
9.98	660	MS, KI	2-Methylbutanal	161,809	106,099	45,579	46,569
12.16	698	MS, KI	Pentanal	88,710	256,119	38,256	198,206
15.13	748	MS, KI	2-Methyl-2-butenal	1,102	906	693	881
15.81	759	MS, KI	2-Pentenal	N.D.	2,342	N.D.	1,866
18.29	801	MS, KI	Hexanal	428,139	927,913	266,321	838,702
20.99	857	MS, KI	2-Hexenal	1,320	4,090	N.D.	1,915
23.16	902	MS, KI	Heptanal	43,361	112,804	40,954	140,483
25.50	960	MS, KI	2-Heptenal	N.D.	4,686	N.D.	8,111
25.89	970	MS, KI	Benzaldehyde	24,830	39,567	13,198	34,931

Table 3 (*Continued*)

RT	KI[a]	Rel[b]	Compounds	CAR/PDMS		DVB/CAR/PDMS	
				Ground	Homogenized	Ground	Homogenized
27.26	1005	MS, KI	Octanal	13,966	40,883	15,190	59,582
29.00	1054	MS, KI	Benzeneacetaldehyde	3,543	6,965	9,217	15,746
30.86	1106	MS, KI	Nonanal	33,578	55,258	47,495	99,229
34.12	1208	MS, KI	Decanal	1,735	1,011	2,560	1,925
35.90	1274	MS, KI	2-Decenal	N.D.	2,188	N.D.	6,078
37.55	1331	MS, KI	2,4-Decadienal	N.D.	N.D.	N.D.	2,287
38.82	1373	MS, KI	2-Undecenal	N.D.	862	N.D.	4,342
			Total	1,236,163	1,854,589	577,261	1,571,134
Ketones							
4.02	–	MS	2-Propanone	254,475	149,626	512,96	30,850
6.43	598	MS, KI	2-Butanone	191,480	294,912	55,477	45,443
9.87	658	MS	1-Hydroxi-2-propanona	72,335	7,829	23,646	1,202
11.51	686	MS, KI	2-Pentanone	186,057	37,671	80,864	25,385
12.95	711	MS, KI	3-Hydroxi-2-butanona	44,794	3,853	13,618	2,248
17.74	791	MS, KI	2-Hexanone	24,545	3,170	11,690	4,929
18.71	810	MS, KI	2-Methyldihydro-3(2H)furanone	N.D.	N.D.	292	N.D.
20.46	846	MS, KI	2-Methylcyclopentanone	1,338	N.D.	471	N.D.
22.52	889	MS, KI	3-Heptanone	N.D.	540	2,284	794
22.67	892	MS, KI	2-Heptanone	132,458	36,121	57,082	35,318
24.00	923	MS	3-Ethylcyclopentanone	1,707	159	1,810	249
25.40	958	MS, KI	5-Methyldihydro-2(3H)-furanone	7,746	632	3,661	N.D.
26.46	984	MS, KI	2,3-Octanedione	1,142	9,365	1,221	20,714
26.62	988	MS, KI	3-Octanone	15,846	5,353	13,065	8,625
26.78	992	MS, KI	2-Octanone	26,011	8,816	18,137	11,050
29.29	1062	MS, KI	5-Ethyldihydro-2(3H)-furanone	15,765	5,428	13,684	N.D.
30.13	1085	MS	8-Nonen-2-one	2,276	869	2,304	1,496
30.42	1094	MS, KI	2-Nonanone	19,893	5,857	10,429	9,062
32.69	1163	MS	5-Propyldihydro-2(3H)-furanone	2,341	1,215	2,710	1,232
33.71	1194	MS, KI	2-Decanone	1,265	N.D.	1,740	514
35.98	1277	MS, KI	5-Butyldihydro-2(3H)-furanone	3,854	2,000	6,019	2,979
39.00	1379	MS, KI	5-Pentyldihydro-2(3H)-furanone	4,774	3,276	12,669	6,890
			Total	1,010,102	576,692	384,169	208,980
Sulfur compounds							
3.20	–	MS	Methanethiol	6,477	6,642	6,626	4,952
4.45	517	MS, KI	Dimethyl sulfide	7,461	2,684	5,641	4,130
4.86	534	MS, KI	Carbon disulfide	2,857	1,647	2,676	795
15.28	750	MS, KI	Dimethyl disulfide	43,424	29,465	3,104	3,884
23.39	908	MS, KI	3-Methylthio propanal	1,461	8,903	1,108	8,165
			Total	61,680	49,341	19,155	21,926
Terpens							
24.79	943	MS, KI	Alpha-pinene	1,965	N.D.	720	N.D.
28.47	1039	MS, KI	Limonene	9,328	436	38,974	881
			Total	11,293	436	39,694	881
Nitrogen compounds							
15.23	749	MS, KI	Pyridine	2,492	3,545	6,450	2,545
19.55	827	MS, KI	Methylpyrazine	2,546	540	1,275	401
23.69	915	MS, KI	2,6-Dimethylpirazine (+ dihydro-2(3H)-furanone)	15,088	2,603	8,072	2,310
27.35	1007	MS, KI	Trimethylpyrazine	N.D.	1,114	3,339	1,103
			Total	20,126	7,802	19,136	6,358
Aliphatic hydrocarbons							
4.03	500	MS, KI	Pentane	33,355	19,669	5,857	2,914
5.46	558	MS, KI	2-Methylpentane	N.D.	N.D.	3,026	N.D.
5.92	577	MS, KI	3-Methylpentane	3,160	941	1,705	748
6.49	600	MS, KI	Hexane	306,635	76,806	101,558	69,798
7.87	624	MS, KI	Methylcyclopentane	618	N.D.	782	N.D.
12.30	700	MS, KI	Heptane	11,552	N.D.	6,224	N.D.

Table 3 (*Continued*)

RT	KI[a]	Rel[b]	Compounds	CAR/PDMS		DVB/CAR/PDMS	
				Ground	Homogenized	Ground	Homogenized
17.78	792	MS, KI	1-Octene	50,327	N.D.	16,254	N.D.
18.63	808	MS, KI	(Z)-2-Octene	2,535	N.D.	1,781	N.D.
18.82	812	MS	Branched alkene	2,868	N.D.	2,183	N.D.
19.06	817	MS, KI	(Z)-2-Octene	3,152	N.D.	1,862	N.D.
24.49	935	MS	Branched alkene	680	N.D.	N.D.	N.D.
24.58	937	MS, KI	Propylcyclohexane	1,653	N.D.	654	N.D.
24.67	940	MS	Branched alkene	1,343	N.D.	N.D.	N.D.
24.93	946	MS	Branched alkene	6,406	1,067	N.D.	N.D.
25.01	948	MS	Branched alkene	10,091	N.D.	N.D.	N.D.
25.65	964	MS	Branched cycloalkane	7,017	2,192	3,830	2,153
27.10	1000	MS, KI	Decane	793,748	6,644	286,645	13,025
29.13	1057	MS	Alkane	2,062	N.D.	N.D.	N.D.
33.44	1186	MS	Branched alkene	875	N.D.	1,548	N.D.
39.63	1400	MS, KI	Tetradecane	1,358	N.D.	N.D.	N.D.
			Total	1,239,435	107,319	433,909	88,638
Furans							
12.48	703	MS, KI	2-Ethylfurano	2,825	N.D.	1,459	1,415
26.88	995	MS, KI	2-Pentylfurane	7,635	1,734	8,554	2,336
			Total	10,460	1,734	10,013	3,751
Phenols							
29.76	1075	MS, KI	4-Methylphenol	2,099	794	2,438	457
Ethers							
4.14	504	MS, KI	Ethyl ether	2,125	1,047	6,519	1,778

N.D.: not detected; RT: retention time.

[a] Kovats index calculated for a DB-5 capillary column.

[b] MS, mass spectrum tentatively identified using NIST, EPA, NDH, and Wiley libraries. KI, Kovats index in agreement with literature.

fibers. Thus, aldehydes were scarcely affected in their proportion in ground samples, and showed a marked decrease with extraction time in homogenized samples. Ground samples extracted for 15 min with DVB/CAR/PDMS fibers showed a much lower proportion of terpenes than those extracted for 30 and 60 min. The effect of extraction time on the profile of volatile compounds identified in dry cured ham is most likely due to their different volatilities and affinities for the fiber coating. Those with longer equilibration time should increase their proportion with longer extraction times, whereas those achieving equilibrium in shorter times should not increase in absolute area with longer extraction times, and therefore, decrease in proportion.

Nevertheless, the extracted compounds were mostly the same with each studied extraction time (data not shown). Decanal and 3-heptanone were not detected using 15 min of extraction with CAR/PDMS fibers in ground samples, but they were in homogenates. DVB/CAR/PDMS fibers showed the same extracted volatiles in ground samples with any time of extraction except for 2-methylcyclopentanone, that was not detected using 15 min of extraction. Compounds extracted with this fiber were the same for 30 and 15 min of extraction, and using 60 min only very small amounts of a couple of additional compounds were detected.

For practical purposes, any of the studied times would provide useful information for studying dry cured ham volatiles. Nevertheless, given that the total amount of extracted compounds is markedly lower using 15 min of extraction, perhaps 30 min would be a reasonable choice for analysis.

3.3. Sampling conditions

The effect of the form in which dry cured ham samples were prepared for extraction, either ground or homogenized with a saturated sodium chloride solution, on the total area of volatile compounds identified using different fiber coatings and for different extraction times is shown in Table 1. Total ion chromatograms of dry cured ham volatiles extracted with DVB/CAR/PDMS and CAR/PDMS fibers from both ground and homogenized with a saturated sodium chloride solution samples are shown in Figs. 1 and 2.

In general, there was a reduction of the amount of extracted volatiles in homogenates for almost all the fibers and for all the times. The CAR/PDMS coating in homogenized samples extracted around 44, 60, and 51% of the total area counts extracted in ground samples for 15, 30, and 60 min, respectively. The effect of sample preparation was lower for the DVB/CAR/PDMS fiber, homogenized samples showing 79, 100, and 65% of the total area identified in ground samples for the three times of extraction. However, given that homogenized samples had been diluted (1:5) with a saturated sodium chloride solution, there was a net increase in

the total amount of volatile compounds extracted per gram of sample. This confirms previous studies in which homogenization with different salts gave rise to higher extraction yields for most compounds due to its salting-out effect [26].

Table 2 shows the proportion of each chemical family of volatile compounds detected using DVB/CAR/PDMS and CAR/PDMS fibers for 15, 30, and 60 min of extraction in both ground and homogenized samples. The profiles obtained with both CAR/PDMS and DVB/CAR/PDMS in homogenates offered a high proportion of aldehydes compared with the rest of chemical groups for every time tested, ranging from 62 to 72% of the total area. This contrasted with the proportion found in ground samples (between 20 and 24% for CAR/PDMS and 24 and 26% for DVB/CAR/PDMS). Some of these aldehydes were not even found in ground samples (2-pentenal; 2-heptenal; 2-decenal; 2,4-decadienal; 2-undecenal) (Table 3). In CAR/PDMS fibers, this relative increase of aldehydes sharply reduced the proportion of aliphatic hydrocarbons, acids, and alcohols in homogenized samples compared to ground ones. In DVB/CAR/PDMS fibers the trends for most families were similar.

Homogenization led to a lack of detection of some compounds, such as propanoic acid or alpha-pinene, but especially of several aliphatic hydrocarbons of high KI, whereas it allowed the detection of a number of compounds, mostly aldehydes. The increase in aldehydes extracted with homogenate samples might be due to their lower solubility in saturated NaCl solution due to the change of polarity of the medium. Stirring may also contribute to higher extraction due to its effect reducing the time to achieve equilibrium [26]. This would mean a higher extracted amount of a given compounds in a shorter time. In addition, the disruption of the cell structure, which is almost intact in dry cured ham, could also favor the extraction of compounds that would be otherwise hold into the cells and extracted with more difficulty. However, all these effects should have been evidenced in most compounds, and not selectively in aldehydes. For example, aliphatic hydrocarbons show a lower polarity than aldehydes, and the salting-out effect should have been clearer for them. Therefore, the higher extraction of aldehydes in homogenized samples could be also due to induced lipid oxidation during extraction, due to both membrane disruption together with the prooxidant effect of sodium chloride.

Therefore, homogenization might have the advantage of increasing aldehyde extraction, together with the possibility of internal standard addition, but further evaluation of its prooxidant effect should be carried out.

In conclusion, both CAR/PDMS and DVB/CAR/PDMS SPME fiber coatings are feasible for studying dry cured ham volatiles. From a practical point of view it seems that 30 min, but even 15 min, are enough for obtaining a volatile profile of this meat product. Finally, homogenization of dry cured ham samples with a saturated sodium chloride solution together with stirring during extraction might allow the detection of lower levels of several volatile compounds, but its influence in formation of lipid oxidation compounds during extraction has to be further evaluated.

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